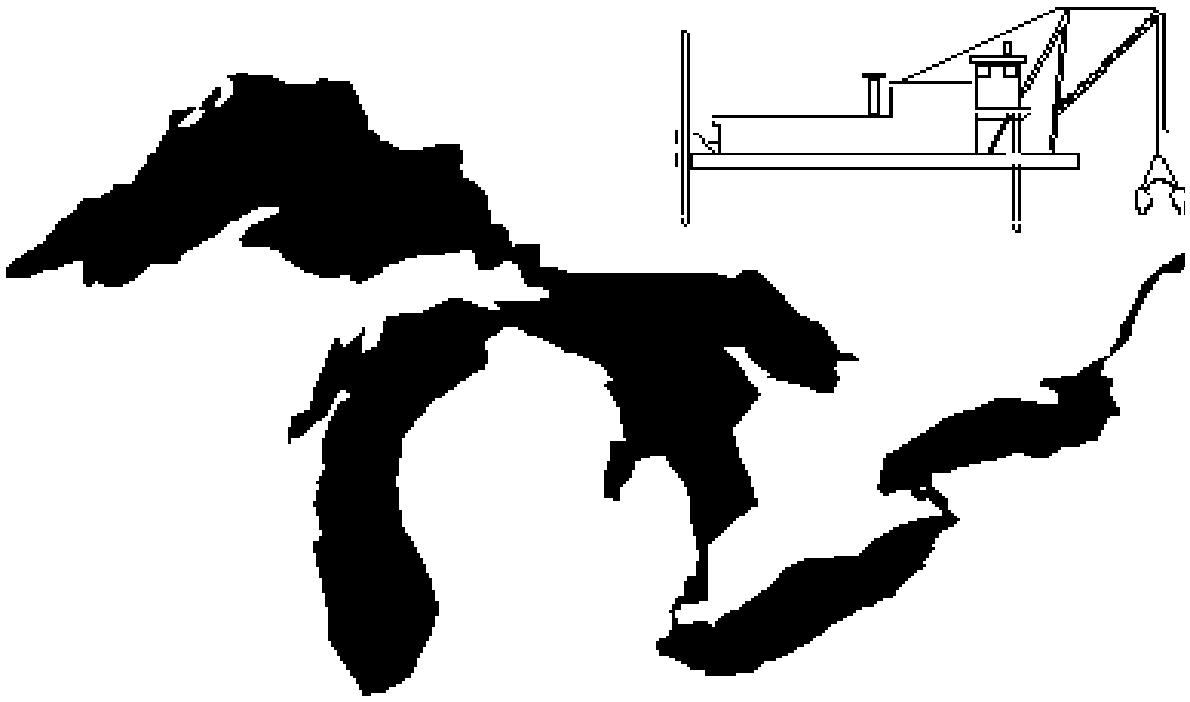
EPA

US Army Corps of Engineers

Great Lakes Dredged Material Testing and Evaluation Manual



GREAT LAKES
DREDGED MATERIAL TESTING AND EVALUATION MANUAL

Prepared by:

U.S. Environmental Protection Agency
Regions 2, 3, 5, and
Great Lakes National Program Office

and

U.S. Army Corps of Engineers
Great Lakes & Ohio River Division

FINAL DRAFT

Version
September 30, 1998

DISCLAIMER

Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the USEPA or USACE.

ACKNOWLEDGEMENTS

This manual was prepared by the USEPA/USACE Great Lakes 404(b)(1) Task Group. The members of this Task Group are as follows:

Mr. Dennis Anderson	USACE, St. Paul District
Dr. Gary Ankley	USEPA, Environmental Research Laboratory - Duluth
Mr. Mark Barath	USEPA, Region 3
Dr. Patricia Boone	USEPA, Region 5
Mr. John Dorkin	USEPA, Region 5
Mr. Al Fenedick	USEPA, Region 5
Mr. Steve Garbaciak	USACE, Great Lakes National Program Office[a]
Ms. Pam Horner	USACE, Detroit District
Mr. Alex Lechich	USEPA, Region 2[2]
Mr. David Melfi	USACE, Buffalo District
Mr. Jan Miller	USACE, Great Lakes & Ohio River Division
Mr. Scott Pickard	USACE, Buffalo District
Mr. Jay Semmler	USACE, Chicago District
Mr. Frank Snitz	USACE, Detroit District[a]
Dr. Marc Tuchman	USEPA, Great Lakes National Program Office
Dr. Thomas Wright	USACE, Waterways Experiment Station[a]
Mr. Howard Zar	USEPA, Region 5

The co-chairs of the Task Group are Dr. Marc Tuchman and Mr. Jan Miller. In addition to the above Task Group members, valuable support was provided by the following:

Dr. Larry Brooke	Lake Superior Research Institute
Dr. Daniel Call	Lake Superior Research Institute
Ms. Joan Clarke	USACE, Waterways Experiment Station
Dr. Tom Dillon	USACE, Waterways Experiment Station[a]
Dr. David Moore	USACE, Waterways Experiment Station[2]
Mr. Mario Paula	USEPA, Region 2
Dr. Brian Schumacher	USEPA, Environmental Monitoring Systems Laboratory
Mr. George Schupp	USEPA, Region 5[a]
Ms. Kathy Schroer	USEPA, Region 5
Ms. Ann Strong	USACE, Waterways Experiment Station
Mr. Richard Sutton	USACE, Chicago District[a]
Mr. Matthew Williams	USEPA, Region 5
Ms. Jennifer Wolf	USEPA, Region 5[a]

[a] Affiliation no longer current.

PREFACE

The availability of the draft Great Lakes Dredged Material Testing and Evaluation Manual for public review and comment was announced in the Federal Register on December 19, 1994. Approximately 500 copies of the draft were distributed. A synopsis of the general comments received is provided below. These comments were evaluated by the USEPA and USACE. In order to save printing and distribution costs, the entire manual will not be reprinted. Only those pages that have been modified to address review comments and the recent revision to the Section 404(b)(1) Guidelines will be distributed to recipients of the draft manual for insertion in the manual. With these changes, the manual is finalized and ready for implementation.

Comments were received from State agencies, lake user groups, consulting firms and environmental interest groups. One comment recommended that the manual provide dredging performance requirements. Another recommended that the manual provide testing guidance for fill materials as well as dredged material. While regional guidance on these areas would be worthwhile, it is beyond the intended scope of this manual. The USEPA and USACE are working toward joint guidance on a variety of issues related to dredged material management on a national level. On a regional level, the USEPA and USACE will continue together to address priority issues related to dredging and dredged material management.

Some comments indicated misunderstandings as to the applicability of the manual. This manual provides guidance that is to be used in evaluations conducted under Section 404(b)(1) of the Clean Water Act. The Clean Water Act does not apply in Canadian waters of the Great Lakes. The guidance in this manual does not bind States as far as their authority under Section 401 of the Clean Water Act, although it was the intent of the developers of this manual that the testing procedures provide the information necessary for States to make decisions regarding Section 401 certification.

A comment was received questioning why the manual did not address the sampling of sediments beneath those to be dredged, as these sediments would be exposed by the dredging operation. The potential impacts of sediments exposed by dredging may be a relevant issue to be addressed in the overall 404(b)(1) evaluation or in an environmental assessment/impact statement. However, this manual has focused on only a part of the 404(b)(1) evaluation, that dealing with contaminant related impacts of dredged material discharges.

Navigation users commented on the length of the document, complexity of the evaluation and costs of biological tests as adversely impacting the maritime industry. In contrast, some environmental interest groups criticized the tiered testing system as sacrificing protection for cost-savings. Throughout the development of this manual, the USEPA and USACE have attempted to balance these conflicting concerns.

The USEPA and USACE concur that there will be some short-term increases in costs with the implementation of this manual, particularly from the use of biological toxicity and bioaccumulation tests. However, we believe that in the long run the manual will help standardize the decision making process, and make the management of dredged material more predictable. This should help navigation interests better plan their dredging activities. The improvements in quality assurance and documentation which are included in the manual should also enable long-term decisions to be made based on test results, and reduce the need to test a project every time it is dredged. Biological effects-based tests have been utilized routinely for ocean disposal decision making for almost 20 years without a significant adverse impact on navigation.

The USEPA and USACE do not believe that a tiered testing approach sacrifices the interests of environmental protection. This approach is more systematic and reasoned than requiring all tests for all materials, and focuses the evaluation at dredged materials that have a greater likelihood of causing contaminant impacts. For those dredged material where there is reason to believe contaminant impacts might result, the biological effects-based tests recommended in this manual represent a scientifically sound and environmentally protective basis for decision making.

A related comment suggested that dredged material be analyzed for, at a minimum, all of the bioaccumulative contaminants of concern (BCC) identified in the Great Lakes Initiative. The manual does, in fact, reference the BCC list for consideration in developing contaminant of concern lists (page 26). But the agencies believe that it is more reasonable to develop site specific lists of contaminants of concern that reflect local conditions and sources of contamination, rather than to apply a "standard list" of contaminants to all sites and situations.

Several comments were received regarding the definition and use of reference sediment. Since the release of the draft manual, the USEPA published proposed rulemaking related to the 404(b)(1) Guidelines in the Federal Register on 1/4/95. The substance of this rulemaking was to include a definition of reference sediment comparable to that already used in ocean

disposal regulations. The draft of this manual had utilized the reference sediment definition, anticipating the rulemaking would occur before this manual was finalized. Finalization of this rulemaking has been delayed. Because of the uncertainty regarding the rulemaking, this regional guidance document will be finalized consistent with the existing Guidelines which do not include a definition of reference sediment. The existing Guidelines specify that dredged material are evaluated compared to sediment from the disposal site. As most open water disposal sites in the Great Lakes are dispersive in nature, this manual will encourage a broad interpretation of "disposal site."

Two reviewers proposed that additional testing methods be incorporated into the manual. It remains the intent of the USEPA and USACE that this manual be a "living document" and that it be updated periodically to incorporate new or modified testing procedures. Before new methods can be incorporated, they must be fully evaluated for appropriateness to this regulatory program. The evaluation and documentation of testing methods currently in the manual required substantial time and effort, and it was not considered appropriate to delay the finalization of this manual while other methods were evaluated. The methodologies proposed by reviewers will be considered for inclusion in the first update to this manual.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

GREAT LAKES
DREDGED MATERIAL TESTING AND EVALUATION MANUAL

1. INTRODUCTION

1.1 Purpose

This manual presents guidance on testing and evaluation for proposed discharges of dredged material into the United States waters of the Great Lakes Basin.

1.2 Authority

The U.S. Environmental Protection Agency (USEPA) Regions 2, 3, and 5, and U.S. Army Corps of Engineers (USACE) North Central Division, have jointly prepared this regional guidance under the authority provided in 40 CFR (Code of Federal Regulations) Section 230.2(c), pursuant to the requirements of Section 404(b)(1) of the Clean Water Act (CWA), Public Law 92-500. This regional guidance is consistent with the national guidance presented in Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. - Testing Manual (USEPA/USACE, 1998), also known as the "Inland Testing Manual."

1.3 Applicability

These guidance are applicable to all proposed discharges of dredged material to the United States waters of the Great Lakes Basin. This includes disposal operations conducted under Section 404 permits issued by the USACE or authorized State agency, as well as Federal projects conducted by the USACE.

Issues relevant to the identification and delineation of wetlands are outside the scope of this manual. In addition, this manual does not provide guidance on the identification of disposal sites for dredged material. Guidance on the selection of disposal sites is provided in "Evaluating environmental effects of dredged material management alternatives--A technical framework" (USACE/USEPA 1992).

This manual will not, in general, address concerns with fill material. The rationale for this omission is that the evaluation and testing described herein is focused upon chemical contaminants. Fill material, such as stone or soil from commercial sources, is not usually a significant carrier of contaminants. Exceptions to this may be specific fill materials which could be carriers of chemical contaminants or when dredged material is used for fill. This manual will also not address the impacts of the excavation or dredging activities during a

dredging and disposal operation.

The testing and evaluation procedures described herein provide only a portion of the information necessary for a complete evaluation of a proposed dredged or fill material discharge, as required by Section 404(b)(1). These testing procedures are directed at the "contaminant determination" portion (40 CFR 230.11(d)) of the larger 404(b)(1) evaluation, although the information obtained through these testing procedures are relevant to other determinations. The final determination of acceptability of any proposed discharge of dredged material also considers the probable impact, including cumulative impacts of the proposed discharge, on the public interest.

The evaluation and testing guidance in this manual will be effective on August 1, 1998 and will be reevaluated at least every five years and revised as necessary by the USEPA in conjunction with the USACE. It is intended that this manual be a "living document" and that additional guidance and updates to evaluation procedures be distributed for incorporation as available.

1.4 Definitions

Acronyms and abbreviations used in this manual are listed in Appendix A. Definitions of terms used are provided in Appendix B. The following definitions are included here because of their importance to understanding the scope and content of this manual.

The **Great Lakes** and **Great Lakes Basin** refers to the United States waters of Lakes Michigan, Superior, Huron, Erie, Ontario, the connecting channels, St. Lawrence River, their tributaries and any other waterbodies within the United States watersheds of these Lakes.

Discharges of dredged material refers to the discharge of dredged material to waters of the United States and includes discharges of water from dredged material disposal operations including beach nourishment, upland, or confined disposal which return to waters of the United States.

Guidelines refers to the Section 404(b)(1) regulations found in 40 CFR 230.

The term **guidance** may refer to either national or regional implementation manuals developed to assist the evaluator in making a contaminant determination as defined in 404(b)(1) Guidelines.

Disposal site is that portion of the United States waters where specific disposal activities are proposed or permitted. It consists of a bottom surface area and all overlying water, if present. If the disposal site is dispersive in nature (e.g., an area subject to currents or wave energies sufficient to transport dredged material), the disposal site might (for purposes of obtaining a sediment sample) be considered to include areas adjoining the immediate disposal location. Regional guidance on the collection of sediment sample(s) from the disposal site is provided in paragraph 4.3.2 and Appendix D.

1.5 History of National Guidelines and Guidance

The discharge of dredged or fill materials to waters of the United States is regulated under Section 404 of the Clean Water Act (CWA), Public Law 92-500. An evaluation of a proposed discharge of dredged or fill materials must be completed in compliance with Section 404(b)(1) of this Act, pursuant to 40 CFR 230.10. Compliance is determined by the Secretary of the Army acting through the Chief of Engineers, and is based upon the 404(b)(1) Guidelines promulgated by the Administrator of the USEPA in conjunction with the USACE.

The first Guidelines were issued in 1975 and, pursuant to these Guidelines, the USACE published an interim guidance manual entitled "Ecological evaluation of proposed discharge of dredged or fill material into navigable waters" (USACE 1976). The amendments to the CWA in 1977 and experience gained between 1975 and 1980 led to a revision of these Guidelines. This revision, at 40 CFR 230, became a final rule on December 24, 1980.

A key component in determining compliance with the Guidelines is the evaluation and testing procedure for the material proposed for discharge pursuant to 40 CFR 230.60 and 230.61. These procedures had been addressed in the interim guidance manual in 1976 and revised procedures were provided on December 24, 1980, as a proposed rule. The final rule specified, at 40 CFR 230.61, that the chemical, physical, and biological evaluation and testing that were based upon the 1975 Guidelines remain in effect until final rule-making. Although a final rule has yet to be issued, additional experience gained since 1980 has indicated that the 1976 manual is in need of revision.

In 1990, the USEPA and USACE began efforts to update the 1976 national guidance manual. The updated national manual has proceeded in parallel with the development of this regional guidance for the Great Lakes.

1.6 History of Regional Guidance

The Guidelines and national guidance are general in nature and lack some of the specificity appropriate for project-specific evaluations. Under 40 CFR 230.2(c), regional guidance on the implementation of 404(b)(1) Guidelines may be developed by the USEPA in conjunction with the USACE. Prior to the development of this regional guidance manual, no previous guidance for testing dredged material for proposed discharge to the Great Lakes had been developed under this authority.

The USEPA and USACE have used criteria and guidelines based on the physical and chemical properties of dredged material to make decisions about discharges to the Great Lakes since the late 1960's. The "Jensen criteria" were a list of numerical levels for seven sediment physical and chemical parameters to be used in the evaluation of dredging projects in fresh and marine waters. These criteria were disseminated by the USEPA Headquarters in early 1971, prior to the 1972 Clean Water Act.

These "Jensen criteria" were used in the Great Lakes to determine which dredged material required disposal to a confined disposal facility (CDF), constructed under Section 123 of PL 91-611 (Rivers and Harbors and Flood Control Act of 1970). These "criteria" were modified by Region 5, USEPA, in 1974, allowing for a determination based on the collective information and not any single pass-fail number. In 1977, the USEPA, Region 5, published "Interim guidelines for the pollutional classification of Great Lakes harbor sediments" (USEPA 1977). These guidelines expanded the "Jensen criteria" to a system for classifying sediments as non-polluted, moderately polluted, and heavily polluted based on 19 physical and chemical parameters.

In 1982, the Dredging Subcommittee to the Great Lakes Water Quality Board of the International Joint Commission published "Guidelines and register for evaluation of Great Lakes dredging projects" (IJC 1982). This report presented recommendations for evaluation of dredged material which were generally consistent with the 404(b)(1) Guidelines and USEPA's 1980 proposed testing procedures. A tiered testing procedure was recommended, utilizing historical information, sediment chemistry and elutriate testing, and sediment bioassessment. This report stated that "standardized procedures must be developed for conducting bioassays and bioaccumulation studies" and "meaningful criteria must be adopted to evaluate bioassay results".

Dredged material evaluations on the Great Lakes have relied almost entirely on sediment chemical testing for many years, largely because of the lack of standardized biological testing procedures or interpretive guidance. The need for regional

guidance on dredged material evaluation and testing, pursuant to 40 CFR 230.2(c) was identified by the USEPA and USACE. A USEPA/USACE task group was formed in 1990 to develop the regional guidance presented in this manual. The members of this task group are listed in the Acknowledgements.

1.7 Use of the Manual

This regional testing and evaluation manual should be used to supplement the national testing and evaluation guidance in accordance with the 404(b)(1) Guidelines (40 CFR 230). **The user of this regional guidance should have read and be familiar with the "Inland Testing Manual" (USEPA/USACE 1998) and the 404(b)(1) Guidelines in their entirety.**

Applicants for Section 404 permits for proposed discharges of dredged material into the United States waters of the Great Lakes should consult the appropriate District office of the USACE before implementing the testing procedures described in this manual. The USACE District will provide assistance on the applicability of this manual to the proposed discharge, the applicability of any regional or nation-wide general permits, in locating existing data, and other requirements of the Section 404 process.

Nation-wide Section 404 permits have been issued for a limited number of specific categories of dredged material and fill discharges that are similar in nature and have minimal impacts (33 CFR 330). The testing requirements for these discharges may differ from those described in this manual. Permit applicants should contact the appropriate USACE District on the applicability of these nation-wide permits to the proposed discharge.

Compliance with the provisions of Section 404 of the Clean Water Act does not eliminate the need to comply with the requirements of other Federal and State environmental laws and regulations.

1.8 Points of Contact for Section 404 permit applications

The Section 404 permit program for the United States waters of the Great Lakes is managed by four district offices of the USACE. The territories of these districts are shown on figure 1. The mailing addresses, telephone and fax numbers for these offices are as follows:

U.S. Army Corps of Engineers Buffalo District, CELRB-CO-R 1776 Niagara Street Buffalo, NY 14207-3199 Phone: (716)-879-4330 Fax: (716)-879-4310	Portions of the Great Lakes Basin within New York, Pennsylvania and Ohio
U.S. Army Corps of Engineers Chicago District, CELRC-CO-R 111 North Canal Street Chicago, IL 60606-7206 Phone: (312)-353-6400 Fax: (312)-353-2141	Portions of the Great Lakes Basin within Illinois
U.S. Army Corps of Engineers Detroit District, CELRE-CO-L P.O. Box 1027 Detroit, MI 48231-1027 Phone: (313)-226-2432 Fax: (313)-226-6763	Portions of the Great Lakes Basin within Indiana and Michigan
U.S. Army Corps of Engineers St. Paul District, CEMVP-CO-R 190 5th Street East St. Paul, MN 55101-1638 Phone: (612)-290-5375 Fax: (612)-290-5330	Portions of the Great Lakes Basin within Wisconsin and Minnesota

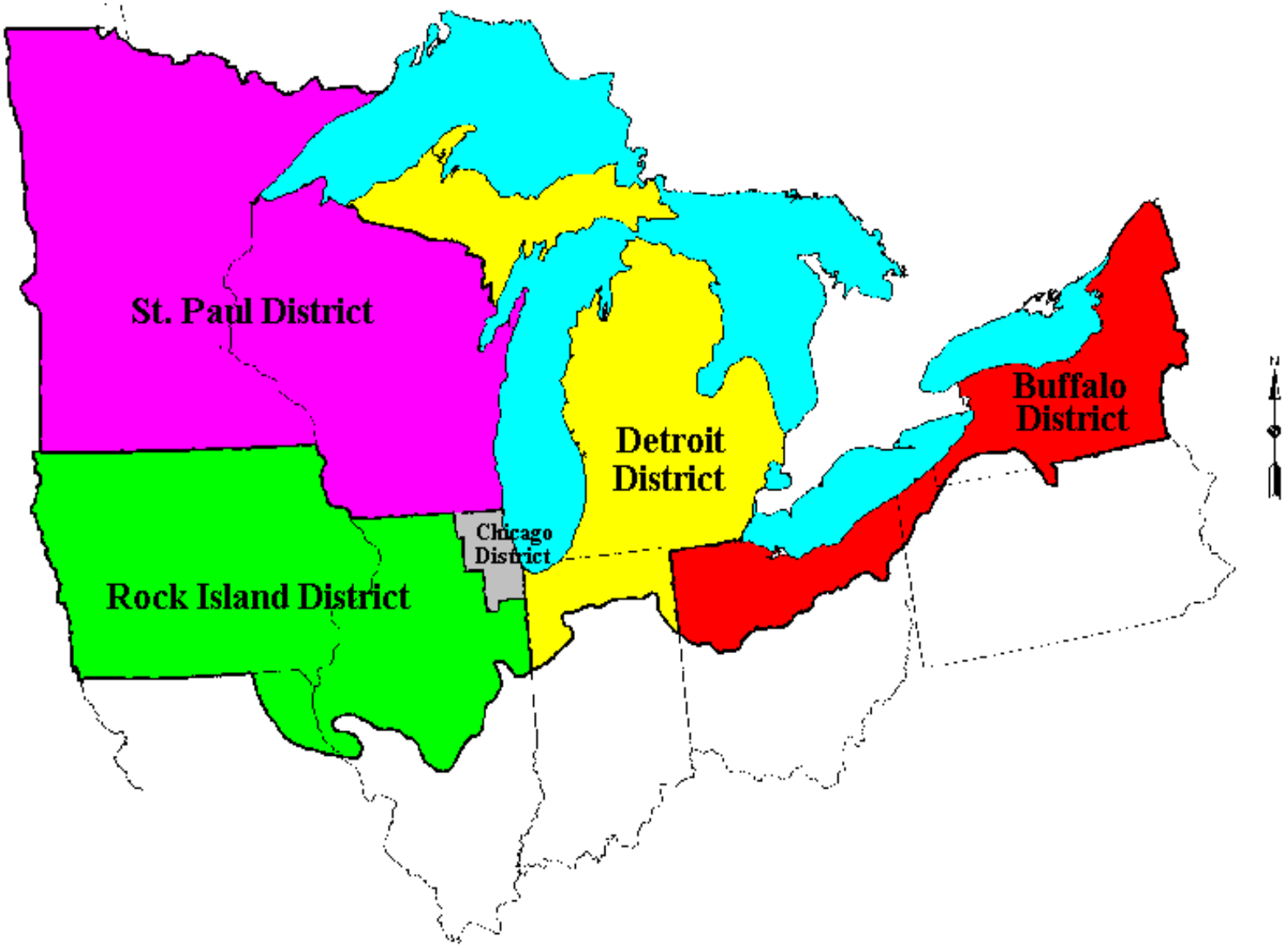


Figure 1. Map of USACE Regulatory Boundaries within the Great Lakes Basin

Section 404(g) of the Clean Water Act allows the USEPA to transfer a portion of the regulatory program for Section 404 to a qualifying State or Indian Tribe. The State or Tribe needs to have sufficient legislative and regulatory infrastructure to be capable of this responsibility. The State or Tribe can assume Section 404 permitting responsibility for any water that is not also a Rivers and Harbor Act Section 10 water based on certain criteria. The USEPA retains oversight authority, and the USACE has some review authority on major permit actions.

The only delegation of Section 404 permitting authority to a Great Lakes State is with the State of Michigan. Memoranda of Agreement between the State of Michigan and the USEPA and USACE were signed in 1983 and 1984, respectively. The Michigan Department of Natural Resources (DNR) issues Section 404 permits for most interior lakes, streams and isolated waters, including wetlands, within the State.

2. TESTING APPROACH

The tiered approach to testing used in this manual is consistent with the national manual (USEPA/USACE 1994), but provides more detailed guidance specifically for the Great Lakes. The reader is referred to the national manual for a more detailed discussion of the tiered approach. The tiered testing approach is consistent with the testing procedures used for ocean disposal of dredged material under Section 103 of the Marine Protection, Research and Sanctuaries Act (MPRSA) (USEPA/USACE, 1991), and is also generally consistent with the "Guidelines for project evaluation" developed by the International Joint Commission (IJC 1982).

The objective of the tiered testing approach is to make optimal use of resources in generating the information necessary to make a contaminant determination, using an integrated chemical, physical, and biological approach. To achieve this objective, the procedures in this manual are arranged in a series of tiers with increasing levels of intensity. The initial tier uses available information that may be sufficient for completing the evaluation in some cases. Evaluation at successive tiers requires information from tests of increasing sophistication and cost.

The basic flow diagram for the tiered testing procedure is shown on figure 2. The most logical and cost efficient approach is to enter Tier 1 and proceed as far as necessary to make a determination. There are two possible conclusions that can be made at each of the first three tiers: 1) available information **is not** sufficient to make a contaminant determination, or 2)

available information **is** sufficient to make a contaminant determination. Where information is sufficient, one of the following determinations may be reached: a) the proposed discharge **will not** have unsuitable, adverse, contaminant-related impacts, or b) the proposed discharge **will** have unsuitable, adverse, contaminant-related impacts

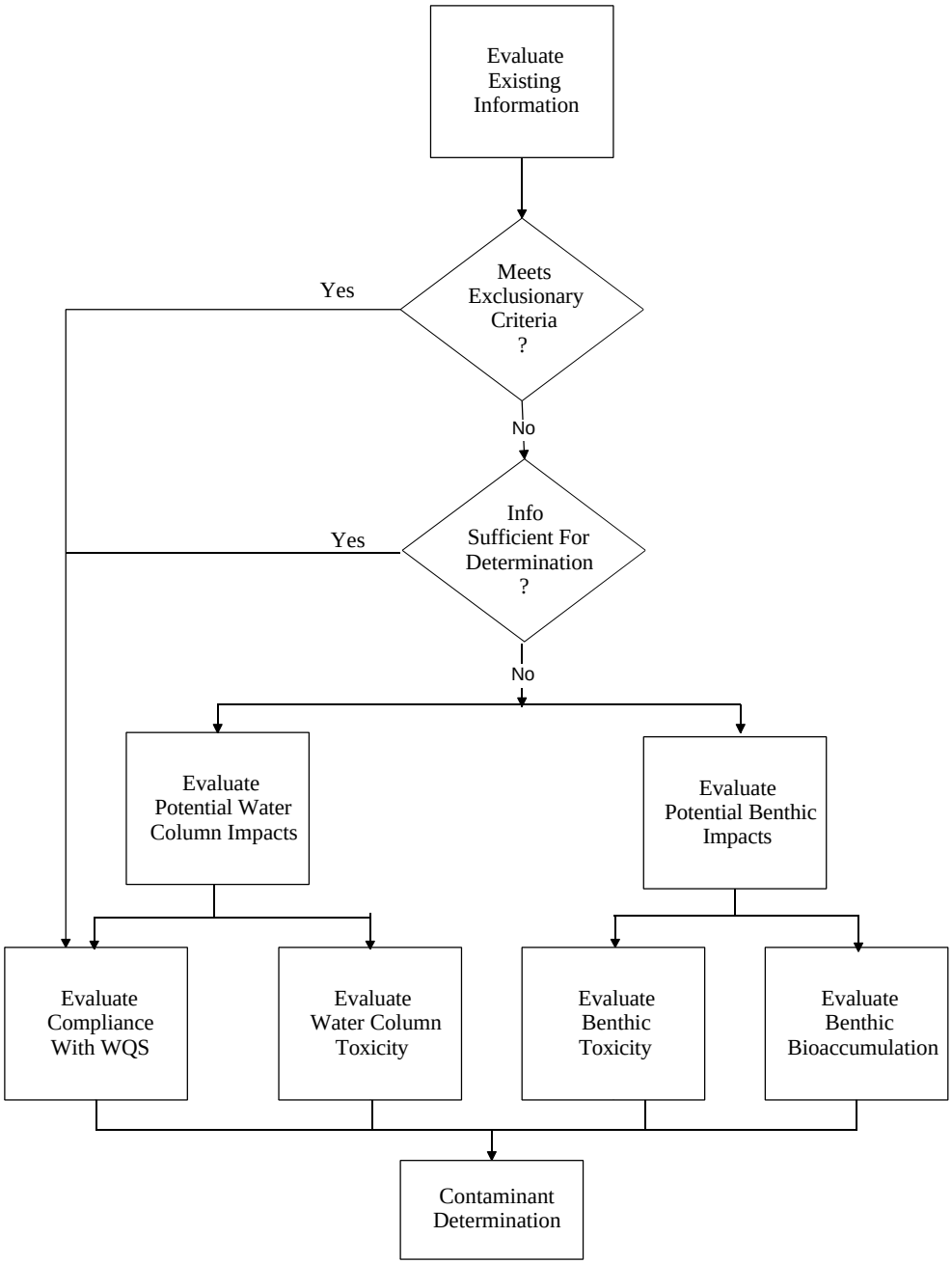


Figure 2. Tiered Testing Flow Diagram

Tier 1 compiles existing information about the potential for contamination in the proposed dredged material. Disposal operations that are excluded from testing or have historic data sufficient for the contaminant determination may proceed to a determination without additional testing.

Tier 2 evaluates the potential impacts of the proposed discharge on water column and benthic environments using sediment physical and chemical data collected for this tier, and applied with computer models to project worst-case conditions for water quality impacts and bioaccumulation. Based on the results of Tier 2 evaluations, additional testing may be reduced or eliminated.

Tier 3 evaluates the potential impacts of the proposed discharge on water column and benthic environments using effects-based biological testing. This manual presents recommended procedures for biological-effects tests with six organisms. These tests have been determined to be appropriate for use in the Great Lakes Basin.

Tier 4 is only entered if the information provided by Tiers 1 through 3 is not sufficient to make a contaminant determination. The procedures used in Tier 4 are keyed to site specific issues not resolved by the standardized procedures of earlier tiers. It is intended that very few situations will require a Tier 4 evaluation.

With this tiered testing structure, it is not necessary to obtain data for all tiers to make a contaminant determination. It may also not be necessary to conduct every test described within a given tier to have sufficient information for a determination. The underlying philosophy is that only that data necessary for a determination should be acquired.

3. TIER 1

3.1 Purposes

One of the purposes of Tier 1 (figure 3) is to determine whether a contaminant determination can be made on the basis of existing information. The compilation of existing information about the dredged material excavation site and proposed disposal site will serve as the basis for determining if a decision can be made without additional testing.

Another purpose of Tier 1 is the identification of the contaminants of concern, if any, in the dredged material. The identification of contaminants of concern will help determine

what, if any, testing should be conducted in subsequent tiers.

The initial focus of the Tier 1 evaluation is to obtain information relevant to sections 230.60 (a), (b), (c), and (d) of the Guidelines, and relevant to the potential for contaminant-associated impacts from the proposed discharge. These four sections of the Guidelines define exclusions from testing. A Tier 1 evaluation should be completed even if these exclusions are not likely to be satisfied, since the information compiled will be needed to determine which, if any, tests should be conducted in subsequent tier(s).

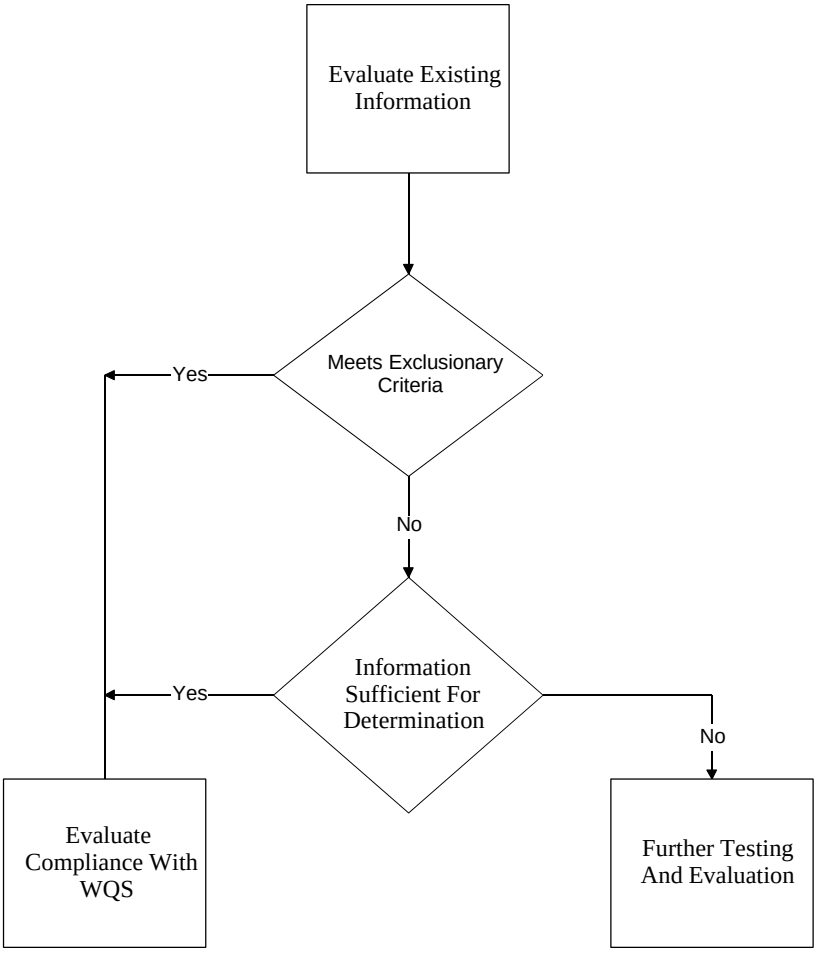


Figure 3. Tier 1 Flow Diagram

3.2 Planning and Coordination

Interagency coordination is essential to the development of a 404(b)(1) evaluation and a legal requirement under the National

Environmental Policy Act of 1969 (PL 91-190). Such coordination is critical in the Tier 1 evaluation process, where available information must be compiled from a variety of sources. Evaluators are encouraged to solicit input from other agencies on data sources, potential contaminants of concern, and proposed sampling and testing. Coordination prior to initiation of sampling and testing will reduce the chance of having to repeat costly procedures and assist in keeping projects on schedule.

3.3 Compilation of Available Information

A survey of contaminant sources and pathways should be conducted for the proposed dredging site. Section 230.60(b) of the Guidelines lists a number of factors that should be considered when evaluating the potential for contamination at the dredging site. These factors represent sources of contamination, pathways of contaminant transport, and naturally occurring substances which may be harmful to aquatic biota. In order to assess the potential for contamination at a proposed dredging site, information on these factors must be evaluated. A more complete inventory of available information will increase the likelihood that decisions concerning the impacts of dredged material may be made without unnecessary testing.

3.3.1 Contaminant Sources and Pathways

There are a number of potential sources of sediment contamination, both anthropogenic and natural. These sources include:

- urban and agricultural runoff,
- sewer overflows/bypassing,
- industrial and municipal wastewater discharges,
- previous dredged or fill material discharges,
- landfill leachate/groundwater discharge,
- spills of oil or chemicals,
- illegal discharges,
- air deposition,
- biological production (detritus), and
- mineral deposits.

Different sources and combinations of sources may contribute differing types and quantities of contaminants to sediments. A matrix of commonly accepted correlations between source types and specific contaminants is provided in figure 4. This matrix is not all inclusive and makes no accounting for current pollution control practices. It should be used as guidance only.

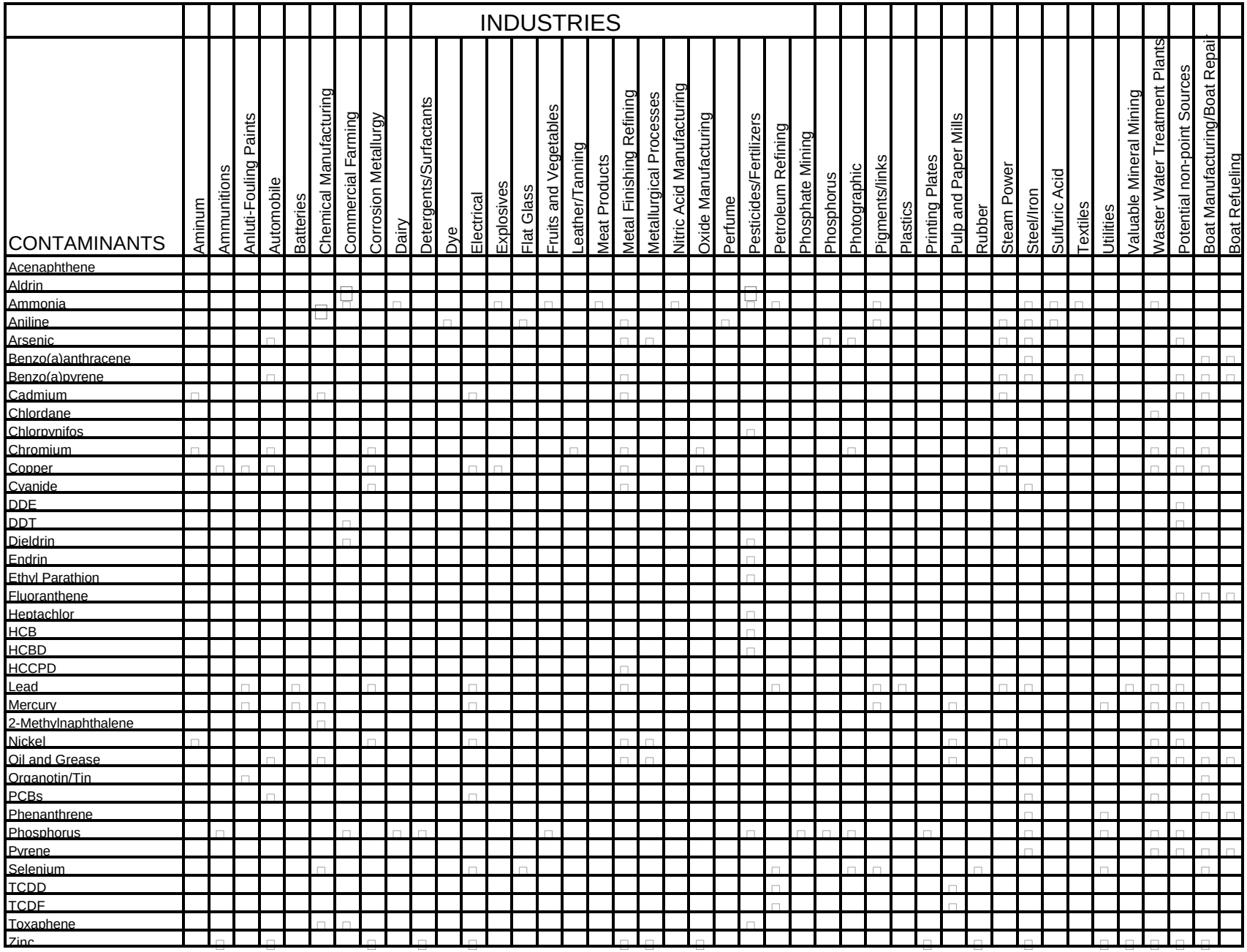


Figure 4. Sediment - Contaminant Source Matrix

There are also a number of factors which influence the pathways between these contaminant sources and the dredging and/or disposal sites. These factors include:

- bathymetry,
- water current patterns,
- wind patterns and local meteorology,
- tributary flows,
- watershed hydrology and land uses,
- sediment and soil types, and
- sediment deposition rates.

3.3.2 Sources of Information

There is a potentially large amount of historical information relevant to sources of sediment contamination available from Federal, State and local agencies, as well as in the open literature. A partial listing of these data sources for areas of the Great Lakes basin are provided in Appendix C.

Sediment quality data are routinely collected by the USACE at many of the authorized navigation projects in the Great Lakes. Much of this database is physical and chemical data with limited biological test results. Sediment data has also been collected by other agencies and investigators. A listing of available data reports is provided in Appendix C.

A number of computer databases are maintained by the USEPA which contain information on known sources of chemical contamination. Most of these databases are maintained by regulatory or clean-up programs such as NPDES and Superfund. Fact sheets for selected computer databases, showing the types of information available and how to access the data are provided in Appendix C. These databases include:

- STORET (STOrage and RETrieval system),
- TRI (Toxic Chemical Release Inventory),
- PCS (Permit Compliance System),
- RCRIS (Resource, Conservation, and Recovery Act Information System),
- ESDC (Environmental Sciences Division Clearinghouse),
- Niagara Frontier Program Office GIS Pilot Project,
- GRIDS (Geographic Resources Information and Data System),
- R5SI (Region 5 Sediment Inventory), and
- other specialized databases.

Ambient water quality data are routinely collected by State resource agencies at a number of locations throughout the Great Lakes and tributaries. These data are commonly reported on an annual or biennial basis in documents published by these

agencies. A listing of these reports and the agency points of contact is provided in Appendix C.

There are 31 Areas of Concern (AOCs) within the United States portion of the Great Lakes basin identified in the Great Lakes Water Quality Agreement. The locations of these AOCs are listed in Appendix C. State resource agencies are developing Remedial Action Plans (RAPs) for these sites. These RAPs are a useful source of information about sources of contamination.

Additional information on sources of contamination in the Great Lakes is provided in the list of published reports provided in Appendix C. These publications may be found in many libraries or through the libraries of some agencies. Local and regional agencies which should be contacted for more site specific information include:

- regional planning commissions,
- county and municipal governments,
- port/marina authorities, and
- State resource/survey agencies.

When utilizing available data, the evaluator should consider the quality of the information and its applicability for making decisions.

3.3.3 Data Acquisition

With the proliferation of computer databases and electronic information capabilities, evaluators may actually face a problem of having too much data rather than not enough. For example, when retrieving data from the STORET computer database, the zone of inquiry may be defined as a circle with the center at the dredging site and a variable radius, a polygon, or a watershed boundary. Other databases may retrieve information along political boundaries (county or State). An excessively large zone of inquiry will often yield an unwieldy amount of data not relevant to the evaluation.

The size of the zone of inquiry should be determined using the information obtained about possible routes of contaminant transport to the dredging and disposal sites. These routes should be defined before initiating computer database searches. In general, the zone of inquiry for potential sources of sediment contamination should be larger for a dredging site in a tributary stream than for a dredging site in the coastal lacustrine area of a Great Lake. Sediments in a riverine setting are more likely to have been exposed to sources of contaminants from different portions of the watershed, many miles from the river channel. This is especially true for non-point sources of contamination

such as urban and agricultural runoff. In contrast, contaminants from most sources in the coastal areas away from tributary outlets are more readily dispersed and diluted, and less likely to impact nearby sediments.

The quality of historic data should be assessed to determine its usability. Limited guidance on the quality assessment of historic data is provided in Appendix E. In general, the weight of evidence can only be determined by best professional judgement.

3.4 Exclusions From Testing

Sections 230.60 (a) and (b) state that if an evaluation of the extraction (dredging) site indicates that the dredged material is not a "carrier of contaminants", the determination of the presence or effects of contaminants can be made without testing. The Guidelines further states that, "Dredged or fill material is most likely to be free from chemical, biological, or other pollutants where it is composed primarily of sand, gravel and other inert materials."

The compilation of existing information described above (paragraph 3.3) will be used to determine the applicability of this exclusion. Dredged material that are most likely to meet this exclusion include sediments from locations which are far removed from most anthropogenic activities or sediments from depths deposited in pre-industrial times and not exposed to modern sources of pollution. However, the potential impacts from natural mineral deposits should also be considered.

Section 230.60 (c) states that testing will not be required "where the discharge site is adjacent to the excavation site and subject to the same sources of contaminants, and materials at the two sites are substantially similar". This exclusion applies even if the dredged material is a carrier of contaminants providing that "dissolved materials and suspended particulates can be controlled to prevent carrying pollutants to less contaminated areas".

A large number of the dredging operations on the Great Lakes remove sediments from the entrances to protected harbors and marinas along the lakefront. In most cases, the material excavated is fine-grained sand that is transported around the near shore areas by littoral processes and deposits in artificially deepened navigation channels. A hypothetical example of this condition is shown on figure 5. In most cases, the dredged material are disposed to the open lake adjacent to the harbor/marina or onto an adjacent beach.

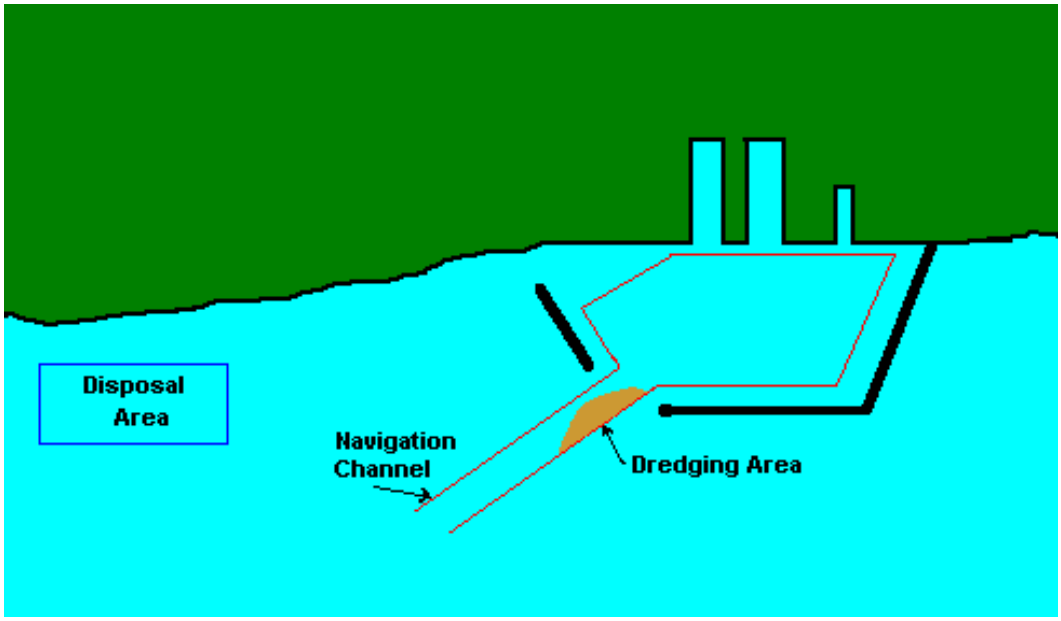


Figure 5. Example Dredging/Disposal Scenario

In this example, the dredging site and disposal site are part of the same littoral system. Where sediments at the dredging site and disposal site are equally exposed to sources of contamination and are shown to be physically and chemically similar, such discharges meet the requirements of the 230.60 (c) exclusion from testing when dredged material pollutants (if any) can be prevented from being transported to less contaminated areas.

Limited physical and chemical testing will generally be necessary to confirm that the sediments from the dredging site and disposal site are physically and chemically similar. Physical testing usually requires a particle size distribution (sieve) analysis. Chemical testing is required for the contaminants of concern identified in paragraph 3.5. Guidance on sediment sampling and analyses is provided in paragraph 4.3.

The 230.60(c) exclusion does not apply when the sediments from the dredging and disposal sites are chemically or physically dissimilar. In the example shown on figure 6, the tributary may have exposed the sediments at the dredging sites to more sources of contamination than the disposal site. It is also possible that the tributary could cause the sediments at the dredging sites to be more fine-grained than the sediments at the disposal site.

Section 230.60 (d) states that testing may not be necessary with material likely to be a carrier of contaminants if constraints acceptable to the USACE District Engineer and USEPA Regional Administrator are available to "reduce contamination to

acceptable levels within the disposal site and to prevent contaminants from being transported beyond the boundaries of the disposal site."

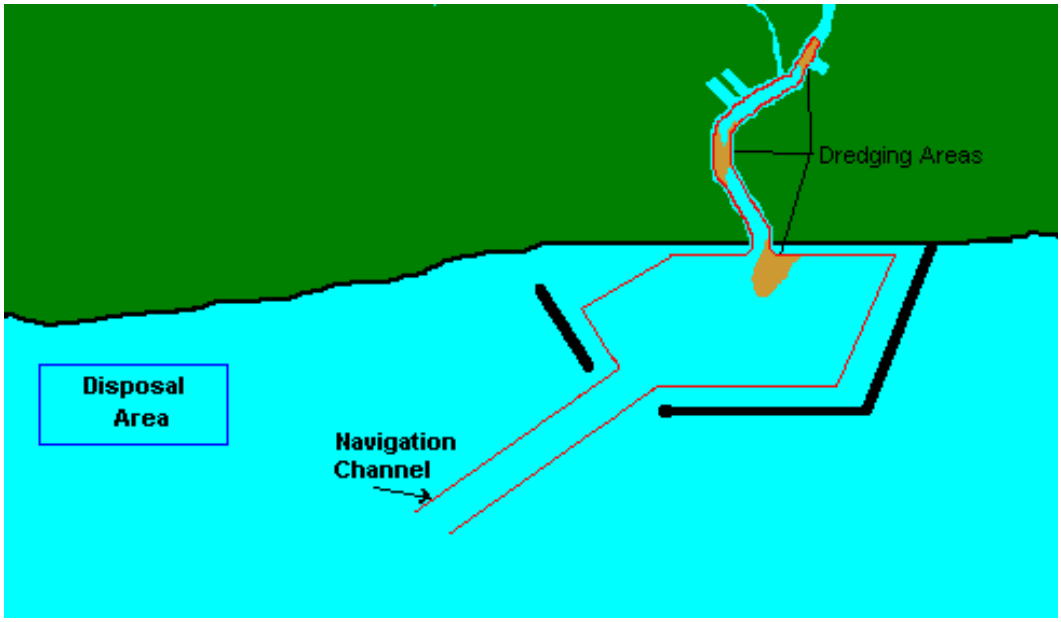


Figure 6. Example Dredging/Disposal Scenario

Technologies for capping and underwater containment of dredged material have been developed and practiced on the east and west coasts for several years (Zeman et al. 1992; Palermo 1991, 1991b). In addition, treatment technologies for contaminated sediments have been evaluated and demonstrated (Averett et al. 1990; USEPA 1994). In order to be subject to an exclusion under 230.6(d), the performance and monitoring requirements for these technologies will need to be developed by the USACE and USEPA on a case-by-case basis. These dredged material management options are outside the scope of this manual.

3.5 Identification of Contaminants of Concern

The purpose of identifying contaminants of concern in each dredged material is to determine parameters for testing in later tiers, if necessary. A contaminant of concern should be identified on the basis of the following factors:

- presence in the dredged material,
- concentration in the dredged material relative to the concentration in sediments at the disposal site,
- toxicological importance,
- persistence in the environment,
- propensity to bioaccumulate from sediments, and
- presence on applicable fish consumption advisory.

To aid in the identification of contaminants of concern for individual projects, the USEPA and USACE have developed the generic list of contaminants shown on table 1. This list is applicable to Great Lakes sediments, but is not all inclusive. The list was developed with consideration of the above factors using historical sediment data, known sources of contamination, and is generally consistent with the IJC guidelines (IJC 1982).

Table 1. Generic list of physical and chemical parameters for characterizing Great Lakes sediments

Arsenic	Total organic carbon (TOC)
Cadmium	Total volatile solids (TVS)
Chromium	Total phosphorous
Copper	Ammonia-nitrogen
Lead	Total petroleum hydrocarbons (TPH)
Mercury	Polychlorinated biphenyls (PCBs)
Nickel	
Zinc	

Routine physical analysis should include grain size and percent solids. All chemical analysis should be reported on a dry weight basis.

This generic list of contaminants of concern should serve as a starting place and not necessarily as the final list. Information compiled on a specific project, as described above (paragraph 3.3) should be used to supplement or reduce the chemical parameters on the generic list. The reasons for supplementing or reducing this list should be fully documented.

As an example, the contaminant of concern list for a dredged material located in a river downstream of a steelmaking plant or coking operation should be expanded to include polynuclear aromatic hydrocarbons (PAHs), which are commonly associated with discharges from these industries. For another project, if there were historical data indicating the absence of mercury in sediments from the project and no suspected sources, this parameter should be removed from the list. With dredging projects covering large areas, it may be possible to have different contaminants of concern for two or more portions of the proposed dredging area.

In situations where there are fish consumption advisories, the responsible bioaccumulative contaminants that are the source of the advisory should be considered for the list of contaminants of concern. A summary of recent State fish advisories and a listing of State agency contacts is provided in Appendix C.

3.6 Contaminant Determination

After consideration of all available information, one of the following two possible **conclusions** can be reached at Tier 1:

1 - Existing information does not provide a sufficient basis for making a contaminant determination. In this case, further evaluation at a higher tier is appropriate.

2 - Existing information does provide a basis for making a contaminant determination. In this case, one of the following three **determinations** may be reached:

a) The dredged material meets the exclusion criteria and no further information on contaminants is necessary to determine compliance (except for information necessary for Section 401 compliance - see paragraph 4.5.3).

b) The dredged material does not comply with the exclusion criteria, but the available information is sufficient to show the material is not a carrier of contamination to a degree which will cause an unsuitable, adverse impact.

c) The dredged material does not comply with the exclusion criteria, but the available information is sufficient to show the material is a carrier of contamination to a degree which will cause an unsuitable, adverse impact.

Sediment data may include results from previous tiered analyses. For many projects, the same areas are dredged routinely and discharged to the same site. In such cases the results of previous tiered testing may be used to reach a decision in Tier 1.

For projects with recurring maintenance dredging, a Tier 1 evaluation is not necessarily required for each dredging and discharge operation. A comprehensive Tier 1 evaluation should require only minor updating on a periodic basis to determine if additional data or evaluation is necessary. This reevaluation of the Tier 1 analysis should consist of the collection and examination of available information on any changes in contaminant sources or pathways to the dredging and discharge sites. It is recommended that the Tier 1 evaluation be updated at least every three years for frequently dredged projects and prior to each operation for projects dredged less frequently.

In navigation projects that cover a large area, it is common that only selected portions are dredged at any one time. While a

full Tier 1 evaluation should initially be conducted for the entire project, a Tier 1 reevaluation and determination of compliance may be performed for only a portion of the larger navigation project for individual dredging operations.

At the completion of Tier 1, even if a decision is made to exclude the dredged material from testing or that existing information is sufficient to make a contaminant determination, additional testing may be necessary to obtain a certification of water quality compliance, as required under Section 401 of the CWA. While the requirements for 401 certification are determined by the applicable State agency, the procedures described in paragraph 4.5 of this manual should address water quality compliance. A scenario under which no additional testing may be necessary for water quality certification is one in which the dredged material meets exclusion 230.60 (a) and (b) because there are no contaminants of concern.

3.7 Reporting

Much of the information gathered under Tier 1 will be condensed in the 404(b)(1) evaluation document. Because a comprehensive Tier 1 evaluation will likely gather far more information than can be presented in the 404(b)(1) evaluation, and because of the importance of the decisions made at this tier, it is recommended that this information be documented in supporting materials and referenced as appropriate in the 404(b)(1) evaluation.

The report of the Tier 1 evaluation should summarize the following information:

- potential sources of sediment contamination identified,
- sources of information investigated,
- historic sediment data (physical, chemical, biological),
- contaminant pathways to dredging and discharge sites,
- reasons for applying exclusions from testing,
- results of any confirmatory testing,
- contaminants of concern list,
- reasons for the final list of contaminants of concern, and
- QA/QC documentation supportive of critical data.

This documentation should be developed into a report that can be distributed for State and Federal agency review and if necessary, inserted as an appendix to the 404(b)(1) evaluation public review document. A well documented Tier 1 evaluation will expedite future 404(b)(1) evaluations at the same project or any new dredging projects in the vicinity.

4. TIER 2 - PHYSICAL AND CHEMICAL TESTING

4.1 Purpose

Within the tiered structure, the purpose of Tier 2 (figure 7) is to make a contaminant determination using dredged material physical and chemical data collected for this tier. However, not all decisions can be made in this tier. Tier 2 utilizes calculations and/or models to predict the potential for dredged material contaminant impacts in the water column and benthic environments, and is intended to provide a reliable, rapid screening tool to determine when the more costly biological testing is necessary.

There are two situations under which the evaluator will enter Tier 2. The first is having completed Tier 1 with insufficient information to reach a determination. The second is having completed Tier 1 with sufficient information for a contaminant determination or exclude material from testing, but additional data is necessary for Section 401 certification.

At the present time, the state-of-the-art of Tier 2 evaluation is rather limited. Our ability to predict toxicological and bioaccumulation impacts based on sediment chemical data is not sufficient to reach a determination in most cases. Despite these limitations, Tier 2 will provide information necessary to determine water quality compliance for Section 401 and may reduce the scope of future testing.

4.2 Planning and Coordination

The purpose of sediment sampling and analysis in Tier 2 is to obtain the necessary physical, chemical and elutriate data for evaluating potential water column and benthic impacts from sediment contaminants with the screening methods of this tier. The existing information compiled in Tier 1 (paragraph 3.3) is the logical starting point for planning a sediment sampling and testing program. This information should, in most cases, be adequate to determine the scope of sediment sampling and analysis. The contaminant of concern list developed in Tier 1 identifies the chemical parameters for analyses.

It is possible to conduct sediment sampling and analysis for Tier 1 (confirmatory testing only), for Tier 2 (physical and chemical testing), for Section 401 compliance, and Tier 3 (biological testing) as either separate or combined activities. The costs of multiple sediment sampling events, allowable sample holding times, and project time and funding constraints should be considered when developing a sampling and analysis plan.

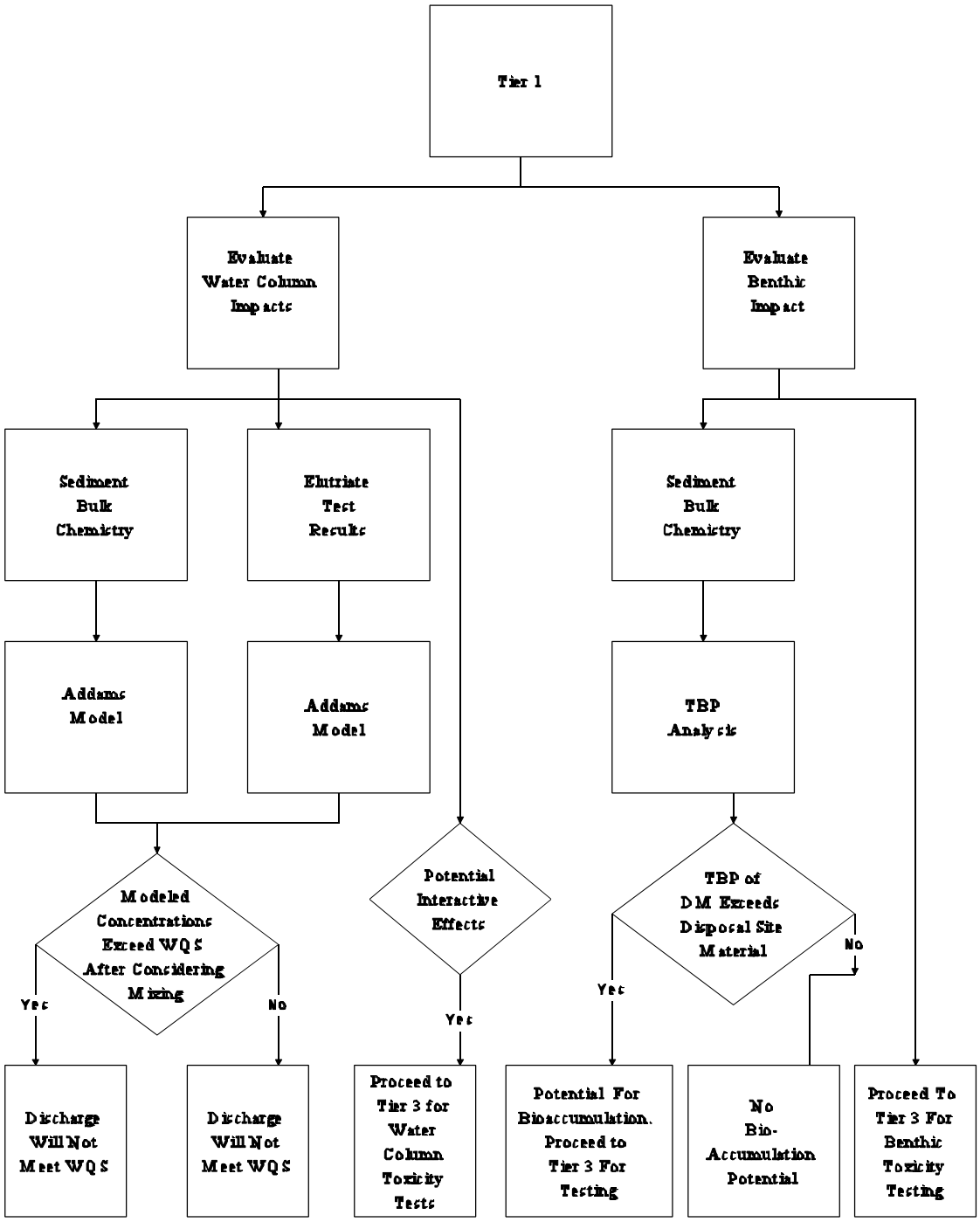


Figure 7. Tier 2 Flow Diagram

Because of the limitations in the ability to reach a decision at Tier 2, it is possible to go directly to Tier 3 testing to develop the information necessary for a contaminant determination. However, the cost of biological testing in Tier 3 will be a major constraint in the number of samples collected.

In order to keep these costs in line, while collecting samples that are representative, physical and chemical data of the type used in Tier 2 may also be needed to develop the framework for a sampling design in Tier 3. Guidance on the sampling design is provided in Appendix D.

Where practicable, it is recommended that a written plan for sediment sampling and analyses be prepared and provided to the appropriate Federal and State agencies for coordination prior to sampling. The Tier 1 evaluation would be a logical attachment to the sampling and analysis plan for agency review and comment. This coordination will reduce the chance of having to repeat costly procedures and assist in keeping projects on schedule.

4.3 Sediment Sampling and Analyses

4.3.1 Sampling Methods and Locations

Detailed guidance on acceptable sediment sampling methods and procedures is provided in Appendix D. Included in this appendix are information on acceptable sediment collection and handling procedures. Also included is guidance on how to plan and execute a sampling program. Sediment sampling plans are so site specific that guidance on the number, type, and location of samples is necessarily quite general.

A sediment sampling program for a 404(b)(1) evaluation should collect samples that are representative of the materials to be dredged, and the sediments at the disposal site. The sampling results will be used to determine if all or part of the dredged material for a proposed project are suitable for open water disposal. The historical information collected in Tier 1 should be used in the formulation of the sediment sampling program. This will focus resources on data gaps and minimize redundant data collection.

In any sampling program, a finite number of samples are used to represent some larger area or volume, possibly with some consideration of time. Factors that should be considered in selecting the number, type and locations of sediment samples include:

- distribution of sediments to be dredged,
- known or suspected contaminant distribution,
- dredging methods, and
- tests to be performed.

The distribution of dredged material may be known from bathymetric soundings or previous dredging records. The distribution of sediment contaminants can be estimated based on

historical data and/or information about contaminant sources and pathways developed in Tier 1. Within a single project, dredged material from different areas may have differing levels of contamination, and may have differing disposal requirements. Sampling plans for these dredging sites should be designed to accommodate irregular dredged material distributions, with a contaminant and/or volume bias. Grids and other statistically derived sampling plans are often not useful in these applications.

The approach used to collect representative samples of the dredged material may differ from that used to collect representative samples from the disposal site. The dredged material is a 3-dimensional mass of sediments to be excavated. The disposal site is a 2-dimensional area which will become covered by a new surface as the dredged material are discharged. Samples collected at the disposal site therefore need only represent the surficical sediments.

The dredging method should also be considered in the sampling design. It is impractical to define lateral or vertical distributions of sediment contamination that are beyond the precision of anticipated dredging equipment and operational constraints. For example, vertical sub-sampling at increments less than two feet is not recommended because of the limitations of dredging accuracy.

The types of analyses to be performed on the sediments are another factor in the sampling program. Some tests require large volumes of sample, which may limit sampling equipment selection. Finally, the costs of laboratory analysis is often an overriding practical consideration limiting the number of samples collected.

4.3.2 Disposal Site Sample Selection

Section 404(b)(1) Guidelines (1976) direct that contaminant determinations be made by comparing the dredged material to the sediments at the disposal site. For purposes of a dredged material discharge permit, the disposal site is typically defined by a "box" on a map, outlining an area where dredged material are to be placed by a barge, pipeline or other method. At a non-dispersive site, dredged material remain within the "box," typically forming a mound.

This concept of a "boxed" disposal site is limited in the Great Lakes, where the majority of dredged material disposal sites regulated under Section 404 are in shallow waters with highly dispersive currents and wave energies. In these conditions, dredged material do not form mounds, but are rapidly dispersed over areas several times as large as the original "box"

within days, weeks, or months. Because of the dispersive nature of most Great Lakes dredged material disposal sites, regional guidance is presented here for selecting the sediment for testing that best reflects the disposal site. This may include collection of samples that are physically outside the "box".

Disposal site sediment is taken from a location chosen to serve as the point of comparison for potential contaminant effects of the proposed dredged material. The sample should reflect the conditions at the disposal site, with the following considerations:

- physical similarity to dredged material;
- proximity to sources of contamination; and
- proximity to disposal site "box".

The selection of a disposal site sediment may be complicated where these considerations are conflicting.

Differences in grain sizes of sediments can affect organisms used in toxicity or bioaccumulation tests, and may confound the interpretation of contaminant effects. To the extent possible, the organisms recommended in this manual for Tier 3 benthic toxicity and bioaccumulation tests were selected because of their tolerance for a wide range of sediment physical properties. Nonetheless, the ideal disposal site sediment is physically similar to the dredged material so that the potential effects of grain size variations are minimized.

Where the dredged material and the sediments at the disposal site are physically dissimilar, it may be appropriate to consider nearby areas for a sediment sample that is more physically similar to the dredged material, while also reflecting the contaminant-related conditions at the disposal site. For example, many dispersive disposal sites have coarse grained sediment. If the dredged material are more fine-grained, it is likely they would not remain within the disposal site "box" for very long. Sediments from a nearby, less dispersive area, if available, might be more coarse grained and better match the particle size characteristics of the dredged material more closely. The fine grained dredged material are also more likely to have a higher residence time in such areas than within the dispersive "box".

The second consideration is intended to discourage the use of a disposal site sediment that has been contaminated to a substantial degree by sources other than dredged material. The selection of a disposal site sediment from areas of localized contamination, such as from spills or point discharges, in order to bias the dredged material evaluation is not acceptable.

However, few areas of the Great Lakes are without any contamination, and some large areas, particularly those near major tributaries, do have sediments with appreciable levels of background contamination. Background contamination that reflects the conditions of a large area, including the disposal site "box", is not an appropriate rationale for dismissing a disposal site sediment from use in the dredged material evaluation.

The last consideration of the disposal site sample selection is that the disposal site sediment be collected from within or as close as practicable to the disposal site "box". If the disposal site has never been used for dredged material disposal, the sample for comparison should, allowing for the other considerations, be collected from within the "box". If it is necessary to move outside the "box" to get a suitable sample, the distance should be kept to a minimum to best reflect the contaminant conditions of the disposal site. The maximum distance for a disposal site sample would be that which dredged material might be transported by normal currents or wave energies in 5-21 days (the duration of bioassay tests).

Beach nourishment, the placement of dredged material above the high water line, is a common disposal practice in the Great Lakes. The runoff of return water from such disposal operations to the adjacent lake or river is a 404 discharge. The water that receives this runoff is the disposal site, and the disposal site sediment selected from this site. However, dredged material that is suitable for beach nourishment typically meets the exclusions from testing.

Additional guidance on the selection of a disposal site sediment sample is provided in Appendix D. The rationale for sample site selection should be documented in the 404(b)(1) evaluation.

4.3.3 Physical and Chemical Analyses

Guidance on laboratory procedures for physical and chemical analysis of sediments is provided in Appendix F. Included in this appendix are acceptable procedures for laboratory analysis of the more common sediment contaminants on the Great Lakes. These procedures were determined to be suitable for achieving detection limits below ambient levels for these sediment contaminants. Any variation from these procedures should be coordinated with the USACE District and USEPA Region.

Also included in Appendix F are the accepted procedures for the preparation and chemical analysis of an elutriate. The elutriate test (USACE 1976) is a procedure developed to simulate the release of dissolved contaminants from a hydraulic dredged

disposal operation in open waters, and may be considered a worst case analysis for the release of dissolved contaminants from a mechanical dredged disposal operation. The elutriate test is used to evaluate water quality compliance for Section 401 certification (see paragraph 4.5.3). Elutriate concentrations should be reduced to reflect dilution resulting from mixing and dispersion at the proposed disposal site.

4.3.4 Quality Assurance

Quality assurance (QA) is a critical element within any 404(b)(1) contaminant evaluation. The importance of QA is not limited to the laboratory, but extends throughout the evaluation. General QA guidance and the data quality objectives (DQOs) for Great Lakes dredged material testing and evaluation is provided in Appendix E. More specific quality control (QC) guidance for dredged material sampling and handling is provided in Appendix D. Minimum QC requirements for analytical procedures are provided in Appendix F for chemical and physical analyses.

4.4 Benthic Impact Evaluations

One objective of the Tier 2 benthic evaluation is to determine if dredged material contaminants have the potential to cause an unacceptable adverse impact on benthic organisms, or on other aquatic organisms through bioaccumulation. This tier uses sediment chemical data with calculations and/or models to predict potential benthic and bioaccumulation impacts. The current state-of-the-art will allow only a partial resolution of this objective.

4.4.1 Potential for Bioaccumulation

Bioaccumulation is the uptake and retention of contaminants by organisms. In aquatic systems, sediment contaminants may bioaccumulate to levels having ecological and human health consequences. Some non-polar organic contaminants and a few metals have been found at elevated levels in the tissues of fish and other organisms, resulting in consumption advisories. Not all sediment contaminants will bioaccumulate. Some are readily metabolized, or degraded, within the organism's body. Others are simply not taken up. A listing of Great Lakes critical pollutants, many of which are bioaccumulative is provided in Appendix C.

The following factors should be considered to determine which (if any) contaminants should be evaluated for bioaccumulation potential:

- presence in the dredged material,
- propensity to bioaccumulate from sediments, and
- presence on applicable fish consumption advisories.

A list of potentially bioaccumulative contaminants should be a subset of the contaminants of concern list developed in Tier 1.

This manual provides a procedure to estimate the potential for bioaccumulation of certain sediment contaminants. Using this procedure, it is possible to determine if bioaccumulation testing is necessary in Tier 3.

4.4.1.1 Theoretical Bioaccumulation Potential (TBP)

TBP analysis was developed by McFarland (1984), based upon the laboratory work of Konemann and Van Leeuwen (1980) and Karickhoff (1981), and the results of later field studies. TBP utilizes the following equilibrium partitioning theory-based algorithm:

$$[\ TBP = pf\ (\ C_s\ /\ TOC\ )\ L\ ]$$

where:

pf = preference factor (a constant set to = 4.0)

C_s = the concentration of non-polar organic chemical in the dredged material or disposal site sediment, usually expressed as dry weight mg/kg (ppm)

TOC = total organic carbon content of the dredged material or disposal site sediment usually expressed as a dry weight decimal fraction (i.e., 2% = 0.02)

L = organism lipid content usually expressed as a decimal (wet weight fraction (i.e., 3% = 0.03)

TBP = wet weight of contaminant concentration in fish or organism tissue in mg/kg (ppm).

This algorithm uses the association between many non-polar organic contaminants and non-polar organic matrices in sediments and biota, known as equilibrium partitioning. In an idealized, closed system composed of sediment, organisms and water, the non-polar organic contaminants held by the sediment TOC will partition over time into the lipid compartment of organisms. At equilibrium, the non-polar organic contaminants will preferentially reside in the organism lipid. The preference factor setting at 4.0 is based upon the results of laboratory and field studies.

To perform a TBP evaluation, the evaluator must obtain data on the concentrations of non-polar organic contaminants and TOC in the proposed dredged material and disposal site material, as discussed in paragraph 4.4.1.1. The lipid content of the selected target organism(s) can be obtained from literature values or direct measurements. A listing of ranges of lipid content typically found in a variety of Great Lakes aquatic organisms is provided in Appendix C. Target organisms for TBP analysis may be selected because of their economic and/or ecological importance. Lipid levels of specific organisms (species) may vary widely with sex, age classes, size classes, and regional populations.

Using the above formula, and the data collected, the TBP may be calculated for every combination of sediment and target organism. For example, a sediment with 2 mg/Kg dry weight PCBs and 3% TOC has the potential to cause a fish with 6% lipid to have a PCB body burden of 16 mg/Kg wet weight.

TBP represents a theoretical condition of equilibrium, which is rarely present in the field. This condition is most closely met by organisms that have constant, direct contact with the sediment, such as a burrowing worm. The use of TBP to predict bioaccumulation from sediment in more mobile organisms, such as migratory fish, can be complicated by a number of factors. At this time, TBP should only be considered a worst-case estimate of potential bioaccumulation in fish.

The TBP for the proposed dredged material should be interpreted by comparison to the TBP of the disposal site material. If the TBP of the dredged material is not greater than that of the disposal site, no bioaccumulation testing for non-polar organic contaminants may be necessary. For any non-polar organic contaminant having a consumption advisory, the TBP for the appropriate species and size/age classes listed should be evaluated.

The TBP algorithm is not suitable for sediments with TOCs of less than 0.5%. If the dredged material or disposal site sediment contain less than 0.5% TOC, the potential for bioaccumulation should be presumed where the concentrations of hydrophobic contaminant(s) in the dredged material are greater than disposal site sediment. Under these circumstances, bioaccumulation testing in Tier 3 would be warranted. The necessity for bioaccumulation testing for other circumstances where TOC is less than 0.5% should be determined on a case-by-case basis.

4.4.1.2 Bioaccumulation Potential of Other Contaminants

Aside from the non-polar organic contaminants, only a limited number of other contaminants have been shown to bioaccumulate from sediments to aquatic organisms. For other bioaccumulative contaminants, there are no well established relationships between concentrations in sediment and organism tissues. The need for bioaccumulation testing for these contaminants may be determined based upon the comparison of the contaminant concentrations in the dredged material and disposal site sediment, taking into consideration any consumption advisories.

Future research may result in chemical relationships and predictive tools, similar to TBP, for other classes of sediment contaminants.

4.4.2 Benthic Toxicity

Procedures for predicting the toxicological response of benthic organisms to dredged material contaminants based on chemical data are not available. Potential applications of national sediment criteria are discussed in paragraph 4.6.

4.5 Water Column Impact Evaluations

Another objective of the Tier 2 evaluation is to determine if the dredged material contaminants will cause an unacceptable adverse impact on organisms within the water column and comply with applicable water quality standards, using chemical data. The state-of-the-art will allow only a partial resolution of water column biological impacts, but will provide sufficient information to address water quality compliance.

4.5.1 Water Quality Screening

There are two approaches used in Tier 2 to evaluate the potential impacts of a dredged material discharge on water quality. The first approach employs a water quality screening model to assess the conservative, worst-case water quality impacts of the proposed discharge. This model assumes 100 percent release of sediment-bound contamination into the water column, and calculates the concentrations of contaminants at the disposal site, allowing for mixing. The second approach utilizes the results of sediment elutriate analyses together with the mixing zone model.

If bulk chemical data representative of the proposed dredged material is available, it is recommended that the first approach be used. If the results with the screening model show that using

worst-case assumptions, the discharge would not exceed State water quality standards, no elutriate testing should be necessary. If the results of the screening model indicate the potential for exceeding State water quality standards, or if no bulk chemical data is available, the elutriate tests should be performed to determine compliance with State water quality standards.

The computer model used in Tier 2 for the evaluation of water quality is a part of a collection of computer models named Automated Dredging Disposal Alternatives Management System (ADDAMS). Floppy discs with the ADDAMS model, and full documentation are provided in the "Inland Testing Manual" (USEPA/USACE, 1998). The module of ADDAMS utilized in the Tier 2 analysis is STFATE (Short Term FATE). This module was developed for predicting the concentration of dredged material contaminants within a specified mixing zone. It can also determine the size of a mixing zone necessary to meet a specified standard. STFATE was developed for simulating disposal from a barge, scow or hopper in relatively deep water. Models for simulating disposal in shallow water (<15 feet) and beach nourishment are in development.

The impacts of a dredged or fill discharge are quite different from those of a permanent, wastewater point-source discharge. Dredged material discharges have not been regulated under the National Pollutant Discharge Elimination System (NPDES) of Section 402 of the Clean Water Act. For these reasons, the evaluation of a mixing zone for a dredged or fill material discharge is generally more complex, requiring consideration of additional factors beyond those used for NPDES mixing zone evaluations. The ADDAMS modules were developed for this more complex evaluation.

Part 230.11(f) of the Guidelines states that, "The mixing zone shall be confined to the smallest practicable zone within each specified disposal site that is consistent with the type of dispersion determined to be appropriate by the application of these Guidelines." The following factors should be considered in determining the acceptability of a proposed mixing zone:

. depth of water;
. current velocity, direction, and variability;
. degree of turbulence;
. stratification attributable to causes such as obstructions, salinity or density profiles;
. discharge vessel speed and direction, if appropriate;
. rate of discharge;
. ambient concentration of constituents of interest;
. dredged material characteristics, particularly

concentrations of constituents, amount of material, type of material (sand, silt, clay, etc.) and settling velocities;
- number of discharge actions per unit of time, and;
- other factors of the disposal site that effects the rates and patterns of mixing.

In order to run the model, the evaluator must obtain information about the disposal site necessary to address the above mixing zone factors, as well as data on the dredged material (bulk chemistry, solid content, void ratio, specific gravity). For the application with sediment bulk chemistry (step 1), the model need only be run for the contaminant of concern that requires the greatest amount of dilution to meet applicable State water quality standards. If this contaminant is shown by the model analysis to meet the State standards, all of the other contaminants would require less dilution and will also meet acceptable concentrations within the mixing zone.

If the application of the model with bulk chemical data shows potential exceedance of State water quality standards outside the mixing zone, the model should be rerun using elutriate data. If the results still exceed applicable standards outside the mixing zone, alternative disposal methods or management measures should be considered.

4.5.2 Water Column Toxicity

Procedures for predicting the toxicological response of water column organisms to dredged material contaminants based on sediment bulk chemical data are not available. Most applicable State water quality standards are derived from aquatic toxicity or human health data in conjunction with other factors. In some cases, State standards are specifically linked to aquatic toxicity tests. Water quality screening that demonstrates compliance with applicable water quality standards may therefore address water column toxicity concerns. However, the potential for interactive (i.e. synergistic, antagonistic) effects of contaminants on aquatic toxicity will necessitate that Tier 3 testing be conducted for most dredged material with more than a single contaminant of concern.

4.5.3 Section 401 Certification

Section 401 of the Clean Water Act requires that any applicant for a 404 permit must provide the permitting agency a certification from the State that the discharge complies with applicable State water quality standards. Part 230.10 (a)(5)(b) of the Guidelines states that, "No discharge of dredged or fill material shall be permitted if it: (1) Causes or contributes,

after consideration of disposal site dilution and dispersion, to violations of any applicable State quality standards".

Section 401 certification is wholly the responsibility of the States. Some States have codified specific testing procedures and requirements for making Section 401 certification determinations, but most have not. The testing and evaluation procedures presented in this manual address all aspects of water quality impacts from dredged material discharges, and should be sufficient for a Section 401 decision.

A letter requesting 401 certification, together with information and data demonstrating compliance with State water quality standards will be sent by the USACE District Engineer to the appropriate State agency at the earliest practicable time. The USACE Final Rule for Operation and Maintenance of Army Corps of Engineers Civil Works Projects Involving the Discharge of Dredged Material into Waters of the U.S. or Ocean Waters (33 CFR Parts 209, 335, 336, 337, and 338) provides timeframes for 401 certification. The USACE will assume the State has waived 401 certification if the State agency does not respond in a timely manner.

If the mixing zone determined in accordance with Part 230.11(f) of the Guidelines is substantially different from the mixing zone defined by the State 401 authority, the evaluator should reconcile the differences in coordination with the State, USACE and USEPA.

4.6 Contaminant Determination

After consideration of all available information, one of the following two possible **conclusions** can be reached at Tier 2:

1 - Existing information does not provide a sufficient basis for making a contaminant determination. In this case, further evaluation at Tier 3 is appropriate.

2 - Existing information does provide a basis for making a contaminant determination. In this case, one of the following two **determinations** can be reached:

a) The proposed dredged material discharge will not cause unsuitable, adverse, contaminant-related impacts.

b) The proposed dredged material discharge will cause unsuitable, adverse, contaminant-related impacts.

The current state-of-the-art will provide adequate information for a contaminant determination at the end of Tier 2

in only a limited number of situations. If the only cause for proceeding into Tier 2 was the presence of a single contaminant, of which the toxicology and bioaccumulation potential are well understood, a determination may be completed in Tier 2. In addition, if Tier 2 testing was performed solely for determining 401 compliance, a determination may be completed here.

When (and if) national sediment quality criteria (SQC) are developed, they may be applied in Tier 2. However, the application of numerical SQC would probably not address potential interactive effects of contaminants for which additional testing may be necessary.

4.7 Reporting

Information gathered under Tier 1 and Tier 2 must be summarized and condensed in the 404(b)(1) evaluation document. Because a comprehensive tiered evaluation will likely gather far more information than can be presented in the 404(b)(1) evaluation, and because of the importance of the decisions made at this tier, it is recommended that this information be documented and filed as a backup to the 404(b)(1) evaluation. This documentation should be developed into a report that can be distributed for State and Federal agency review and if necessary, inserted as an appendix to the 404(b)(1) evaluation public review document.

A summary of the results from Tier 2 analysis should include the following, along with the summary of results developed from the Tier 1 analysis discussed above in paragraph 3.7:

- sampling results of sediment bulk chemistry and physical testing program;
- QA/QC documentation;
- water column impact evaluations (where appropriate), including; water quality screen/model results, or elutriate/model results, mixing zone determination, and;
- benthic impact evaluations (where appropriate), including; list of potentially bioaccumulative contaminants, TBP calculation results, and evaluation of non-hydrophobic, bioaccumulative contaminants.

5. TIER 3 - BIOLOGICAL TESTING

5.1 Purpose

The purpose of Tier 3 is to make contaminant determinations through the use of effects-based biological tests (figure 8). It is anticipated that the vast majority of contaminant

determinations will be reached at Tier 1 or Tier 3. As outlined in Tier 1, dredged material which are not a carrier of contaminants, which satisfy the exclusions from testing, or which have sufficient historical data will require no additional testing for a determination.

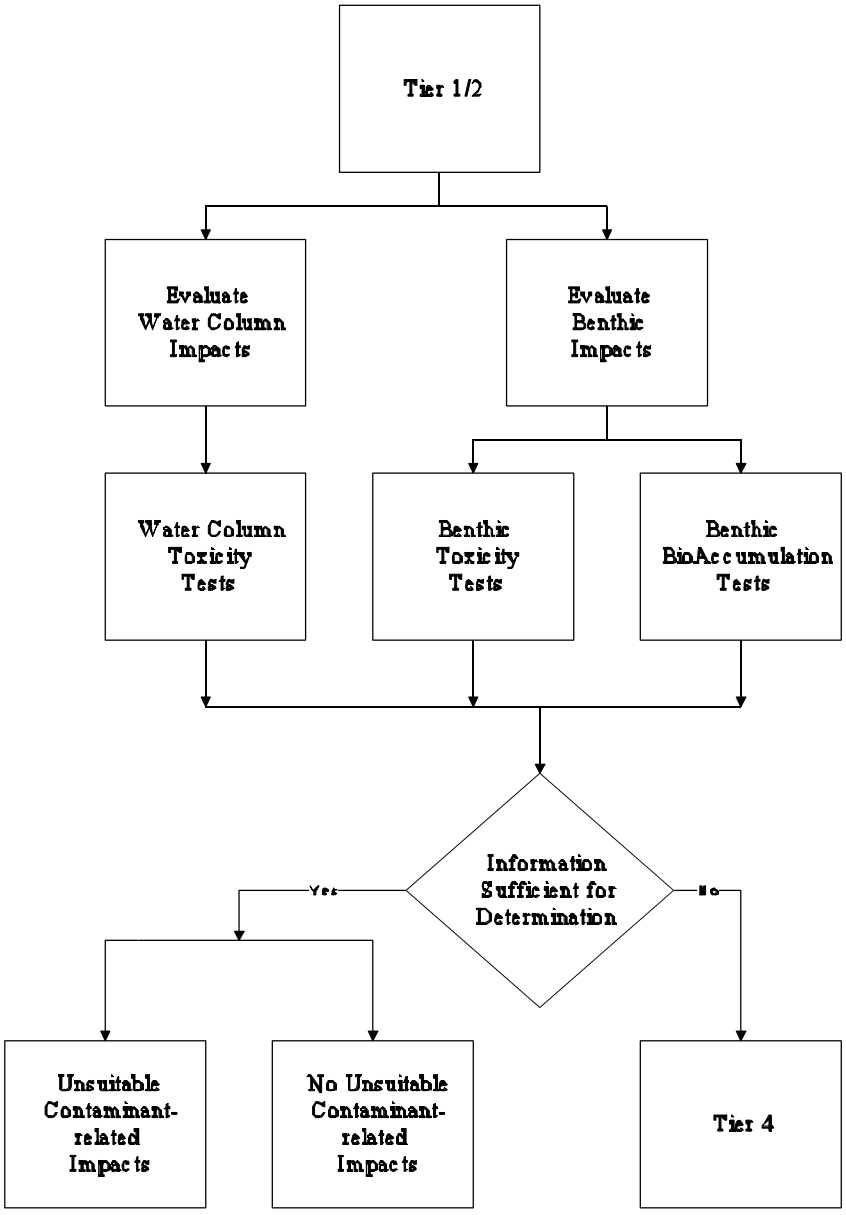


Figure 8. Tier 3 Flow Diagram

In Tier 2, it was intended that a determination be made using sediment physical and chemical data alone. However, there are relatively few biological effects that can be correlated with

specific contaminants in sediments. In addition, sediments typically contain complex mixtures of contaminants and the interactive effects of these contaminants on biological organisms cannot yet be predicted based upon physical and chemical data alone. As a result, there are very few situations where a determination can be reached in Tier 2.

Appendix G presents six effects-based biological tests for dredged material evaluation. Potential water column and benthic impacts of the discharge of dredged material are evaluated through Tier 3 biological tests. It is expected that the completion of these tests will result in information sufficient for making a contaminant determination. Only in unusual cases should further testing in Tier 4 testing be necessary.

5.2 Planning and Coordination

Planning and coordination is needed in all stages of a 404(b)(1) evaluation, but the need is especially critical in Tier 3 because of the high costs of biological effects testing. For most dredging projects, these high costs will necessitate that each sample represent a larger portion (e.g., management unit) of the area to be dredged. Coordination with other agencies conducted in earlier tiers should be continued in Tier 3. A written plan for sediment sampling and analyses should be prepared and provided to the appropriate Federal and State agencies for coordination prior to sampling.

5.3 Sediment Sampling and Analysis

5.3.1 Sediment Sampling

Detailed guidance on acceptable sediment sampling methods and procedures is provided in Appendix D. Included in this appendix are information on acceptable sediment collection and handling procedures. Also included is guidance on how to plan and execute a sampling program. Sediment sampling plans are so site specific that guidance on the number, type, and location of samples is necessarily quite general. The guidance provided in paragraph 4.3 is generally applicable to Tier 3 sampling.

Because of the limitations of Tier 2 in reaching a contaminant determination without further testing, the evaluator may decide to collect data for Tier 2 and Tier 3 at the same time. This may be appropriate where Tier 1 has yielded an extensive amount of information about the physical and chemical properties of the dredged material and their distribution. If there is very limited information about the physical and chemical nature of the dredged material, it is recommended that a Tier 2 evaluation be completed before proceeding with Tier 3 sampling.

Because of the cost of effects-based testing and the volume of dredged material sample needed for a suite of tests, the total number of samples tested in Tier 3 will generally be much fewer than in Tier 2. As a result, the dredging site must be divided into subareas, or "management units". The test results from a single sample (or composite) are used to make a determination about the contaminants in each management unit.

It is possible to have different results for different samples from a single dredging project. The management unit concept was developed for this condition. The contaminant determination for a single dredging project composed of several management units may be that one area is acceptable for open water disposal, another area is unacceptable, and a third has an inconclusive determination after Tier 3.

The delineation of management units for a proposed dredging project is an important step, and should be made through consideration of:

- information from Tier 1 evaluation,
- data from Tier 2 testing (if available), and
- proposed dredging and disposal method.

While the linkages between physical/chemical characteristics of sediments and biological effects are not well understood, the physical and chemical homogeneity of dredged material in a specific area of a river or harbor is considered appropriate rationale for management unit delineation. The sediments within a management unit will typically be dredged and disposed within a limited timeframe, mixed and homogenized to some degree during handling, and will likely be disposed in the same portion of the disposal site.

5.3.2 Effects-Based Tests

Effects-based biological tests are laboratory procedures in which organisms are exposed to a contaminated medium. Most of the water quality standards and criteria for specific contaminants were developed from effects-based tests. These types of tests used direct exposures of organisms to known levels of a single contaminant. Example of test exposures include a mouse fed a contaminant in its food, or a fish placed in a tank with the contaminant dissolved in its water. The biological effects which may be measured by such tests include mortality (death) of the organism, growth, reproduction, and others.

A number of methodologies for the bioassessment of freshwater sediments have been developed (Dillon and Gibson, 1990; Dillon and Gibson, 1986). Some of the existing

methodologies were developed to measure biological effects related to specific contaminants. Some were designed to simulate specific exposure conditions. Most are tests intended to measure the response of a sensitive organism to a mixture of sediment contaminants.

The type of organism, exposure media, exposure conditions, and measured effects or end-points are all specific to the questions being addressed. In the context of a 404(b)(1) evaluation, the question being asked is not what impacts the sediment contaminants are having in-place, but what impacts they would have if the sediments were dredged and discharged somewhere else.

Biological-effects tests for dredged material testing and evaluation must represent the physical and chemical conditions of contaminant exposure during dredged material disposal. For a 404(b)(1) evaluation, there are two exposure conditions to be tested; water column and benthic. The water column exposure is directed at the impacts of contaminants released into the water from dredged material as they are discharged and settle to the bottom. The benthic exposure is directed at the impacts of contaminants in the dredged material after they have deposited on the bottom at the disposal site.

For this manual, the USEPA and USACE have developed six effects-based biological tests for dredged material evaluation. Three of the tests developed for this manual are water column tests, which utilize sediment elutriate preparations. The other three are benthic tests, which utilize whole sediment as test media. Complete methodologies for the six tests are provided in Appendix G. The six biotests are summarized on table 2.

These six test organisms were selected for a number of reasons. All are easily cultured and handled in a laboratory setting, and are relevant from an ecological standpoint. The three species for the water column (elutriate) tests (*Daphnia magna*, *Ceriodaphnia dubia* (which are both cladocerans), and *Pimephales promelas* (fathead minnow) have been used extensively in the NPDES permitting program. These three species are relatively sensitive to a variety of contaminants, and standard test methods are available for both short-term and long-term exposures with these organisms (USEPA 1989, 1991). With a few modifications, these methods have been adapted for dredged material elutriate testing (Appendix G).

The midge *Chironomus tentans* and the amphipod *Hyalella azteca* are used to estimate the toxicity of solid phase dredged material. Both species have been widely used for sediment assessments, and standard test methods developed (ASTM 1992,

Ankley et al. 1993). We recommend that both species be tested in routine dredged material assessments, as they vary in their sensitivity to different contaminants, e.g., *H. azteca* is quite sensitive to metals, while *C. tentans* tends to be more sensitive to pesticides.

Table 2. Effects-based biological tests

Species	Test Type[1]	Endpoint(s)	Test Duration (days)[2]
Daphnia magna	E	Survival/Survival and reproduction	2/21
Ceriodaphnia dubia	E	Survival/Survival and Reproduction	2/7
Pimephales promelas	E	Survival/Survival and Growth	4/7
Chironomus tentans	S	Survival and Growth	10
Hyalella azteca	S	Survival and Growth	10
Lumbriculus variegatus	S	Bioaccumulation	28

[1] Elutriate (E) or solid phase (S)
[2] Only short-term tests recommended for Tier 3 application.

The oligochaete *Lumbriculus variegatus* is used to assess the potential bioaccumulation of contaminants from dredged material. Unlike many other freshwater macroinvertebrates that have been used for sediment tests, *L. variegatus* is large enough to ensure that adequate tissue mass is available to perform chemical analysis for bioaccumulative contaminants. Standard methods have been developed for testing *L. variegatus* (Phipps et al. 1993), and the test has performed well in field validation studies (Ankley et al. 1992).

While there are many other biological tests which have been developed for sediments, only those presented in this manual are recommended for Tier 3 evaluation at the present time. Other tests, which are not considered ready for regional use in 404(b)(1) evaluations are discussed briefly in Tier 4. The USEPA and USACE will continue to consider other effects-based tests for their applicability to Great Lakes 404(b)(1) evaluations. Future updates of this manual may include modifications to the test procedures presented here and new tests for inclusion in Tier 3.

5.3.3 Quality Assurance

Quality assurance is a critical element in all tiers of a 404(b)(1) contaminant evaluation. General QA guidance and the

data quality objectives for Great Lakes dredged material testing and evaluation is provided in Appendix E. Quality control guidance for dredged material sampling and handling is provided in Appendix D. Minimum QC requirements for the performance of specific effects-based tests are provided in Appendix G.

5.4 Benthic Impact Evaluations

The Tier 3 benthic evaluation will determine if dredged material contaminants have the potential to cause an unacceptable adverse impact on benthic organisms. Two toxicity tests and one bioaccumulation test have been developed for regional use in this manual.

5.4.1 Benthic Toxicity Tests

The methodologies for the benthic toxicity tests with *Chironomus tentans* and *Hyalella azteca* are detailed in Appendix G. *Chironomus tentans* is the insect known as the midge fly. Midge fly larvae are often referred to as "bloodworms" because of the hemoglobin pigment in their bodies gives them a distinctive red coloration. This species is a non-biting form whose larvae are typically found burrowing in sediments of eutrophic ponds and lakes, and is an important food item in the diets of bottom feeding fish.

H. azteca is an amphipod (also called a scud or sideswimmer) which is a small freshwater crustacean which inhabits the water column and sediment surface, feeding on detritus. This species is an important food item for bottom feeding and water column fish in the Great Lakes.

The benthic toxicity tests are conducted by placing the test organisms into small (300 ml) beakers which are filled with water and have a layer of the test sediment at the bottom. The water overlying the sediment is renewed periodically. Organisms are fed during the exposure. The tests are completed in ten days, at which time the organisms are examined for response.

Both of these toxicity tests have been developed to measure lethal or sublethal responses. The lethal response is measured as mortality or survival of organisms. The sublethal response measured is growth. The results of these toxicity tests for the dredged material and the disposal site sediment are compared statistically for the contaminant determination.

The USEPA and USACE recommend that both of these toxicity tests be used within Tier 3 for 404(b)(1) evaluations of Great Lakes dredged material and measured for survival. The USEPA and USACE recommend that growth be measured for Tier 3 evaluations

only for *C. tentans.* Interpretation guidance on sublethal responses for *H. azteca* is currently under development. When this guidance has been completed and accepted by the USEPA and USACE, it will be incorporated into the Tier 3 evaluation.

The results of the benthic toxicity tests must first be evaluated in light of the QA objectives defined in Appendix E. If the responses of organisms in control exposures are within acceptable limits, the test results with the dredged material and the disposal site sediment may be evaluated using the statistical methods described in the Inland Testing Manual (USEPA/USACE 1994).

Dredged material is considered **not** to meet the Guidelines when the mortality of test organisms exposed to the dredged material is more than 10 percent greater (20 percent for *C. tentans*) than the mortality of test organisms exposed to the disposal site sediment **and** is statistically different at the 95 percent confidence level.

Dredged material is considered **not** to meet the Guidelines when the mean weight of *C. tentans* exposed to the dredged material is less 0.6 mg per organism (dry weight), **and** the mean weight of organisms exposed to the dredged material is more than 10 percent less than the mean weight of organisms exposed to the disposal site sediment, **and** this difference in mean weights is statistically significant at the 95 percent confidence level.

Determinations based on survival of *C. tentans* and *H. azteca* and growth of *C. tentans* are considered independently. If the results of any of these three evaluations are negative, the dredged material discharge is considered not to meet the Guidelines. If negative test results are suspected to be the result of non-contaminant impacts, additional benthic toxicity testing using sublethal end points or other organisms may be considered in Tier 4.

5.4.2 Bioaccumulation Test

The methodology for the benthic bioaccumulation test with *Lumbriculus variegatus* is detailed in Appendix G. *L. variegatus* is a freshwater oligochaete worm (aquatic earthworm) that is 1-1.5 mm in diameter and 40-90 mm long. It burrows in sediments, is an important food item for bottom feeding fish, and is commonly cultured and harvested for fish food in pet stores.

The benthic bioaccumulation test is conducted by placing a large number (500-1000) of organisms into a 5.5 liter aquarium with a layer of sediment and overlying water. The water is

renewed periodically, but the organisms are not fed during the exposure (other than organic matter already in the sediments). The tests are completed in 10-28 days, at which time the organisms are prepared for chemical analysis.

Benthic bioaccumulation testing is **not** necessary if the proposed dredged material has no bioaccumulative contaminants of concern (as determined in Tier 1) or if the TBP analysis conducted in Tier 2 conclusively indicates that there is no potential for bioaccumulation of contaminants relative to the disposal site sediment.

If the contaminant of concern list for the dredged material includes bioaccumulative contaminants, and if analysis for potential bioaccumulation conducted in Tier 2 was inconclusive, the dredged material should be tested using the benthic bioaccumulation test. The results of bioaccumulation tests with the dredged material are compared statistically to the results with the disposal site sediment.

Dredged material is considered **not** to meet the Guidelines when the mean concentration of bioaccumulative contaminant(s) in test organisms exposed to the dredged material is statistically greater than the concentration of these contaminant(s) in test organisms exposed to the disposal site sediment.

5.5 Water Column Impact Evaluations

The Tier 3 evaluation will determine if the dredged material contaminants cause an unacceptable adverse impact on organisms within the water column. Three water column toxicity tests (elutriate-based tests) have been developed for this manual.

The methodologies for the water column toxicity tests with *Daphnia magna*, *Ceriodaphnia dubia*, and *Pimephales promelas* are detailed in Appendix G. *D. magna*, commonly called a water flea, is a freshwater cladoceran common in Great Lakes plankton. It, and its smaller cousin *C. dubia* have been cultured in the laboratory and used in a variety of bioassays. The daphnids are an important food item of small and young fish.

P. promelas is also called the fathead minnow. It is a small fish (about 10-14 cm at maturity) which is commonly used for fishing bait. It is a prolific breeder, has been used for toxicity testing both as an adult and as larvae. The fathead minnow is ubiquitous throughout the Great Lakes and its tributaries, and is a forage food for larger fish.

All three water column toxicity tests use elutriate preparations prepared by mixing sediment and water (on a 1:4

ratio) into a slurry. The slurry is allowed to settle and the supernatant decanted. The supernatant is then centrifuged to remove suspended particles. This supernatant is the elutriate, which is diluted in series and used as the test solution for water column toxicity tests.

The test organisms are exposed to the elutriate in beakers or small aquaria. The elutriate is renewed periodically and the organisms are fed during the exposure. The elutriate tests were developed to measure lethal and sub-lethal responses, with short- and long-term exposures. The *D. magna* tests are completed in two (short-term) or 21 (long-term) days. The *C. dubia* tests are run in two or seven days, and the *P. promelas* test in seven or 21 days. The lethal response is measured as mortality or survival of organisms. The sublethal response measured is reproduction for *D. magna* and *C. dubia* and growth for *P. pimephales*. The results of these toxicity tests for the dredged material are evaluated to determine if an unacceptable toxicity risk will occur outside the mixing zone. Water column testing of the disposal site sediment is not appropriate.

The water column tests simulate exposure conditions that may be very transient in the field. The majority of open-water disposal of dredged material in the Great Lakes occurs from barges, scows and hoppers which "dump" the material through bottom doors. These discharges are instantaneous, rather than continuous, and the time between discharges may be as short as 30 minutes to as long as several hours. The water column exposure period is limited to the time required for the dredged material to settle to the bottom (a matter of minutes or seconds). The discharge from a hydraulic dredge is more continuous, and can produce water column exposures more closely resembling the toxicity tests. However, hydraulic discharge is not commonly used in the Great Lakes except for beach nourishment disposal of dredged material.

Experience with effects-based testing of dredged material conducted for ocean disposal (Section 103) regulation has demonstrated that the benthic impacts of dredged material contaminants are more ecologically significant than water column impacts. Water column toxicity testing has been greatly reduced or eliminated in some regional 103 testing manuals.

For the above reasons, the USEPA and USACE recommend that only one of the water column toxicity tests be used within Tier 3 for 404(b)(1) evaluations of Great Lakes dredged material and measured for lethal responses with a short-term exposure. Interpretation guidance on sublethal responses for these tests is currently under development. When this guidance has been

completed and accepted by the USEPA and USACE, it may be incorporated into the Tier 3 evaluation.

One potential cost-saving measure during the implementation of water column tests that might be considered is to perform the test only with the full-strength elutriate, and not conduct the dilution series. Experience with similar tests and marine sediments has shown that undiluted elutriates infrequently produced mortality greater than 50 percent. While it must be recognized that there is a risk of having to repeat the test, the potential cost-savings outweigh this risk in most cases.

The results of the water column toxicity test must first be evaluated in light of the QA objectives defined in Appendix E. If the responses of organisms in control exposures are within acceptable limits, the test results with the dredged material may be evaluated using the statistical methods in the "Inland Testing Manual" (USEPA/USACE 1998) and the water quality screen model employed in Tier 2.

Dredged material is considered **not** to meet the Guidelines when the concentration of dredged material contaminants at the boundary of the mixing zone statistically exceeds 0.01 of the concentration (LC_{50}) causing 50 percent mortality of test organisms exposed to the dredged material elutriate. The screening model (paragraph 4.5.1) is used to calculate the dilution of the elutriate within the mixing zone.

5.6 Contaminant Determination

After consideration of all available information, one of the following two possible **conclusions** can be reached at Tier 3:

1 - Existing information does not provide a sufficient basis for making a contaminant determination. In this case, further evaluation at Tier 4 may be appropriate.

2 - Existing information does provide a basis for making a contaminant determination. In this case, one of the following **determinations** can be reached:

a) The proposed dredged material discharge will not cause unsuitable, adverse, contaminant-related impacts.

b) The proposed dredged material discharge will cause unsuitable, adverse, contaminant-related impacts.

The information obtained in Tier 3 and earlier tiers should be sufficient to reach a contaminant determination in almost all cases. Therefore, the first conclusion (information not

sufficient) should be reached only in unusual circumstances.

5.7 Reporting

Information gathered during Tiers 1, 2 and 3 must be summarized and condensed in the 404(b)(1) evaluation document. Because a comprehensive tiered evaluation will likely gather far more information than can be presented in the 404(b)(1) evaluation, and because of the importance of the decisions made at this tier, it is recommended that this information be documented and filed as a backup to the 404(b)(1) evaluation. This documentation should be developed into a report that can be distributed for State and Federal agency review and if necessary, inserted as an appendix to the 404(b)(1) evaluation public review document.

6. TIER 4 - CASE-SPECIFIC TESTING

6.1 Purpose

The purpose of Tier 4 is to make contaminant determinations through the use of case-specific testing and evaluation. It is anticipated that the information obtained from testing and evaluations in Tiers 1, 2 and 3 will not be sufficient for a contaminant determination in very few cases. For example, Tier 4 testing may be appropriate where Tier 3 test results are conflicting or inconclusive.

In these rare cases, testing procedures that have not been adopted for regional application, and those that are more research-oriented may be employed, as necessary. Because any testing and evaluation conducted in Tier 4 is entirely case-specific, limited guidance can be offered. Further, it must be recognized that Tier 4 is not an invitation to conduct basic research, but a mechanism for obtaining the information necessary to address case-specific dredged material contaminant impacts.

Tier 4 testing should be focused on contaminant issues not resolved in earlier tiers. If Tier 3 testing for water column toxicity and benthic bioaccumulation were conclusive but the benthic toxicity testing was not, Tier 4 testing should be limited to the unresolved benthic toxicity impacts of dredged material contaminants. Similarly, if Tier 3 testing produced conclusive determinations for some management units of a proposed dredging area, but not others, Tier 4 evaluations should be limited to those management units in question.

6.2 Planning and Coordination

Because there are no hard-and-fast rules in Tier 4, it is imperative that the testing and evaluation be coordinated with other agencies up front. When using testing procedures which have no established interpretive guidance, case-specific evaluative criteria must be developed in advance.

6.3 Testing and Evaluation Procedures

The tools that are used in Tier 4 to evaluate dredged material contaminant impacts may include toxicity and bioaccumulation tests which differ from the Tier 3 tests in both the level of intensity and in cost. Examples of these differences include:

- different end points,
- different test species, and
- varying exposure conditions to reflect case-specific field conditions.

The USEPA and USACE have developed methodologies for the sub-lethal benthic toxicity tests with *Chironomus tentans* and *Hyalella azteca* and sub-lethal water column toxicity tests with *Daphnia magna*, *Ceriodaphnia dubia*, and *Pimephales promelas*. These tests are developed for measurement of growth as a sublethal response, and the procedures are provided in Appendix G. Since the interpretation guidance for these tests has not been completed and accepted by the USEPA and USACE, the use of these sub-lethal toxicity tests remains an option under Tier 4 When the interpretation guidance is completed, these sub-lethal toxicity tests may be incorporated into the Tier 3 evaluation.

The "Inland Testing Manual" (USEPA/USACE 1998) lists a number of organisms for which toxicity and bioaccumulation tests have been developed. Although few of these tests were developed or used for regulatory decision making, this list can be used to identify potential species for Tier 4 testing.

Tier 4 may also require tools to evaluate the exposure and impacts of dredged material contaminants in the field, away from the disposal site, or on higher trophic levels. Examples of these tools include:

- field biota collection,
- field exposures (caged organisms),
- contaminant transport/contaminant fate modeling, and
- human health/ecological risk analysis.

When planning a Tier 4 evaluation, it is recommended that the evaluator review the Guidelines and keep the following principles in mind throughout:

- a benthic evaluation is made of contaminant impacts relative to the disposal site sediment,
- a water column evaluation must consider the effects of mixing, and
- a contaminant determination is directed at whether or not an impact will occur, and not why.

6.4 Contaminant Determination

At the conclusion of Tier 4, there are two possible **determinations** which can be reached:

a) The proposed dredged material discharge will not cause unsuitable, adverse, contaminant-related impacts.

b) The proposed dredged material discharge will cause unsuitable, adverse, contaminant-related impacts.

Dredged material management considerations, treatment options, or other actions which might be used to abate contaminant-related impacts are outside of the scope of this guidance manual.

7. REFERENCES

American Society for Testing and Materials (ASTM). 1992. "New standard guide for conducting solid phase sediment toxicity tests with freshwater invertebrates," in ASTM Annual Book of Standards, Volume 11.04, E1383-92, ASTM, Philadelphia, Pennsylvania.

Ankley, G.T., Benoit, D.A., Hoke, R.A., Leonard, E.N., West, C.W., Phipps, G.L., Mattson, V.R., and L.A. Anderson. 1993. "Development and evaluation of test methods for benthic invertebrates and sediments; effects of flow rate and feeding on water quality and exposure conditions," Arch. Environ. Contam. Technol., in press.

Ankley, G.T., Cook, P.M., Carlson, A.R., Call, D.J., Swenson, J.A., Corcoran, H.F., and R.A. Hoke. 1992. "Bioaccumulation of PCBs from sediments by oligochaetes and fishes: a comparison of laboratory and field studies," Can. J. Fish. Aquat. Sci. 49:2080-2085.

Averett, D.E., Perry, B.D., Torrey, E.J., and J.A. Miller. 1990. "Review of removal, containment and treatment technologies for remediation of contaminated sediment in the Great Lakes," Miscellaneous Paper EL-90-25, prepared for USEPA Great Lakes National Program Office, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

Dillon, T.M. and A.B Gibson. 1986. "Bioassessment methodologies for the regulatory testing of freshwater dredged material; Proceedings of a Workshop," Miscellaneous Paper EL-86-6, prepared for U.S. Army Engineer District, St. Paul and the Wisconsin Department of Natural Resources, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

Dillon, T.M. and A.B Gibson. 1990. "Review and synthesis of bioassessment methodologies for freshwater contaminated sediments," Miscellaneous Paper EL-90-2, prepared for USEPA Great Lakes National Program Office, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

Eckenfelder, W.W., Jr. 1980. Principles of Water Quality Management, CBI Publishing, Boston, MA.

International Joint Commission (IJC). 1982. "Guidelines and register for evaluation of Great Lakes dredging projects," Report of the Dredging Subcommittee to the Water Quality Programs Committee of the Great Lakes Water Quality Board, Windsor, Ontario.

IJC. 1986. "Evaluation of sediment bioassessment techniques," Report of the Dredging Subcommittee to the Great Lakes Water Quality Board, Windsor, Ontario.

Karickhoff, S. 1981. "Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils," Chemosphere, 9:3-10.

Konemann, H. and C. van Leeuwen. 1980. "Toxicokinetics in fish: Accumulation and elimination of six chlorobenzenes by guppies," Chemosphere, 9:3-19.

McFarland, V.A. 1984. "Activity-based evaluation of potential bioaccumulation from sediments," in Dredging '84, Proceedings of ASCE 1:461-467.

Merck. 1989. Merck Index, 11th edition, Merck & Co., Inc., Rahway, NJ.

NOAA. 1991. "Contaminant trends in the Southern California Bight: Inventory and assessment," NOAA Technical Memorandum NOS ORCA 62, National Oceanic and Atmospheric Administration, Seattle, WA.

Palermo, M. 1991. "Design requirements for capping," Dredging Research Technical Note DRP-5-03, U.S. Army Waterways Experiment Station, Vicksburg, Mississippi.

Palermo, M. 1991b. "Site selection considerations for capping," Dredging Research Technical Note DRP-5-04, U.S. Army Waterways Experiment Station, Vicksburg, Mississippi.

Phipps, G.L., Ankley, G.T., Benoit, D.A., and V.R. Mattson. 1993. "Use of aquatic oligochaete *Lumbriculus variegatus* for assessing the toxicity and bioaccumulation of sediment-associated contaminants," Environ. Toxicol. Chem. 11:269-280.

U.S. Army Corps of Engineers (USACE). 1976. "Ecological evaluation of proposed discharge of dredged or fill material into navigable waters; Interim guidance for implementation of Section 404(b)(1) of Public Law 92-500," DMRP Miscellaneous Paper D-76-17, Waterways Experiment Station, Vicksburg, Mississippi.

USACE/USEPA. 1992. "Evaluating environmental effects of dredged material management alternatives--A technical framework," EPA842-B-92-008, Washington, D.C.

U.S. Environmental Protection Agency (USEPA). 1977. "Guidelines for the pollutional classification Great Lakes Harbor sediments", Region 5, Chicago, Illinois.

USEPA. 1987. "Quality assurance/quality control (QA/QC) for 301(h) monitoring programs: Guidance on field and laboratory methods," EPA 430/9-86-004, prepared by Tetra Tech, Inc., Bellevue, WA, for the USEPA Office of Marine and Estuarine Protection, Washington, DC.

USEPA. 1989. "Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms," EPA/600/4-89/001, Cincinnati, Ohio.

USEPA. 1991. "Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms," EPA/600/4-90/027, Washington, DC.

USEPA. 1992. "Tiered testing issues for freshwater and marine sediments - workshop proceedings," Office of Science and Technology, Washington, DC.

USEPA. 1994. "ARCS remediation guidance document," EPA 905-R94-003, Great Lakes National Program Office, Chicago, Illinois.

USEPA/USACE. 1990. "Ecological evaluation of proposed discharge of dredged material into ocean waters," EPA-503-8-90/002, Washington DC.

USEPA/USACE. 1998. "Evaluation of material proposed for discharge to waters of the U.S. - Testing Manual (Inland Testing Manual)," EPA-823-B-98-004, Office of Water, Washington DC.

WDNR/USGS. 1992. "Sources of pollutants in Wisconsin stormwater," unpublished report prepared by Wisconsin Department of Natural Resources and U.S. Geological Survey, Madison, WI.

Zeman, A.J., Sills, S., Graham, J.E. and K.A. Klein. 1992 (Draft). "Subaqueous capping of contaminated sediments: Annotated bibliography," NWRI Contribution 92-XX, National Water Research Institute, Burlington, Ontario, Canada.

GREAT LAKES
DREDGED MATERIAL TESTING AND EVALUATION MANUAL

APPENDIX A
LIST OF ACRONYMS AND ABBREVIATIONS

ADDAMS - Automated Dredging Disposal Alternatives Management System
AOC - Area of Concern
ASTM - American Society of Testing and Materials

CDF - Confined Disposal Facility
CFR - Code of Federal Regulations
CWA - Clean Water Act

DMRP - Dredged Material Research Program
DNR - Department of Natural Resources
DO - dissolved oxygen
DQI - Data Quality Indicator
DQO - Data Quality Objective

EA - Environmental Assessment
EIS - Environmental Impact Statement
ER - Engineering Regulation

FDA - Food and Drug Administration
FONSI - Finding Of No Significant Impact
FY - Fiscal Year

GC - Gas Chromatography
GIS - Geographic Information Systems
GLTEM - Great Lakes Testing & Evaluation Manual

HTW - Hazardous and Toxic Wastes
HQUSACE - Headquarters, U.S. Army Corps of Engineers

IJC - International Joint Commission

Kg - Kilogram

LC_{50} - lethal concentration (50% mortality)

mg - milligram
MPRSA - Marine Protection, Research and Sanctuary Act
MS/MSD - Matrix Spike/Matrix Spike Duplicate

NEPA - National Environmental Policy Act
NOAA - National Oceanic and Atmospheric Administration
NPDES - National Pollutant Discharge Elimination System

PAH - Polynuclear Aromatic Hydrocarbon
PCB - Polychlorinated Biphenyl
PCS - Permit Compliance System
PL - Public Law
ppm - parts per million

QA - Quality Assurance
QAMP - Quality Assurance Management Plan
QAPP - Quality Assurance Project Plan
QC - Quality Control

RAP - Remedial Action Plan
RCRA - Resource Conservation and Recovery Act
RCRIS - RCRA Information System

SOP - Standard Operating Procedure
SQC - Sediment Quality Criteria
STFATE - Short Term Fate
STORET - STOrage and RETrieval system

TBP - Theoretical Bioaccumulation Potential
TKN - Total Kjeldahl Nitrogen
TOC - Total Organic Carbon
TPH - Total Petroleum Hydrocarbons
TRI - Toxic chemical Release Inventory
TSCA - Toxic Substances Control Act
TVS - Total Volatile Solids

USACE - U.S. Army Corps of Engineers (Corps)
USEPA - U.S. Environmental Protection Agency (EPA)
USFWS - U.S. Fish & Wildlife Service
USGS - U.S. Geological Survey

VOC - Volatile Organic Compound

WES - Waterways Experiment Station
WQS - Water Quality Standards

GREAT LAKES
DREDGED MATERIAL TESTING AND EVALUATION MANUAL

APPENDIX B
GLOSSARY OF TERMS

Absorbance: A measure of the decrease in incident light passing through a sample into the detector.

Accuracy: The closeness of agreement between an observed value and an accepted reference value. When applied to a set of observed values, accuracy will be a combination of a random component (precision) and of a common systematic error (or bias) component.

Action level: Criteria for taking action for the environmental variables or characteristics being measured.

Acute toxicity: Short-term toxicity to organism(s) that have been affected by the properties of a substance, such as contaminated sediment.

Adjacent: Bordering, contiguous or neighboring.

Aliquot: Measured portion of a field sample taken for analysis.

Analyte: Specific component measured in a chemical analysis.

Analytical sample: Any solution or media introduced into an instrument on which an analysis is performed excluding instrument calibration, initial calibration verification, initial calibration blank, continuing calibration verification and continuing calibration blank.

Assessment: Evaluation process used to measure the performance or effectiveness of a system and its elements.

Audit: Planned and documented investigative evaluation of an item or process to determine the adequacy and effectiveness as well as compliance with established procedures, instructions, drawings, QAPPs, and or other applicable documents.

Batch: A group of samples which behave similarly with respect to the sampling or the testing procedures and which are processed as a unit.

Bioaccumulation: The accumulation of contaminants in the tissue of organisms.

Bioaccumulation factor: The degree to which an organism accumulates a chemical compared to the source. A dimensionless factor derived by dividing the concentration in the organisms by that in the source.

Bioassay: A test using a biological system, involving the exposure of an organism to a test material and determining a response.

Bioavailable: Can be taken up by organisms (i.e., from water, sediment, food, etc.).

Blanks: Field and laboratory quality control samples that are processed with the samples.

Calibration: Systematic determination of the quantitative, linearity and dynamic range of response of a test to the concentration of the analyte of interest.

Certified reference material: A reference material whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate of other documentation which is issued by a certifying body.

Chromatography: A process of selectively separating a mixture into its component compounds.

Chronic (sub-lethal) toxicity: Biological tests which use such factors as abnormal development, growth and reproduction, rather than solely lethality, as end-points.

Coefficient of variation: Standard deviation as a percent of the arithmetic mean.

Comparability: Reflects the confidence with which one data set can be compared with others.

Completeness: Measure of the amount of valid data obtained as compared to the amount of data intended to be collected.

Contaminant: A chemical or biological substance in a form that can be incorporated into, onto or be ingested by and harms aquatic organisms, consumers of aquatic organisms, or users of the aquatic environment, and includes but is not limited to the substances on the 307(a)(1) list of toxic pollutants promulgated on January 31, 1978 (43 CFR 4109).

Control limit: Range within which specified measurement results must be within to be compliant/valid.

Control sediment: A sediment essentially free of contaminants and compatible with the biological needs of the test organisms such that it has no discernable influence on the response being measured in the test. Control sediment may be the sediment from

which the test organisms are collected or a laboratory sediment, providing the organisms meet control standards.

Corrective action: Measures taken to rectify conditions adverse to quality and, where necessary, to preclude their recurrence.

Correlation coefficient: Number (r) which indicates the degree of dependence between two variables (e.g. concentration and response).

Data quality indicators: Measurable attributes of the attainment of the necessary quality for a particular environmental decision, including precision, bias, completeness, representativeness, reproducibility, comparability, and statistical confidence.

Data quality objectives: Qualitative and quantitative statements of the overall uncertainty that a decision make is willing to accept in results or decisions derived from environmental data.

Data validation: Process of evaluating available data against project DQIs and DQOs to make sure that the objectives were met.

Detector: Device used in conjunction with an analytical instrument to measure, and sometimes identify, the components of a sample.

Digestion: Process used prior to analysis that breaks down samples using acids (or bases). The end product is called a digestate.

Discharges of dredged material: Any addition of dredged material into waters of the United States and includes discharges of water from dredged material disposal operations including beach nourishment, upland, or confined disposal which return to waters of the United States. Material resuspended during normal dredging operations is considered "de minimis" and is not regulated under Section 404 as a dredged material discharge.

Disposal site: That portion of the United States waters where specific disposal activities are proposed or permitted. It consists of a bottom surface area and all overlying water, if present. Given that most disposal sites within the Great Lakes may be dispersive in nature, professional judgment may be necessary in the collection of sample(s) representing the disposal site (see discussion in section 4.3.3).

District: A USACE administrative area.

Dredged material: Material that is excavated or dredged from waters of the United States.

EC_{50}: The median effective concentration. The concentration of a substance that causes a specific effect in 50% of the organisms tested.

Elutriate: A suspension prepared by mixing specific volumes of sediment and water, used for chemical analysis and toxicity testing.

Estimated quantitation limit: Lowest concentration that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions.

Evaluation: A process of judging data in order to reach a decision.

Extraction: A chemical or mechanical procedure to remove organic compounds from a sample matrix. The end product of extraction is called an extract.

Factual determination: A determination in writing of the potential short-term and long-term effects of a proposed discharge of dredged or fill material on the physical, chemical and biological components of the aquatic environment in light of Subparts C-F of the Guidelines.

Federal standard: The dredged material disposal alternative(s) identified by the USACE that represent the least costly, environmentally acceptable alternative(s) consistent with sound engineering practices and which meet the environmental standards established by the 404(b)(1) evaluation process.

Fill material: Any material used for the primary purpose of replacing an aquatic area with dry land or changing the bottom elevation of a water bottom for any purpose. The term does not include any pollutant discharge into the water primarily to dispose of waste, as that activity is regulated under Section 402 of the Clean Water Act.

Great Lakes and **Great Lakes Basin:** The United States waters of Lakes Michigan, Superior, Huron, Erie, Ontario, the connecting channels, St. Lawrence River, their tributaries and any other waterbodies within the United States watersheds of these Lakes.

Guidance: National or regional implementation manuals developed to assist the evaluator in making a contaminant determination as defined in 404(b)(1) Guidelines.

Guidelines: The Section 404(b)(1) final rule (40 CFR 230) dated December 24, 1980.

Holding time: Elapsed time expressed in days from the time of collection until the date of its processing and/or analysis.

Instrument detection limit: Smallest signal above background noise that an instrument can detect reliably.

LC_{50}: The median lethal concentration. The concentration of a substance that kills 50% of the organisms tested.

Limit of detection: Lowest concentration that can be determined to be statistically different from a blank.

Limit of quantitation: Level above which quantitative results may be obtained with a specified degree of confidence.

Management unit: A manageable, dredgeable unit of sediment which can be differentiated by sampling and which can be separately dredged from a larger dredging area.

Matrix: Component or substrate (e.g. water, sediment, tissue) which contains the contaminants or constituents of interest. Matrix refers to the physical structure of a sample and how contaminants are bound within this structure.

Matrix duplicate: A type of laboratory duplicate used for organic analyses.

Matrix effect: Physical or chemical interactions between the sample material and the chemical of interest that can bias measurements in either a negative or positive direction.

Matrix spike: Quality control samples prepared by adding known amounts of contaminants to actual samples, usually prior to processing. Analysis of matrix spikes estimates the bias due to matrix effects.

Method: A body of procedures and techniques for performing an activity systematically presented in the order in which they are to be executed.

Method blank: An analyte-free matrix to which all reagents are added in the same volumes or proportions as used in sample processing. The method blank is used to document contamination resulting from the analytical process.

Method detection limit: Minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix type containing the analyte.

Method of standard additions: Addition of three increments of a standard solution (spikes) to sample aliquots of the same size.

Mixing zone: A limited volume of water serving as a zone of initial dilution in the immediate vicinity of a discharge point where receiving waters quality may not meet quality standards or other requirements otherwise applicable to the receiving water.

Practicable: Available and capable of being done, after taking into consideration cost, existing technology and logistics in light of overall project purposes.

Performance evaluation: A type of audit in which the quantitative data generated in a measurement system are obtained independently and compared with routinely obtained data to evaluate the proficiency of an analyst or laboratory.

Precision: Agreement among a set of replicate observations or measurements of the same property, usually obtained under similar conditions, without assumption of knowledge of the true value.

Procedure: Documented set of steps or actions that systematically specifies or describes how an activity is to be performed.

Process: Orderly system of actions that are intended to achieve a desired end or result. Examples of processes include analysis, design, data collection, operation, fabrication, and calculation.

Quality assurance: The total integrated program for assuring the reliability of data. It is a system for integrating the quality planning, quality control, quality assessment, and quality improvement efforts to meet user requirements and defined standards of quality within a stated level of confidence.

Quality assurance project plan: Detailed, project-specific document specifying guidelines and procedures to assure sufficient data of sufficient quality to meet project needs during data collection, analysis, and reporting.

Quality control: The overall system of technical activities for obtaining prescribed standards of performance in the monitoring and measurement process to meet user requirements.

Quality improvement: A management program for improving the quality of operation. Such management programs generally entail a formal mechanism for encouraging worker recommendations with timely management evaluation and feedback or implementation.

Quality management plan: A formal document that describes the quality system in terms of the organizational structure, functional responsibilities of management and staff, lines of authority, and required interfaces for those planning, implementing, and assessing all activities conducted.

Quality system: A structured and documented management system describing the policies, objectives, principles, organizational authority, responsibilities, accountability and implementation plan of an organization for ensuring quality in its work processes products (items), and services. The quality system provides the framework for planning, implementing, and assessing work performed by the organization and for carrying out required QA and QC.

Reference sediment: A term whose definition applies to the evaluation of dredged material proposed for discharge to the ocean. This term, and "reference site" do not have any legal standing in a 404(b)(1) evaluation at the time this manual is finalized.

Region: An USEPA administrative area.

Regulations: Procedures and concepts published in the Code of Federal Regulations Title 40, Part 230 for evaluating the discharge of dredged material into waters of the United States.

Replicate: One of several identical samples.

Representativeness: The degree to which sample data depict an existing environmental condition. A measure of the total variability associated with sampling and measuring that includes the two major error components: systematic error (bias) and random error.

Sediment: A soil material which has settled on the bottom of a water body. The term *dredged material* refers to sediments which have been dredged from a water body (see definition of dredged material), while the term sediment generally refers to material in a water body prior to the dredging process.

Semivolatile organic compound: Organic compound with moderate vapor pressure that can be extracted from samples using organic solvents and analyzed by gas chromatography.

Sensitivity: Amount of instrument response to a change in sample concentration which can be expressed as the slope of a curve of concentration versus instrument response.

Scope of work: A document used to define work to be performed by a contractor as part of a legally binding agreement.

Spectrometer: Instrument which measures the physical constants of materials (e.g. mass, index of refraction).

Spectrophotometer: Instrument which measures the relative intensities of light absorbed or emitted by chemical species.

Split samples: Aliquots of sample taken from the same container and analyzed independently.

Standard curve: Plot of concentrations of known analyte standards versus the instrument response to the analyte.

Sublethal: Not directly causing death; producing less obvious effects on behavior, biochemical and/or physiological function, histology of organisms.

Surrogate organic compound: Compounds with characteristics similar to those of compounds of interest that are added to all samples prior to processing. They are used to estimate recovery of organic compounds in a sample.

Standard operating procedure: Written document which details the method for an operation, analysis, or action whose mechanisms are thoroughly prescribed techniques and steps, and which is commonly accepted as the method for performing certain routine or repetitive tasks.

Technical systems audit: A thorough, systematic, on-site, qualitative audit of facilities, equipment, personnel, training, procedures, record keeping, data validation, data management, and reporting aspects of a system.

Trip blank: Sample of analyte-free media taken from the laboratory to the sampling site and returned to the laboratory unopened. A trip blank is used to document contamination attributable to shipping and field handling procedures.

Validation: Activity that demonstrates or confirms that a process, item, data set, or service satisfies the requirements defined by the user.

Volatile organic compound: Organic compound with a high vapor pressure that tend to evaporate readily from a sample.

Water quality certification: A statement or affirmation that the proposed discharge of dredged material will comply with applicable State water quality standards.

Water quality standard: Law or regulation that consists of the beneficial designated use or uses of a water body, the numeric and narrative water quality criteria that are necessary to protect the use or uses of that particular water body, and an anti-degradation statement.

Waters of the U.S.: In general, all waters landward of the baseline of the territorial sea and the territorial sea. Specifically, all waters defined in Section 230.3(g) of the Guidelines.

Wet weight: Weight of a sample aliquot including moisture (undried).

GREAT LAKES
DREDGED MATERIAL TESTING AND EVALUATION MANUAL

APPENDIX C
INFORMATION FOR TIER 1 AND TIER 2 EVALUATIONS

Note: This Appendix contains numerous sources of information and names of individuals who were points-of-contact at the time this Appendix was last updated (1997). These sources of information and contacts are subject to change, and may no longer be current. An effort will be made to update this Appendix after the manual is finalized.

compiled by:

John L. Dorkin
USEPA, Region 5

Jan A. Miller
USACE, Great Lakes & Ohio River Division

TABLE OF CONTENTS

SECTION 1
Computer Data Bases

This section provides information about existing environmental databases maintained by the USEPA that a 404(b)(1) evaluator might use to identify historical sediment quality data or information about potential sources of contamination in Tier 1. Some of these databases are multi-purpose, and others are maintained by the USEPA to support a specific program, such as RCRA permitting program. Fact sheets are provided for each data base with brief descriptions and access information.

The databases can be accessed through the USEPA contacts provided. In most cases, a database manager will perform the search and provide the evaluator with a report. Some lead time for coordination with the database managers should be anticipated in order to determine the format and the capabilities of the database being searched. The type of information needed to retrieve data may vary with the database. For example, STORET can query for data within a circle around a point of known latitude and longitude, or within polygon formed of points of known latitude and longitude. Other databases can make queries based on political boundaries (state, county, etc.).

When responding to certain more extensive data requests by the general public or a private 404(b)(1) permit applicant or contractor for the applicant, the USEPA database manager may occasionally ask that the request be formally transmitted through the agency's Freedom of Information Act (FOIA) office. If so instructed, the requestor should describe the desired information clearly and succinctly in a letter addressed to the "Freedom of Information Officer" at the appropriate address below. Other Federal agencies should request USEPA database information through normal coordination channels.

Region 2	Region 3	Region 5
USEPA Region 2 26 Federal Plaza New York, NY 10278	USEPA Region 3 841 Chestnut Bldg. Philadelphia, PA 19107	USEPA Region 5 77 West Jackson Street Chicago, Il 60604

STORET (STOrage RETrieval Database)

STORET Database Description

Initiated by the U.S. Public Health Service in 1961 for managing water quality data, the STORET database has grown from 140 sampling locations in 1964 to over 800,000 sampling stations. There are now more than 150 million water quality observations stored in the current database. While most data relate to surface water, sediment or groundwater quality, users can access a variety of other information, including; USGS flow data, point source effluent monitoring, locations of industrial sites, municipal waste sources and disposal systems, stream gage locations, pollution- caused fish kills, and biological survey information on distribution, abundance, and physical condition of aquatic organisms.

The two largest component systems of STORET are the Water Quality System (WQS) and the BIOS Field Survey System. The WQS contains extensive data on physical and chemical characteristics of water and sediment. WQS parameters are organized into categories such as "organics", "pesticides", and "metals". The system contains information on site description and can produce a variety of maps.

The BIOS Field Survey System is the national biological survey information repository and contains information on over 60,000 species. BIOS includes powerful analytical tools to facilitate assessments of water quality and biological integrity such as diversity indices and community structure analyses. Further, BIOS can relate biological information with physical and chemical data in the WQS file.

The STORET database can be searched in a variety of geography-based manners. Polygons can be specified that encompass the area where data is requested, or the user can search for specific localities.

STORET User Accessibility

The STORET database is accessible by anyone who needs to analyze, store or retrieve water quality related data, including:

State and local government personnel
Federal government agencies
Interstate Commissions
Commercial clients
Universities
General public through Freedom of Information Act (FOIA) requests to government agencies

STORET Points of Contact

STORET access fees are dependent upon the user category. An account can be established by contacting:

STORET
U.S. Environmental Protection Agency
Washington, D.C. 20460
(800) 424-9067
(703) 883-8861

Local assistance can be provided by contacting the following USEPA regional STORET managers:

Region 2	Region 3	Region 5
Bill Jutis	Chuck Kanetsky	Stuart Ross
STORET Manager	STORET Manager	STORET Manager
USEPA Region 2	USEPA Region 3	USEPA Region 5
26 Federal Plaza	841 Chestnut Bldg.	77 West Jackson Street
New York, NY 10278	Philadelphia, PA 19107	Chicago, IL 60604
(212) 637-3334	(215) 566-2735	(312) 353-0299

TRI (Toxic Chemical Release Inventory)

TRI Database Description

Mandated by Title III of SUPERFUND Amendments and Reauthorization Act (SARA) of 1986, the TRI database is an inventory of required reporting by industry of the releases of over 300 toxic chemicals into the air, water and land. Called the "Emergency Right-to-Know Act", Title III requires that EPA collect the information and that it be made publicly available through a computer database. This file is accessible by the public on the National Library of Medicine's (NLM) Toxicology Data Network (TOXNET).

The database is structured for menu-driven retrieval of data arranged in the broad categories:

Facility Identification
Substance Identification
Environmental Release of Chemical
Off-site Waste Transfer

This data includes the names, addresses and public contacts of plants manufacturing, processing or using the reported chemicals, the estimated quantity emitted into the air, discharged into water bodies, injected underground, or released to land, methods used in waste treatment, and data on off-site transfer of chemicals for treatment or disposal.

TRI User Accessibility

TRI is accessible 24 hours/day and 7 days/week via telephone computer modem connection. Public users must contact the TRI Representative for an account number to access the NLM online services and must pay for line charges and computer CPU time. TRI users will automatically have access to other TOXNET database services and other NLM files including the:

HSDB	Hazardous Substances Data Bank
RTECS	Registry of Toxic Effects of Chemical Substances
CCRIS	Chemical Carcinogenesis Research Information System
DBIR	Directory of Biotechnology Information Resources
ETICBACK	Environmental Teratology Info. Center Backfile
EMICBACK	Environmental Mutagen Info. Center

Other NLM files contain over 25 million references on literature related to toxic chemicals. Registered users can access TOXNET and TRI by direct dial or through TELENET, TYMNET, INFONET, or COMPUSERVE telecommunications networks.

TRI Points of Contacts

National Library of Medicine - USER SERVICES

Specialized Information Services
National Library of Medicine
8600 Rockville Pike
Bethesda, MD 20894
(301) 496-6531

Local assistance can be provided by contacting the following USEPA regional TRI managers:

Region 2	Region 3	Region 5
Nora Lopez	Craig Yussen	Thelma Codina
TRI Consultant	TRI Specialist	TRI Consultant
USEPA Region 2	USEPA Region 3	USEPA Region 5
	841 Chestnut Bldg.	77 West Jackson Street
Edison, NJ	Philadelphia, PA 19107	Chicago, Il 60604
(908) 906-6890	(215) 566-2151	(312) 886-6219

PCS (Permit Compliance System)

PCS Database Description

The Permit Compliance System (PCS) database is the national computerized tracking system for NPDES (National Pollutant Discharge Elimination System) permit compliance and enforcement status. NPDES permits are issued by the State or EPA Regions under the Clean Water Act authorization. The PCS database contains extensive records on more than 65,000 active NPDES permits issued across the nation.

The PCS database records identify and describe the permittees, specify the pollutant discharge limits for each permit, record the amounts of pollutants measured in its waste water discharge on a monthly basis, track compliance history, construction schedules, permit limits and other reporting requirements.

PCS User Accessibility

PCS software may be accessed by anyone with an account at EPA's National Computer Center located at Research Triangle Park, North Carolina. The PCS database must be accessed using the PCS Generalized Retrieval Language, so some training or assistance may be necessary. The general public can request printouts of PCS data by writing to the appropriate USEPA regional office contact listed below, or to the Freedom of Information Office of the region. Nominal fees are charged for Freedom of Information requests. Requests for PCS data should be as specific as possible.

PCS Points of Contact

Local assistance can be provided by contacting the following USEPA regional PCS managers:

Region 2	Region 3	Region 5
Roger Vann	Edna Jones	Arnold Leder
PCS Manager	PCS Manager	PCS Manager
USEPA Region 2	USEPA Region 3	USEPA Region 5
26 Federal Plaza	841 Chestnut Bldg.	77 West Jackson Street
New York, NY 10278	Philadelphia, PA 19107	Chicago, IL 60604
(212) 637-3321	(215) 566-5795	(312) 886-0133

RCRIS
(Resource, Conservation, and Recovery Act Information System)

RCRIS Database Description

The RCRIS database contains data from a variety of media at and associated with hazardous waste generating, storage and disposal facilities permitted under the Resource Recovery and Conservation Act by EPA and the States. This database is less comprehensive than STORET or PCS, but contains site-specific information about RCRA facilities.

The RCRIS system contains five data modules. The Notification module contains the names, addresses, and hazardous waste activity description. The Part A Permit Application module contains data on other permits at the site, processes and specific wastes associated with each process under permit, and facility map information. The Permit module tracks the status of closure/post-closure activity. The Compliance module contains the scope of inspections and information resulting from inspections, such as violations and enforcement actions. The Corrective Action module contains facility status and results of assessment.

The RCRIS database can be accessed through a menu-driven system on a facility or geographic basis. Zip codes, county borders and address searches are supported.

RCRIS User Accessibility

Federal users can access the system directly through contact with the regional database managers listed below. The public can initiate a database accession through a Freedom of Information Act request of the USEPA regional office.

RCRIS Points of Contacts

Region 2	Region 3	Region 5
Barry Kaye	Gmerice Wilson	Jane Ratcliffe
Database Manager	RCRIS Manager	Database Manager
USEPA Region 2	USEPA Region 3	USEPA Region 5
26 Federal Plaza	841 Chestnut Bldg.	77 West Jackson Street
New York, NY 10278	Philadelphia, PA 19107	Chicago, IL 60604
(212) 637-3323	(215) 597-6505	(312) 886-7449

GLIC (Great Lakes Initiative Clearinghouse)

GLIC Database Description

This Region 5 database tracks all water quality data reports received from State and local agencies by the State, stream name, discharger, publication date and an entry number. Copies of the listings and reports are available from the point of contact listed below.

GLIC User Accessibility

The point of contact listed below will provide the requester (agency or public) with the most expedient and appropriate method to receive copies of the required documents.

GLIC Point of Contact

Robert Pepin
USEPA Region 5
77 West Jackson Street
Chicago, IL 60604
(312) 886-1505

Niagara Frontier Program Office GIS Pilot Project

Niagara Frontier Database Description

The Niagara River Basin Geographic Information System (GIS) Pilot Project is an adjunct to a bi-national effort by Canada and the U.S. to reduce toxic loadings to the Niagara River and Lake Ontario. The GIS pilot project is a special database aids in managing, accessing and displaying data from all sources involved in this effort. This is a multi-media database and it facilitates comparisons from ambient data to all contaminant sources including point sources, non-point sources, hazardous waste sites, sediment, groundwater contamination, surface water run-off, and air deposition.

This database study area includes the USGS 11-digit site boundary code 04120104 and the majority of the area in USGS boundary code 04120103. Data includes 205 geodetic control points, hydrography data, elevations, soils, tunnels and major conduits, water quality and flow data, point source pipe and facility locations, hazardous waste site boundaries, landuse and landcover data, transportation and census data, groundwater flow data, and municipal boundaries.

Niagara Frontier User Accessibility

Contact the database manager listed below. Access will be similar to STORET but database system is not yet completed.

Niagara Frontier Point of Contact

Linda Timander (WMD-NFPO)
USEPA Region 2
26 Federal Plaza
New York, NY 10278
(212)-637-3596

ESDLS (Environmental Spatial Data Library System)

ESDLS Database Description

The Environmental Spatial Data Library System (ESDLS) created by the USEPA Systems Development Center is now available for use in Region 5. ESDLS is an Arc/INFO Library comprised of TIGER/Line 1992, Geographic Names Information System 2(GNIS2), ENVIROFACTS facilities, and USGS Digital Line Graph (DLG) data.

Unlike our current TIGER files and population tables, ESDLS stores the census population tables in Oracle, requiring that the user connect to the Oracle database and relate from the TIGER census boundary coverages to the appropriate Oracle tables. Also, note that ESDL does not provide census tract boundaries.

Likewise, the attributes of the ENVIROFACTS facility point coverages are also stored in Oracle as a component of Gateway/ENVIROFACTS. The ENVIROFACTS facility point coverage feature attribute tables contain items with the EPA identification number, and a code for the EPA data system.

The libraries are composed of Arc/INFO Version 7.0 coverages (in 8.3 file naming format) using the standard US Albers projection parameters, but using datum NAD83. (see sample projection file below.)

There are 7 libraries available for use; one at a National scale, and 6 at 1:100,000 scale (one per Region 5 state.) Libraries for NY and PA will be loaded at a later date.

ESDLS User Accessibility

http://www.epa.gov/reg5ogis/esdls.htm

ESDLS Point of Contact

Ed Partington (202) 260-3106

Fish Advisory SIG (Special Interest Group)

Fish Advisory SIG Database Description

The Fish Advisory SIG, located on the Nonpoint Source Information Exchange Bulletin Board System (NPS BBS) will provide state and local agencies, private organizations, businesses, and concerned individuals with timely information, a forum for open discussion, and the ability to exchange computer text and program files. The service has a number of "doors" through which a user can pass. Door 1 contains three files that may be searched: a table of State fish advisories, a list of contacts, and a bibliography of fish advisory related documents.

Fish Advisory Accessibility

This BBS is open to all public and agency users that have the required hardware and software:

Personal computer or terminal
Telecommunications software
1200/2400 baud Modem
Phone line that supports modem communications

The phone number to access the system is (301) 589-0205

Telecommunications parameters are:
(N-8-1), No Parity, 8 Bits, 1 Stop-bit

You will be asked to register the first time you attempt access. Then simply type **J2** at the system prompt.

Fish Advisory SIG Point of Contact

For further assistance or to receive a copy of the user's manual, call Barbara Burke at (202) 260-7136.

Other Databases

The following is a listing of other more specialized databases that can be utilized in a 404(b)(1) evaluation or associated NEPA assessment as special data requirements may arise.

AIRS (Aerometric Information Retrieval System)

This database contains national air quality, point source emissions, and area/mobile source data. Monitoring is required for critical pollutants based on population density, pollutant source types, and geographical area.

AQUIRE (Aquatic Information Retrieval System)

This database contains information on the toxicity of chemicals (excluding oils) to fresh and saltwater organisms (excluding bacteria and amphibians). It contains acute, chronic and bioaccumulation effects published in the literature that has been reviewed before results are accepted into the database.

ASTER (Assessment Tools for the Evaluation of Risk)

This database is designed to assist ecological risk assessments. ASTER integrates the AQUIRE (Aquatic toxicity Information Retrieval) database and the QSAR (Quantitative Structure Activity Relationships) expert system. ASTER provides high quality data for discrete chemicals when available, or QSAR estimates chemical behavior when data is lacking. ASTER outputs are structured in Hazard Identification, Ecological Exposure Assessment, and Risk Characterization sections.

BRS (Biennial Reporting System)

This USEPA system provides overviews and progress reports on the status of the RCRA program through trend tracking of hazardous waste generation and management.

CERCLIS (Comprehensive Environmental Response, Compensation and Liability Act Information System)

This database contains site specific information used for project planning and scheduling for all Superfund programs, Site Assessment, Remedial, Removal, and Enforcement. The system contains an automated inventory of abandoned, inactive, or uncontrolled hazardous waste sites. The system contains some enforcement sensitive information and must be accessed through the FOIA Office of the USEPA Region.

CHRIS/HACS (Chemical Hazards Response Information System and the Hazard Assessment Computer System)

This database system provides information essential to decision-making by responsible Coast Guard personnel and others during emergencies involving the water transport of hazardous chemicals. CHRIS consists of a set of manuals and two computerized components, the Hazard Assessment Computer System and MicroHACS. The manuals provide detailed information on the chemical, physical and biological properties of over 1,000 chemicals. Hazards for each chemical are identified, as are appropriate responses in the event of accidental release.

CICIS (Chemicals in Commerce Information System)

This USEPA database contains an inventory of TSCA-regulated chemicals manufactured for commercial purposes. It allows the USEPA to maintain a comprehensive listing of over 70,000 chemical substances that are manufactured or imported.

EMMI (Environmental Monitoring Methods).

This system is the USEPA source of chemical lists and catalog of standard EPA analytical methods.

ERNS (Emergency Response Notification System)

This national computer database system is used for tracking information about releases of oil and hazardous substances.

FIATS (Freedom of Information Action Tracking System)

This database is an administrative system used by Federal agency FOIA officers. The system tracks the status of requests for data under the requirements of the Freedom of Information Act.

FINDS (Facilities Index Tracking System)

This is a computerized inventory of facilities regulated or tracked by the USEPA. All facilities are assigned a unique facility identification number by the system.

FISHTEMP (National Compendium of Freshwater Fish and Water Temperature Data)

This database contains historical information on freshwater fish with accompanying water temperature data from about 1930 through 1972 for over 100 species of fish from over 574 locations in the U.S.

FRDS (Federal Reporting Data System)

This database maintains an inventory of compliance data (violations and follow-up actions) reported by primary agents under the supervision of the Public Water Supplies program.

IRIS (Integrated Risk Information System)

This database contains summary information related to human health risk assessments performed by the USEPA. This system is updated monthly and is the USEPA's primary vehicle for the communication of health hazard information representing USEPA consensus positions.

ISI (EPA Information Systems Inventory)

This USEPA database tracks 500 major information systems and facilitates sharing of information across media.

LAMS (Lake Analysis Management System)

This is a set of databases that includes water quality data collected by the USEPA Office of Research and Development program conducted by Large Lakes Research Station.

LPOW (List of Plants that Occur in Wetlands)

The Wetlands Plant List database contains plants associated with wetlands, as defined by the USFWS wetland definition and classification system. It lists scientific and common names of plants, their distribution, and the regional wetland indicator status of about 6,700 species. It can be accessed by plant name, region, State, and wetland indicator status. The database is updated as additional information is received.

NAPAP (National Acid Precipitation Assessment Program Emission Inventory)

This database contains point source emissions data and supportive quality assurance information. It is capable of generating a number of special purpose reports to support modeling and data comparison efforts.

NES_PHYTO (National Phytoplankton DataBase)

This database contains the classification and enumeration of phytoplankton algae in lakes for the National Eutrophication Survey initiated in 1972 and carried out at the Environmental Monitoring Systems Laboratory (EMSL) ever since.

NWI (National Wetlands Inventory)

This is an automated geo-referenced database containing wetlands data utilizing GIS technology. To date, more than 5,700 maps have been digitized.

NWRCDB (National Wetlands Research Center DataBase) This database provides information related to the USFWS mission in wetland and coastal areas. The database is used by the USFWS to provide natural resource inventories for selected geographic areas which are displayed as statistical maps developed by using a geographic information system (GIS).

NWUDS (National Water Use Data System)

This database contains water use information collected and maintained by the USGS. The system is comprised of two parts: the Site Specific Water Use Data System (SSWUDS) and the Aggregated Water Use Data System (AWUDS). The SSWUDS contains water use information for individual users or systems, and includes five types of data files; water use, measurement point, conveyance, annual measurements, and extended data.

OHMTADS (Oil and Hazardous Substance Material Technical Assistance Data System)

This database contains hazardous chemical identification information, such as chemical name, manufacturer's name for a chemical trade name, chemical abstract service numbers, physical properties chemicals.

OLS (On-line Library System)

This USEPA library system contains information to assist in accessing the 28 Headquarters, Regional and laboratory libraries.

PPIS (Pesticide Product Information System)

This database contains information concerning all pesticide products registered in the US. It includes registrant name and address, chemical ingredients, toxicity, brand name and other information about each pesticide.

QSAR (Quantitative Structure Activity Relationships)

This is a chemical structure/activity-based expert system that includes a database of measured physicochemical properties of chemicals such as, melting points, boiling points, vapor pressures, and water solubilities.

RODS (Record of Decision Tracking System)

This database provides the justification for the remedial action chosen under the SUPERFUND program. It was developed to track site clean-ups and stores information on the technologies being used for site remediation.

SSTS (Section Seven Tracking System)

This USEPA database tracks the registration of all pesticide producing establishments and annually tracks the types and amounts of pesticides, active ingredients, and devices that are produced, sold, or distributed in the nation.

TSCATS (Toxic Substances Control Act Test Submissions Online database)

This USEPA database contains unpublished, non-confidential test data used to monitor health, ecological, and safety effects of the toxic chemicals used by industries.

UICS (Underground Injection Control System)

This database contains an inventory of underground injection wells with facility, well, inspection, violation, compliance and permit information.

WASTELAN (Wastelands)

This is a PC LAN version of the CERCLIS database used by USEPA Regions for data input and local analysis needs.

WATSTORE (Water Data Storage/Retrieval System)

This database contains location, chemical and flow information on surface and groundwater, collected by the Water Resources Division of the US Geological Survey (USGS).

WBS (Waterbody System)

This USEPA database contains information gathered under Section 305(b) CWA on the water quality status of specific waterbodies as reported to the agency by the States. The data includes causes, sources and monitoring basis.

WVCDB (Wetlands Value Citation DataBase)

This database contains a bibliographic listing of over 14,000 scientific articles concerning the functions and values of wetlands. The database includes information on the author, year, sequence, title, source and subject of each article.

SECTION 2
USACE Data from Navigation Projects

The first attachment is a map showing the locations of the Congressionally authorized navigation projects within the USACE North Central Division. This area includes the upper Mississippi River basin as well as the Great Lakes. The USACE collects data on sediments relative to maintenance dredging activities at many of these projects.

The second attachment is a bibliography of sediment investigations conducted at navigation projects within the Buffalo District. This includes all U.S. navigation projects on Lake Erie and Lake Ontario.

The third attachment is a bibliography of sediment investigations conducted at navigation projects within the Chicago District. This includes the Illinois and Indiana portions of Lake Michigan.

The fourth attachment is a tabular summary of sediment investigations conducted at navigation projects within the Detroit District. This includes Lake Huron, Lake Superior, and the Michigan and Wisconsin portions of Lake Michigan.

Most of the bulk chemical data from studies conducted by or for the USACE districts are available on STORET. Copies of reports or data summaries are available upon written request. Requests should be directed to the following contacts:

Buffalo District	Chicago District	Detroit District
Steve Yaksich	Jay Semmler	Carla Fisher
CELRB-PE-A	CELRC-ED-HE	CELRE-CO-O
USACE, Buffalo District	USACE, Chicago District	USACE, Detroit District
1776 Niagara Street	111 North Canal Street	P.O. Box 1027
Buffalo, NY 14207-3199	Chicago, IL 60606-7206	Detroit, MI 48231-1027

Map of GL Navigation Projects

(Not yet scanned)

Bibliography of Sediment Investigations
at USACE Navigation Projects
within the Buffalo District

Ashtabula River & Harbor

"Ashtabula Harbor, Ohio," USEPA, June 1974.
4 river, 3 harbor samples
bulk chemical, benthos

"Ashtabula Harbor, Ohio - Report on the Degree of Pollution of Bottom Sediments - 1975 Harbor Sediment Sampling Program," USEPA, Region 5, Great Lakes Surveillance Branch, February 1975
16 harbor samples
bulk chemical, physical characteristics, particle size

"Ashtabula, Ohio, Report on the Degree of Pollution of Bottom Sediments," USEPA, Region 5, June 1977
5 river, 8 harbor sediment samples
bulk chemistry, elutriate, macroinvertebrate, particle size

"Report on the Degree of Pollution of Bottom Sediments, Ashtabula, Ohio - Sampled June 22, 1977," US EPA, Region 5, March 1978
5 river, 8 harbor samples
bulk chemical, elutriate, particle size, macroinvertebrate

"Aquatic Disposal Field Investigations - Ashtabula River Disposal Site, Ohio -- Evaluative Summary," WES Technical Report D-77-42, Great Lakes Laboratory, June 1978

"Aquatic Disposal Field Investigations - Ashtabula River Disposal Site, Ohio -- Appendix A: Planktonic Communities, Benthic Assemblages, and Fisheries," WES Technical Report D-77-42, Great Lakes Laboratory, July 1978

"Aquatic Disposal Field Investigations - Ashtabula River Disposal Site, Ohio -- Appendix B: Investigation of the Hydraulic Regime and Physical Nature of Bottom Sedimentation," WES Technical Report D-77-42, Great Lakes Laboratory, December 1977

"Aquatic Disposal Field Investigations - Ashtabula River Disposal Site, Ohio -- Appendix C: Investigation of Water-Quality and Sediment Parameters," WES Technical Report D-77-42, Great Lakes Laboratory, July 1978

"Ninety-Six Hour Toxicity Bioassay Tests of Ashtabula Harbor (Ohio)," Heidelberg College, September 1978
5 river, 8 harbor, 1 disposal, 1 reference samples
bioassay

"A 96-Hour Sediment Bioassay of the Ashtabula River," Heidelberg College, June 1979
6 river, 1 disposal, 1 reference, 1 beach samples
bioassay

"Field Sampling Analysis of Core Sediment Samples, Ashtabula River, Ohio," Environmental Research Group Inc., June 1979
8 river core samples
bulk chemistry, particle size

"Sampling and Analyses of Sediments from Ashtabula, Ohio," Recra Research Inc., September 1980
6 harbor, 1 disposal, 1 reference samples
bulk chemistry

"Revision of Analytical Results - Ashtabula Sediments," Recra Research Inc., December 1980
revision of results of September report

"Ashtabula Site 16 Groundwater Sampling and Analysis - Samples Collected December 15, 1982," Aqua Tech Environmental Consultants Inc., January 1983
site 16 - 7 wells
bulk chemistry

"Ashtabula Harbor Site 16 Well Sampling December 15, 1982," Floyd Browne Associates Limited, January 1983
site 16 well water quality
bulk chemistry

"Ashtabula Harbor Site 16 Well Sampling January 17 & 18, 1983," Floyd Browne Associates Limited, February 1983
site 16 well water quality bulk chemistry

"Ashtabula Harbor Site 16 Well Sampling February 21, 1983," Floyd Browne Associates Limited, March 1983
site 16 well water quality bulk chemistry

"Ashtabula Harbor Site 16 Well Sampling March 28, 1983," Floyd Browne Associates Limited, April 1983
site 16 well water quality bulk chemistry

"Ashtabula Harbor Site 16 Well Sampling April 18, 1983," Floyd Browne Associates Limited, May 1983
site 16 well water quality bulk chemistry

"Ashtabula Harbor Site 16 Well Sampling May 16, 1983," Floyd Browne Associates Limited, May 1983
site 16 well water quality bulk chemistry

"Analysis of Sediments from Ashtabula River, Ashtabula, Ohio [1983]," Technical Report No. G0072-02, Floyd Browne Associates Limited, June 1983
5 core samples - river bulk chemistry, settling test, leaching test

"Ashtabula Harbor Site 16 Well Sampling June 13, 1983," Floyd Browne Associates Limited, July 1983
site 16 well water quality bulk chemistry

"Ashtabula Harbor Site 16 Sediment Analysis November 30 and December 2, 1982," Floyd Browne Associates Limited, July 1983
5 samples bulk chemistry, particle size

"Ashtabula Harbor Site 16 Well Sampling July 18, 1983," Floyd Browne Associates Limited, August 1983
site 16 well water quality bulk chemistry

"Benthic Macroinvertebrate Sampling - Ashtabula Harbor, Ohio - August 17-19, 1983," Swanson Environmental Inc., 1983
6 harbor samples
benthic macroinvertebrate analysis

"Ashtabula Harbor Site 16 Well Sampling August 31 & September 19, 1983," Floyd Browne Associates Limited, September 1983
site 16 well water quality bulk chemistry

"Ashtabula Harbor Site 16 Well Sampling October 17, 1983," Floyd Browne Associates Limited, November 1983
site 16 well water quality bulk chemistry

"Analysis of Sediment from Ashtabula River - Ashtabula, Ohio [1983]," Technical Report No. G0130-01, Floyd Browne Associates Limited, November 1983.
14 harbor samples
bulk chemical

"Ashtabula Harbor Site 16 Well Sampling November 21, 1983," Floyd Browne Associates Limited, December 1983
site 16 well water quality bulk chemistry

"Slurry Clarification and Column Leachate Tests on Polluted Harbor Sediments," E.S. Seger and R.P. Leonard, US Army Corps of Engineers, Buffalo, 1984

"Ashtabula Harbor Core Sediment Samples Analyses," Environmental Research Group Inc., February 1984
6 sediment cores
bulk chemistry

"Column Settling and Column Leachate Tests on Polluted Harbor Sediments," Edward Segar & Richard Leonard (USACE, Buffalo), 27th Annual Conference on Great Lakes Research, May 1984

"Analysis of Sediment from Ashtabula Harbor - Ashtabula, Ohio [1984]," Technical Report No. G0130-08, Floyd Browne Associates Limited, July 1984
11 harbor & river, 3 reference, 1 disposal samples
bulk chemical, particle size, elutriate, bioassay

"Summary of Documents in the Fields Brook File that Mention Radioactive Waste at the RMI-Extrusion Plant," CH2M Hill Inc., October 1985

"The Analyses of Sediment and Water Samples from Ashtabula Dewatering Pilot Plant Project," Technical Report No. G0176-02, Aqua Tech Environmental Consultants Inc., January 1986.
28 samples (5 trial pilot plant runs)
bulk chemical

"The Analyses of Sediments from Ashtabula Harbor," Technical Report No. 10175-13, Aqua Tech Environmental Consultants Inc., November 1988
3 river, 8 harbor, 1 disposal, 3 reference samples
bulk chemistry, elutriate, bioassay, particle size

"The Analyses of Sediments from Ashtabula Harbor [1988]," TP Associates International Inc., December 1988.
11 harbor & river, 3 reference, 1 disposal samples
bulk chemical, elutriate, particle size, bioassay

"Sediment Analyses - Ashtabula Harbor - Ashtabula, Ohio [1989]," Technical Report No. G0193-09, Aqua Tech Environmental Consultants Inc., November 1989
11 samples disposal area
bulk chemical, particle size

"Monitoring of Open-Lake Disposal Area at Ashtabula Harbor 1989 - 1990," Buffalo District in-house report, 1990
benthic & sediment surveys, chemical analysis

"Sediment Analyses - Ashtabula Harbor - Ashtabula, Ohio [1990]," Technical Report No. G0218-09B, Aqua Tech Environmental Consultants Inc., July 1990
9 samples disposal area
bulk chemical, particle size

"Analysis of Bottom Sediment Placement and Movement at Ashtabula Harbor Open-Lake Disposal Area," Buffalo District in-house memorandum, September 1990

migration of sediment at disposal area

"Ashtabula, OH Site, ARDL Report Nos. 6029 and 6030," June 1992
4 river, 11 harbor, 1 disposal site, 3 reference sites
bulk chemistry, particle size, elutriate

"Evaluation of Sediments from the Ashtabula Harbor Area, Ashtabula, OH," Environmental Science & Engineering Report No. 593-1060G-0200, October 1993
6 river, 6 harbor, 3 reference
bioassays

"Pilot Scale Demonstration of Thermal Desorption for the Treatment of Ashtabula River Sediments", EPA 905-R94-021, Buffalo District, 1994

"Phase I Upper Ashtabula River Interim Dredging Monitoring", Ohio River Division Laboratory, March 22, 1994
17 river samples
PCB testing

"Phase II Upper Ashtabula River Interim Dredging Monitoring", Ohio River Division Laboratory, August 12, 1994
17 river samples
PCB testing

"1995 Sampling and PCB Bioaccumulation Study for the Ashtabula River", Engineering and Environment, Inc., October 1995
10 river samples
physical, chemical, biological, PCBs

Barcelona Harbor

"Barcelona and Rocky River Sediment Chemistry," Federal Water Pollution Control Agency memorandum, June 1970
4 harbor samples
bulk chemistry

"Barcelona Harbor - 1970 & 1972 Chemistry," EPA, 1972
3 harbor samples
bulk chemistry

"Barcelona, New York - Report on the Degree of Pollution of Bottom Sediments - Sampled: August 1, 1977," USEPA, Region 5, 1977
5 harbor samples
bulk chemistry, elutriate, benthos

Buffalo River & Harbor

"Buffalo Harbor - Sediment Sampling," (not the title - extract from an unknown report), USEPA, 1972
4 harbor, 4 river sediment samples
bulk chemistry

"Water Quality Monitoring Programs at the Buffalo Harbor Dike Disposal Site #4," Calspan Report No. 6619-M-1, Calspan Advanced Technology Center, February 1980
3 lake, 3 dike samples 8 times
bulk chemistry

"Analysis of Sediment, Water and Elutriate Water Collected and Processed from Buffalo Harbor, New York sampling Sites," Great Lakes Laboratory, October 1981
7 harbor, 3 river, 1 reference, 2 Niagara River samples
bulk chemistry, elutriate

"Buffalo Harbor Fish Study", USEPA, Region 5, 1982
14 sites
fish chemistry

"Interpretive Summary, Evaluation of Availability and Plant Uptake of Contaminants from Dredged Material from Buffalo, New York, Toledo, Ohio, and Cleveland, Ohio," WES, January 1982

"Chemical Analysis, Buffalo Harbor Sediments," Actes Testing Labs Inc., December 1983
10 harbor samples
bulk chemistry

"1981 Buffalo, New York, Area Sediment Survey (BASS)," Great Lakes National Program Office, USEPA, December 1983
103 site sediment samples - Buffalo & Niagara River
bulk chemistry, description

"Analysis of Suspended Sediment and Water Samples from Buffalo River - Buffalo, New York [1984] (Overflow Dredging)," Technical Report No. G0130-07, Aqua Tech Environmental Consultants Inc., June 1984
13 overflow water samples, 2 suspended sediment samples
bulk chemistry

"Musselwatching in the Buffalo River, Times Beach and Lake Erie," TNO, The Netherlands, November 1986
PCB & pesticide concentrations in mussels

"Investigations Conducted at the Confined Disposal Facilities, Buffalo, New York," Technical report No. 10175-07A, Aqua Tech

Environmental Consultants Inc., November 1987

water samples -	9 wells, 1 surface	Times Beach
	4 wells	Small Boat Harbor
soil samples -	4 soil	Times Beach
	3 soil	Small Boat Harbor
	4 soil	CDF

bulk chemistry, TCLP, Particle size

"Buffalo Harbor - Cladophera and Iso-octane Extract Sampling," TP Associates International Inc., November 1987
16 algal, 25 extract samples
bulk chemistry

"Investigations Conducted at the Confined Disposal Facilities, Buffalo, New York," Technical Report No. 10175-07B, Aqua Tech Environmental Consultants Inc., May 1988
12 column leachate tests

"Contaminant Mobility at Buffalo Harbor," John R. Adams, Buffalo District, July 1988

"Sediment Analyses, Buffalo River and Harbor, Buffalo, New York," Technical Report No. G0193-06A, Aqua Tech Environmental Consultants Inc., August 1989
13 river, 7 harbor, 2 Niagara River sediment samples
bulk chemistry, particle size

"The Analyses of Sediments from Buffalo Harbor," Technical Report Bioassay, Aqua Tech Environmental Consultants Inc., December 1989
8 river, 4 harbor samples
bioassay

"Transmittal of Clam Tissue Mercury Analysis", AScI Corporation, October 1996
24 samples
mercury analysis

"Black Rock Channel Laboratory Analysis Report", Laboratory Resources Inc, January 1996
1 sample from lower approach channel
chemistry testing

"Chemical and Particle Size Analyses, Sediment Samples, Black Rock Lock, Buffalo, New York", Engineering and Environment, Inc., May 1996
6 river samples
chemical and particle size analyses

"Special Sediment Study for Strawberry Island-Buffalo Harbor, NY", Engineering & Environment, Inc., October 1996

4 offshore sand samples
chemistry & particle size analysis

"Sediment Sampling for Chemical and Particle Size Analysis - Buffalo Harbor, NY", Volumes I & II, Engineering and Environment, Inc., October 1996
13 river, 7 harbor, 2 reference samples
chemistry & particle size

"Special Bioaccumulation Study for Sediment from Sampling Location 31 - Buffalo Harbor, NY" (Chemistry on sediments only), Engineering and Environment, Inc., October 1996
1 sample
chemistry & particle size

Butternut Creek

"The Analyses of Sediments from Butternut Creek," Technical Report No. G0159-11, Aqua Tech Environmental Consultants Inc., October 1985
8 creek samples
bulk chemistry, particle size

Canandaigua Lake Outlet

"Analysis of Sediment from Canandaigua Lake," Technical Report No. G0130-02, Aqua Tech Environmental Consultants Inc., December 1983
6 stream sediment samples
bulk chemistry, particle size

Cape Vincent Harbor

"Cape Vincent Harbor - 1972 Chemistry," USEPA, 1972
3 harbor sediment samples
bulk chemistry, benthos

"Laboratory Results - Harbor Dredge Samples 6/27/75," USEPA, Region 2, July 1975
5 harbor sediment samples
bulk chemistry - metals

"Cape Vincent, New York - Report on the Degree of Pollution of Bottom Sediments - Samples: November 9, 1977," USEPA, Region 5, 1977
5 harbor sediment samples
bulk chemistry, particle size, benthos, bottom

characteristics

Cattaraugus Creek Harbor

"The Analysis of Sediments from Cattaraugus Creek Harbor," Technical Report No. G0193-03A, Aqua Tech Environmental Consultants Inc., December 1988
3 river/harbor samples
bulk chemistry, elutriate, particle size

"Cattaraugas Harbor, ARDL Report Nos. 6343-6345," September 1993
4 harbor/creek, 2 nearshore sites
bulk chemistry, particle size, elutriate

Cleveland Harbor

"Cleveland Harbor - Cuyahoga River Sediment Study - November 3, 1972," (not the title - extract from an unknown report), USEPA, 1972
7 river, 8 harbor sediment samples
bulk chemistry

"Water Quality Baseline Assessment for Cleveland Area - Lake Erie, Volume I - Synthesis," EPA Project G005107, City of Cleveland, May 1974
environment impact assessment, planning and evaluation in urban water pollution abatement for the Cleveland metropolitan area

"Cuyahoga River, Ohio - Report on the Degree of Pollution of Bottom Sediments - Sampled: August 25 - November 5, 1977," USEPA, Region 5, Great Lakes National Program Office, May 1978
14 river samples
bulk chemistry, elutriate, particle size, benthos
7 river, 7 harbor samples for 1972
bulk chemistry

"Cleveland, Ohio - Report on the Degree of Pollution of Bottom Sediments - Sampled: August 24 - November 7, 1977," USEPA, Region 5, Revised October 1978
13 harbor samples
bulk chemistry, elutriate, particle size, benthos

"Analysis of Sediment and Water from the Diked Disposal Facility at Cleveland, Ohio (Prior to Disposal Operation)," State University College at Buffalo, July 1981
1 sediment, 12 water samples - Dike 14
bulk chemistry

"Water Quality in and Adjacent to and Performance of the Cleveland Diked Disposal Area 14," State University College at Buffalo, November 1981
monitor water quality during & after 7 inside & 7 outside
bulk chemistry

"Interpretive Summary, Evaluation of Availability and Plant Uptake of Contaminants from Dredged Material from Buffalo, New York, Toledo, Ohio, and Cleveland, Ohio," WES, January 1982

"Bulk Chemical Analysis of Sediment Samples Collected from Cleveland Harbor, Ohio," Bionomics No. D99-450, EG&G Bionomics, 1982
3 sediment, 3 water samples
bulk chemistry

"An Assessment of the Chemical and Toxicological Properties of Dredged Sediments Collected from Cleveland Harbor, Ohio," EG&G Bionomics, 1982
14 river, 15 harbor sediment samples
bulk chemistry, elutriate, bioassay, particle size

"Cleveland Harbor Navigation Study - ORD Test Results on River Sediment Samples," Ohio River Division, November 1982
visual & grain size

"Analysis of Sediment from Cuyahoga River, Cleveland, Ohio [1983]," Technical Report No. G0130-03, Floyd Browne Associates Limited, December 1983
15 river/harbor samples
bulk chemistry, particle size

"Sampling and Chemical Analyses of Sand from the Cuyahoga Navigation Channel and the West Basin - April 1984," Buffalo District in-house memorandum, 1984

"Analysis of Sediment from Cuyahoga River, Cleveland, Ohio [1984]," Technical Report No. G0130-06, Floyd Browne Associates Limited, May 1984
14 river/harbor samples
bulk chemistry, particle size

"Cuyahoga River, Sediment Sampling Program," Buffalo District in-house report, 1986

"The Analyses of Sediments from Cleveland Harbor, Cleveland, Ohio," Technical Report No. G0176-11, Aqua Tech Environmental Consultants Inc., August 1986
5 Edgewater Park, 6 Burke Lakefront Airport samples
bulk chemistry, benthos, particle size

"Monitoring Program Conducted at Cleveland, Ohio [1986]," Technical Report No. G0176-15, Aqua Tech Environmental Consultants Inc., September 1986
30 sampling stations - Edgewater Park
DO, nitrogen, phosphorus, fecal coliform, circulation patterns

"The Analyses of Sediments from Cleveland Harbor," Technical Report No. G0176-13A, Aqua Tech Environmental Consultants Inc., September 1986
9 river, 9 harbor sediment samples
bulk chemistry, elutriate, bioassay, particle size

"The Analyses of Sediments from the Cuyahoga River, Cleveland Ohio," Technical Report No., G0176-13B, Aqua Tech Environmental Consultants Inc., October 1986
27 river sediment samples
bulk chemistry, elutriate, bioassay, particle size

"The Analyses of Sediments for LTV Steel," Technical Report No. I0217- 01, Aqua Tech Environmental Consultants Inc., January 1988
5 upper river sediment samples
bulk chemistry, elutriate, particle size

"Sediment Analyses, Cleveland Harbor, Cleveland, Ohio," Technical Report No. G0193-07, Aqua Tech Environmental Consultants Inc., February 1990
1 harbor, 2 river samples
bulk chemistry, column settling, column leachate

"Sediment Analyses, Cuyahoga River, Cleveland, Ohio," Technical Report No. G0218-01, Aqua Tech Environmental Consultants Inc., May 1990
5 River bend samples - study
bulk chemistry, particle size

"Sediment Analyses, Cuyahoga River, Cleveland Harbor, Ohio," Technical Report No. G0218-09, Aqua Tech Environmental Consultants Inc., January 1991
1 harbor, 2 river samples
bulk chemistry, column settling, column filtration, CDF core material

Cleveland Harbor, ARDL Report Nos. 6331, 6333-6341, August 1993
22 river/channel, 8 harbor, 4 nearshore, 3 reference
bulk chemistry, particle size, elutriate

Conesus Creek

"Selected Chemical Properties of Sediments from Conesus Creek, Livingston County, New York," Great Lake Laboratories, State University College at Buffalo, March 1981
10 creek sediment samples
bulk chemistry

Conneaut Harbor

"Conneaut Harbor Sediment Sample Chemistry," (not the title - extract from an unknown report), Federal Water Pollution Control Agency, 1969
7 harbor, 3 creek samples
bulk chemistry

"Conneaut Harbor Ohio - Harbor Sampling Program," (not the title - extract from an unknown report), USEPA, 1974
3 river, 7 harbor samples
bulk chemistry, benthos

"Conneaut, Ohio - Report on the Degree of Pollution of Bottom Sediments - Sampled: June 22, 1977," USEPA, Region 5, 1977
4 river, 4 harbor samples
bulk chemistry, elutriate, particle size, benthos

"A 96-Hour Sediment Bioassay of the Conneaut Harbor - Pittsburgh & Conneaut Dock Co. Area," Technical Report 21 (Case 79009), Aqua Tech Environmental Consultants Inc., September 1979
10 sediment samples
bioassay

"Sediment Bioassays of Harbors of Lake Erie and Lake Ontario," Technical Report 79200A, Aqua Tech Environmental Consultants Inc., March 1980
12 harbor sediment samples
bioassay

"Sediment Bioassay of Harbors of Lake Erie and Lake Ontario - Work Order No. 6: Huron and Conneaut Harbors," Technical Report No. 79200D, Aqua Tech Environmental Consultants Inc., November 1980
4 river, 5 harbor, 6 reference samples
bioassay

"Analysis of Sediments from Conneaut Harbor, Conneaut, Ohio [1980]," Ecology & Environment Inc., January 1981
9 harbor, 6 reference samples
bulk chemical, elutriate, particle size

"A 96-Hour Sediment Bioassay of the Conneaut Harbor - Pittsburgh and Conneaut Dock Company Area," Technical Report 22, Aqua Tech Environmental Consultants Inc., January 1981
11 dock area sediment samples
bioassay

"The Analyses of Sediments from Conneaut Harbor, Ohio [1985]," Technical Report No. G0159-07, Aqua Tech Environmental Consultants Inc., August 1985
4 river, 6 harbor, 3 disposal, 3 reference samples
bulk chemistry, elutriate, bioassay, particle size

"Sediment Analyses, Conneaut Harbor, Conneaut, Ohio," Technical Report No. G0218-06, Aqua Tech Environmental Consultants Inc., August 1990
4 river, 6 harbor, 3 disposal, 3 reference samples
bulk chemistry, elutriate, particle size

"The Analyses of Sediments from Conneaut Harbor," Technical Report Bioassay, Aqua tech Environmental Consultants Inc., December 1990
3 river, 6 harbor, 1 disposal, 3 reference samples
bioassay

"ARDL Report Nos. 6331, 6333-6341, Corps of Engineers, Buffalo District, Cleveland Harbor Site", Volumes 1 and 2, ARDL Inc., September 1993
19 Cuyahoga channels, 3 Old River channels, 8 harbor,
4 nearshore, and 3 reference samples
physical, chemical, elutriate

"Sediment Sampling for Chemical and Particle Size Analysis - Conneaut Harbor, OH", Volumes I & II, Engineering & Environment, Inc., October 1996
10 harbor, 2 disposal, 4 reference samples
chemistry & particle size

<u>Dunkirk Harbor</u>

"Dunkirk Harbor Sampling Program," (not the title - extract from an unknown report), USEPA, Rochester Field Office, 1972
2 harbor samples
bulk chemistry

"Dunkirk Harbor Sampling - 1975," (not the title - extract from an unknown report), USEPA, 1975
7 harbor samples
bulk chemistry

"Dunkirk, New York - Report on the Degree of Pollution of Bottom Sediments - Sampled: August 2, 1977," USEPA, Region 5, 1977
5 harbor samples
bulk chemistry, elutriate, benthos

"Sediment Bioassay of Harbors of Lake Erie and Lake Ontario - Work Order No. 4: Dunkirk Harbor," Technical Report 79200B, Aqua Tech Environmental Consultants Inc., 1980
8 harbor, 4 disposal, 4 reference samples
bioassay

"The Analyses of Sediments from Dunkirk Harbor, New York," Technical Report No. G0176-06, Aqua Tech Environmental Consultants Inc., June 1986
7 harbor, 3 disposal, 3 reference samples
bulk chemistry, elutriate, bioassay, particle size

"Results of 96-Hour Sediment Bioassay Results of Sediment from Dunkirk Harbor, New York, and Erie Harbor, Pennsylvania," Supplement to Technical Reports Nos. G0176-06 and G0176-07, Aqua tech Environmental Consultants Inc., October 1986
7 harbor, 3 disposal, 3 reference samples
bioassay

"ARDL ID No. 6005, US Army District - Buffalo, Site: Dunkirk Harbor, Ohio," ARDL Inc., October 1991
7 harbor, 3 disposal, 3 reference samples
bulk chemistry, particle size

"ARDL ID No. 6006, US Army District - Buffalo, Site: Dunkirk Harbor, Ohio," ARDL Inc., October 1991
7 harbor, 3 disposal, 3 reference samples
elutriate

<u>East Harbor</u>

"Sediment Analyses, East Harbor, Ohio," Technical Report No. G0193-11, Aqua Tech Environmental Consultants Inc., November 1989
5 harbor samples
bulk chemistry, elutriate, particle size

"Sediment Analyses, East Harbor, Ohio," Technical Report No. G0193-12, Aqua Tech Environmental Consultants Inc., December 1989
2 new proposed disposal site samples
bulk chemistry, elutriate, particle size

Erie Harbor

"Erie Harbor, Pennsylvania - Report on the Degree of Pollution of Bottom Sediments - 1975 Harbor Sampling Program," USEPA, Region 5, 1975
18 harbor samples
bulk chemistry, elutriate, particle size

"Elutriate Test Data - Erie Harbor," (not the title - extract from an unknown report), Ecology & Environment, June 1980
7 sediment samples
elutriate

"Chemical, Physical and Bioassay Analysis of Sediment Samples, Erie Harbor, Erie, Pennsylvania," Applied Biology Inc., December 1982
14 Harbor sediment samples
bulk chemistry, elutriate, bioassay, particle size

"Priority Pollutants, Lake Erie and Presque Isle Bay, 1985," Erie County Department of Health, 1985
17 harbor, 2 lake sediment samples, 9 water samples
bulk chemistry

"The Analyses of Sediments from Erie Harbor, Erie, PA," Technical Report No. G0176-07, Aqua Tech Environmental Consultants Inc., June 1986
16 harbor samples
bulk chemistry, elutriate, bioassay, particle size

"Results of 96-Hour Sediment Bioassay Results of Sediment from Dunkirk Harbor, New York, and Erie Harbor, Pennsylvania," Supplement to Technical Reports Nos. G0176-06 and G0176-07, Aqua Tech Environmental Consultants Inc., October 1986
12 sediment samples
bioassay

"Sediment Sampling and Testing, Erie Harbor, PA," Bowser-Morner Inc., September 1992
11 harbor, 1 disposal, 1 lake reference samples
bulk chemical, particle size, elutriate, bioassay

"1995 Sampling of Erie harbor, Erie, Pennsylvania", Volumes I & II, Engineering & Environment, Inc., November 1996
3 channel, 4 upland, 2 CDF samples
chemistry & particle size

Euclid Creek

"Bulk Chemical Analysis of Sediment Samples Collected from Euclid Creek, Ohio," Bionomics No. D39260, EG&G Bionomics, November 1981
5 stream sediment samples
bulk chemistry

Fairport Harbor

"Fairport Harbor and Grand River Sediment Study - November 6, 1972," USEPA, 1972
3 river, 3 harbor samples
bulk chemistry

"Fairport Harbor, Ohio," (not the title - extract from an unknown report), USEPA, 1974
12 river samples
bulk chemistry

"Fairport Harbor, Ohio - Report on the Degree of Pollution of Bottom Sediments - 1975 Harbor Sampling Program," USEPA, Region 5, 1975
11 harbor samples
bulk chemistry, elutriate, particle size, bottom characteristics

"Fairport, Ohio - Report on the Degree of Pollution of Bottom Sediments - Sampled: June 24, 1977," USEPA, Region 5, 1977
6 river, 5 harbor samples
bulk chemistry, elutriate, particle size, benthos

"Sediment Bioassays of Harbors of Lake Erie and Lake Ontario," Technical Report 79200A, Aqua Tech Environmental Consultants Inc., March 1980
6 river, 5 harbor sediment samples
bioassay

"The Toxicity of dredged Materials from Fairport Harbor, Ohio to Aquatic Organisms," Bionomics Report No. Bw-82-3-1142, EG&G Bionomics, March 1982
6 river, 5 harbor sediment samples
bioassay

"Report on the Analysis of Surface Sediment Grab Samples for the U. S. Army Corps of Engineers' Lake Erie Harbor Dredging Program," Wadsworth Testing Laboratories Inc., November 1981
6 river, 5 harbor, 1 disposal, 1 reference samples
bulk chemistry, elutriate, bioassay, particle size

"Fairport, Ohio Harbor (memorandum)," Empire Soils Investigation, May 1982
7 samples
particle size

"The Analyses of Sediments from Fairport Harbor - Fairport Harbor, Ohio," Technical Report No. G0176-09, Aqua Tech Environmental Consultants Inc., August 1986
6 river, 5 harbor, 3 disposal, 3 reference samples
bulk chemistry, elutriate, bioassay, particle size

"ARDL ID No. 6017, Corps of Engineers - Buffalo District, Site: Fairport Harbor - Bulk Sediment," ARDL Inc., November 1991
6 river, 5 harbor samples
bulk chemistry, particle size

"ARDL ID No. 6014, Corps of Engineers - Buffalo District, Site: Fairport Harbor - Elutriate," ARDL Inc., November 1991
6 river, 5 harbor samples
elutriate

"Sediment Sampling for Chemical and Particle Size Analysis - Fairport Harbor, OH", Volumes I & II, Engineering & Environment, Inc., October 1996
11 harbor/river, 2 disposal, 4 reference
chemistry & particle size

Fort Drum, New York

"The Analyses of Sediments from Ft. Drum," Technical Report No. G0176- 01, Aqua Tech Environmental Consultants Inc., December 1985
2 sediment samples - Remington Pond (St. James Lake)
bulk chemistry, particle size

"EP Toxicity Testing from Ft. Drum," Technical Report No. G0176-08, Aqua Tech Environmental Consultants Inc., June 1986
2 sediment samples - Remington Pond (ST. James Lake)
EP toxicity test data

Great Sodus Harbor

"Great Sodus Bay," (not the title - extract from an unknown report), USEPA, 1972
3 channel, 1 bay, 1 disposal sample
bulk chemistry

"Analysis of Sediment from Oswego Harbor, Great Sodus Harbor and

Little Sodus Harbor," Macola Inc., August 1981
3 harbor samples
bulk chemistry, elutriate, bioassay, particle size

"The Toxicity of Dredged Materials from Oswego, Great Sodus, and Little Sodus Harbors to Aquatic Organisms," Report No. BW-81-10-1034, EG & G, Bionaomics, October 1981
3 samples
bioassay

Huron Harbor

"Huron Harbor Ohio," (not the title - extract from an unknown report), USEPA, 1973
3 channel samples
bulk chemistry, benthos

"Huron, Ohio - Report on the Degree of Pollution of Bottom Sediments - Sampled: September 10, 1975," USEPA, Region 5, 1975
9 harbor sediment samples
bulk chemistry, particle size, benthos, bottom characterization

"Huron, Ohio - Report on the Degree of Pollution of Bottom Sediments - Sampled: September 8 and September 10, 1976," USEPA, Region 5, 1976
5 river/harbor, 12 channel samples
bulk chemistry, elutriate, particle size, benthos

"Huron, Ohio - Report on the Degree of Pollution of Bottom Sediments - Sampled: September 10, 1975," USEPA, Region 5, 1976
5 river, 4 channel samples
bulk chemistry, particle size, benthos, bottom character

"1976 Huron Harbor, Ohio - Dike Study," USEPA, Region 5, 1977
3 inside, 6 outside dike samples
bulk chemistry

"Sediment Bioassays of Harbors of Lake Erie and Lake Ontario," Technical Report 79200A, Aqua Tech Environmental Consultants Inc., March 1980
8 harbor sediment samples
bioassay

"Sediment Bioassay of Harbors of Lake Erie and Lake Ontario - Work Order No. 6: Huron and Conneaut Harbors," Technical Report No. 79200 Aqua Tech Environmental Consultants Inc., November 1980
11 river/harbor, 6 reference samples
bioassay

"Analysis of Sediments from Huron Harbor, Huron, Ohio [1980]," Ecology & Environment Inc., January 1981
11 harbor, 6 reference samples
bulk chemistry, elutriate, particle size

"Sampling of Navigation Channel Sediments at Sandusky and Huron Harbors as Potential Sand Sources for Littoral Nourishment," Buffalo District memorandum, 1984
6 grab, 1 core samples
particle size

"Analysis of Sediments from Huron Harbor, Ohio [1985]," Technical Report No. G0159-06, Aqua Tech Environmental Consultants Inc., July 1985
10 river/harbor, 3 disposal, 3 reference samples
bulk chemistry, elutriate, bioassay, particle size

"ARDL ID No. 6003, US Army District - Buffalo, Site: Huron Harbor, Ohio," ARDL Inc., October 1991
16 sediment samples
bulk chemistry, particle size

"ARDL ID No. 6004, US Army District - Buffalo, Site: Huron Harbor, Ohio," ARDL Inc., October 1991
16 sediment samples
elutriate

"Sediment Sampling for Chemical and Particle Size Analysis - Huron Harbor, OH", Volumes I & II, Engineering & Environment, Inc., October 1996
10 harbor, 2 disposal, 4 reference samples
chemistry & particle size

<u>Keuka Lake Outlet</u>

"The Analyses of Sediments from Keuka Lake Outlet," Technical Report No. G0176-18, Aqua Tech Environmental Consultants Inc., September 1986
4 outlet sediment samples
bulk chemistry, particle size

<u>Little River, Niagara Falls NY</u>
"Analysis of Sediment from Little River - Niagara Falls, New York [1983]," Technical Report No. G0130-04, Floyd Browne Associates Limited, December 1983
12 river samples bulk chemistry
EP toxicity

Little Salmon River/Mexico Bay

"Little Salmon River/Mexico Bay, New York - Report on the Degree of Pollution of Bottom Sediments - Sampled July 27, 1976," USEPA, Region 5, 1976
3 river, 1 lake sediment samples
bulk chemistry, benthos, particle size, bottom characterization

Little Sodus Harbor

"1969 Bottom Sediment Analysis - Little Sodus Harbor," (not the title - extract from an unknown report), FWPCA, 1969
4 harbor samples
bulk chemistry

"Little Sodus Bay," (not the title - extract from an unknown report), USEPA, July 1972
3 harbor samples
bulk chemistry, benthos

"Analysis of Sediment from Oswego Harbor, Great Sodus Harbor and Little Sodus Harbor," Macola Inc., August 1981
2 harbor samples
bulk chemistry, elutriate, bioassay, particle size

"The Toxicity of Dredged Materials from Oswego, Great Sodus, and Little Sodus Harbors to Aquatic Organisms," Report No. BW-81-10-1034, EG & G, Bionaomics, October 1981
1 sediment sample
bioassay

"Final Data Report Sediment Sampling and Testing Little Sodus Bay Harbor, New York", Volumes 1 and 2, Acres International Corporation, December 1994
7 harbor, 2 reference, 2 disposal samples
chemical, physical, and elutriate testing

Lorain Harbor

"Sediment Analysis of Lorain Harbor," (not the title - extract from an unknown report), USEPA, July 1974
9 river, 1 harbor samples
bulk chemistry

"Lorain Harbor, Ohio, Report on the Degree of Pollution of Bottom Sediments, 1975 Harbor Sediment Sampling Program," USEPA, Region 5, 1975

5 river, 9 harbor samples
bulk chemistry, elutriate, particle size

"Bulk Chemical Analysis of Sediment Samples Collected from Lorain Harbor, Ohio," EG&G Bionomics, January 1982
4 harbor sediment samples
bulk chemistry

"Analysis of Sediment from Lorain Harbor, Lorain, Ohio," Technical Report No. G0128-01, Floyd Browne Associates Limited, December 1983
4 river, 9 harbor, 4 reference samples
bulk chemistry

"The Analyses of Sediments from Lorain Harbor," Technical Report No. 10175-10, T.P. Associates International Inc., July 1988
11 river, 10 harbor, 3 reference samples
bulk chemistry, elutriate, particle size

"Lorain Harbor, OH, ARDL Report Nos. 6346-6349," October 1993
10 channel, 7 harbor, 3 reference
bulk chemistry, elutriate, particle size

Niagara River

"Black Rock Canal - Sediment Sampling 1972," (not the title - an extract from an unknown report), USEPA, 1972
3 Black Rock Canal samples
bulk chemistry

"Niagara River Harbors at Tonawanda and Cayuga Islands - Sediment Sampling 1972," (not the title - an extract from an unknown report), USEPA, 1972
4 sediment samples
bulk chemistry

"Niagara Frontier Sampling Results," USEPA, March 1983
33 samples
bulk chemistry

Oak Orchard Harbor

"Oak Orchard, New York - Report on the Degree of Pollution of Bottom Sediments - Sampled: November 8, 1977," USEPA, Region 5, 1977
4 river, 2 harbor samples
bulk chemistry, elutriate, particle size, benthos

"Analysis of Sediment - Oak Orchard Harbor, Oak Orchard, New York," Technical Report No. G0130-09, Aqua Tech Environmental Consultants Inc., July 1984
7 river & harbor samples
bulk chemistry, elutriate, bioassay, particle size

"The Analyses of Sediments from Oak Orchard Harbor," Technical Report No. I0175-05, T. P. Associates International Inc., August 1987
4 river, 3 harbor samples
bulk chemistry, elutriate, particle size

"Results of 96-Hour Sediment Bioassay Tests, Sediment from Oak Orchard Harbor," Technical Report No. I0175-05, T. P. Associates International Inc., September 1987
4 river, 3 harbor samples
bioassay

"Sediment Sampling and Testing, Oak Orchard Harbor, NY," Bowser-Morner Inc., July 1992
5 harbor, 1 disposal, 1 reference samples
bulk chemical, particle size, elutriate, bioassay

Ogdensburg

"Laboratory Results - Harbor Dredge Samples 6/27/75," (not the title - extract from an unknown report), USEPA, Region 2, July 1975
5 harbor sediment samples
bulk chemistry, bottom characteristics

"Ogdensburg Harbor, New York - Report on the Degree of Pollution of Bottom Sediments - Sampled: July 27, 1976," USEPA, Region 5, 1976
3 harbor samples
bulk chemistry, elutriate

"Sediment Quality Testing at Ogdensburg Harbor, New York," Great Lakes Laboratory, State University College at Buffalo, September 1981
11 channel, 3 reference samples
bulk chemistry, elutriate, particle size

"The Toxicity of Dredged Materials from Ogdensburg Harbor to Aquatic Organisms," Report No. BW-81-11-1040, EG & G, Bionomics, November 1981
10 harbor samples
bioassay

"The Toxicity of Dredged Materials from Odgensburg Harbor to Aquatic Organisms," Report No. BW-82-1-1094, EG&G Bionomics, January 1982
10 harbor samples
bulk chemistry, bioassay

Olcott Harbor

"Olcott Harbor," (not the title - extract from an unknown report), USEPA, 1972
6 harbor samples
bulk chemistry

"Olcott Harbor, New York - Report on the Degree of Pollution of Bottom Sediments - Sampled: November 7 - 10, 1977," USEPA, Region 5, 1977
6 river, 5 lake samples
bulk chemistry, elutriate, particle size, benthos, bottom characteristics

"Sediment Quality Testing at Olcott Harbor, New York and Wilson Harbor, New York Sampling Sites," Great Lakes Laboratory, State University College at Buffalo, September 1981
5 harbor, 1 reference, 1 disposal samples
bulk chemistry, elutriate, bioassay, particle size

"The Toxicity of Dredged Materials from Olcott Harbor, New York to Aquatic Organisms," Bionomics Report No. BW-82-1-1098, EG&G Bionomics, January 1982
4 river/harbor, 2 disposal, 3 reference samples
bioassay

"Great Lakes National Program Office - Harbor Sediment Program - Lake Ontario 1981: Rochester New York, Oswego New York, Olcott New York," USEPA, Great Lakes Program Office, April 1984
5 river samples
bulk chemistry

"The Analyses of Sediments from Olcott Harbor," Technical Report No. I0175-04, Aqua Tech Environmental Consultants Inc., July 1987
6 river/harbor, 1 reference samples
bulk chemistry, elutriate, particle size

"Results of 96-Hour Sediment Bioassay Tests - Sediments from Olcott Harbor," Technical Report No. I0175-04, T. P. Associates International Inc., September 1987
6 river/harbor, 1 reference samples
bioassay

"Sediment Analyses - Olcott Harbor, Olcott, New York," Technical Report No. G0193-08A, Aqua Tech Environmental Consultants Inc., September 1989
11 river samples
bulk chemistry, elutriate, particle size

"The Analyses of Sediments from Olcott Harbor," Aqua Tech Environmental Consultants Inc., December 1989
7 river samples
bioassay

Oswego Harbor

"Sediment Quality Oswego Harbor, New York [1967, 1968, 1969]," Federal Water Pollution Control Agency memorandum, May 1969
5 harbor samples
bulk chemistry, benthos, bottom description

"1969 Bottom Sediment Analysis - Oswego Harbor," (not the title - extract from an unknown report), Federal Water Pollution Control Agency, 1969
8 harbor samples
bulk chemistry

"Oswego Harbor," (not the title - extract from an unknown report), USEPA, 1972
2 river, 4 harbor samples
bulk chemistry

"Oswego Harbor Sediments," (not the title - extract from an unknown report), USEPA, Rochester Field Office, February 1975
2 river, 4 harbor samples
bulk chemistry

"Oswego Harbor, New York - Report on the Degree of Pollution of Bottom Sediments - Sampled: April 22, 1976," USEPA, Region 5, 1976
12 harbor samples
bulk chemistry, elutriate, particle size, benthos

"Analysis of Sediment from Oswego Harbor, Great Sodus Harbor and Little Sodus Harbor" Macola Inc., August 1981
13 harbor samples
bulk chemistry, elutriate, bioassay, particle size

"The Toxicity of Dredged Materials from Oswego, Great Sodus, and Little Sodus Harbors to Aquatic Organisms," Report No. BW-81-10-1034, EG & G, Bionaomics, October 1981
6 sediment samples

bioassay

"Great Lakes National Program Office - Harbor Sediment Program - Lake Ontario 1981: Rochester New York, Oswego New York, Olcott New York," USEPA, Great Lakes Program Office, April 1984
4 river samples
bulk chemistry

"The Analyses of Sediments from Oswego Harbor," Technical Report No. I0175-02, T.P. Associates International Inc., June 1987
3 river, 12 harbor, 2 disposal, 3 reference samples
bulk chemistry, elutriate, bioassay, particle size

"Chemical and Biological Assessments of Sediments from Oswego Harbor, 1990," USEPA, July 1990
4 river samples
bioassay

"1995 Sediment Sampling and Testing Oswego Harbor", Volumes 1 and 2, Engineering and Environment, Inc., January 1996
10 harbor, 2 disposal, 4 reference locations
grain size, bulk chemistry

<u>Port Clinton</u>

"Port Clinton, Ohio - Report on the Degree of Pollution of Bottom Sediments - 1975 Harbor Sediment Sampling Program - April 8, 1975," USEPA, Region 5, 1975
4 harbor sediment samples
bulk chemistry, elutriate, particle size, benthos, bottom characterization

"April 1975 EPA Report on the Degree of Pollution of Bottom Sediments, Port Clinton, Ohio, Appendix C," USEPA, 1975
4 river/harbor samples
bulk chemistry, elutriate, particle size

"Analysis of Sediment Port Clinton/West Harbor - Port Clinton, Ohio," Technical Report No. G0130-10, Aqua Tech Environmental Consultants Inc., September 1984
5 river, 3 reference samples
bulk chemistry, elutriate, bioassay, particle size

"Sediment Sampling and Testing, Port Clinton Harbor, OH," Bowser-Morner, Inc., September 1992
5 harbor samples
bulk chemical, particle size, elutriate, bioassay

Port Ontario

"Port Ontario, New York - Report on the Degree of Pollution of Bottom Sediments - Sampled July 27, 1976," USEPA, Region 5, 1976
3 river, 1 lake sediment samples
bulk chemistry, particle size, benthos, bottom characterization

Rochester Harbor/Irondequoit Bay

"1969 Bottom Sediment Analysis - Rochester Harbor," (not the title - extract from an unknown report), Federal Water Pollution Control Agency, 1969
4 river, 1 disposal samples
bulk chemistry

"Rochester Harbor," (not the title - extract from an unknown report), USEPA, 1973
sampling in 1967 - 1969, 1972, 1973
4 harbor samples
bulk chemistry

"Rochester Dredging Spoils Sediment Samples [1974]," O'Brien & Gere, 1975
6 harbor, 4 lake, 2 upland samples
bulk chemistry

"Rochester Harbor, New York - Report on the Degree of Pollution of Bottom Sediments - Sampled: April 20, 1976," USEPA, Region 5, 1976
7 river, 3 harbor, 1 reference samples
bulk chemistry, elutriate, particle size, benthos

"Irondequoit Bay, New York - Report on the Degree of Pollution of Bottom Sediments - Sampled: July 26, 1976," USEPA, Region 5, 1976
13 Bay sediment samples
bulk chemistry, particle size, benthos, bottom characterization

"Irondequoit Bay, New York - Report on the Degree of Pollution of Bottom Sediments - Sampled: July 26 and 29, 1976," USEPA, Region 5, 1976
13 bay sediment samples
bulk chemistry, particle size, benthos, bottom characterization

"Sediment Bioassays of Harbors of Lake Erie and Lake Ontario - Work Order 5: Rochester Harbor," Technical Report 79200C, Aqua Tech Environmental Consultants Inc., August 1980

7 river, 3 harbor, 3 disposal, 4 reference samples
bioassay testing

"Sampling and Analyses of Sediments from Rochester Harbor, New York," Heidelberg College, March 1981
10 harbor, 3 disposal, 4 reference samples
bulk chemistry, elutriate, particle size

"Great Lakes National Program Office - Harbor Sediment Program - Lake Ontario 1981: Rochester New York, Oswego New York, Olcott New York," USEPA, Great Lakes Program Office, April 1984
14 river samples
bulk chemistry

"The Analyses of Sediments from Rochester Harbor, Rochester, New York," Technical Report No. G0159-08, Aqua tech Environmental Consultants Inc., September 1985
10 river, 2 disposal, 2 reference samples
bulk chemistry, elutriate, bioassay, particle size

"Monitoring Project at Rochester Harbor - Rochester, New York," Aqua Tech Environmental Consultants Inc., August 1986
3 events, dredge bin 3 inflow, 3 overflow, bin contents samples
bulk chemistry, bioassay, particle size

"Sediment Analyses - Rochester Harbor - Irondequoit Bay - New York," Technical Report No. G0218-02, Aqua tech Environmental Consultants Inc., August 1990
Rochester: 10 river, 2 disposal, 2 reference samples
Irondequoit: 3 harbor, 1 beach nourishment samples
bulk chemistry, elutriate, particle size

"The Analyses of Sediments from Rochester and Irondequoit Harbors," Technical Report Bioassay, Aqua Tech Environmental Consultants Inc., December 1990
Rochester - 10 river, 1 disposal, 1 reference samples
Irondequoit - 3 river/harbor, 1 beach nourishment samples
bioassay

"Analysis of Sediments for Dioxins and Dibenzofurans, Rochester Harbor, New York," New York State Department of Health, December 1991
10 river/harbor, 1 reference samples
dioxin, dibenzofuran, PCB

"Evaluation of Sediments from the Rochester Harbor Area", Environmental Science and Engineering, Inc., September 22, 1994
8 river/harbor, 2 reference, 2 disposal samples
bioassay

"Final Data Report Sediment Sampling and Testing Rochester Harbor, New York", Volumes 1 and 2, Acres International Corporation, December 1994
9 river, 2 reference, 2 disposal samples
physical and chemical testing

"Final Data Report Sediment Sampling and Testing Rochester Harbor Breakwater", Volumes 1 and 2, Acres International Corporation, January 1995
8 harbor samples
physical and chemical testing

"1995 Sampling of the Proposed Dredged Material Open-Lake Disposal Site Rochester Harbor", Volumes 1 and 2, Engineering and Environment, Inc., September 1995
4 open-lake disposal samples
physical, chemical, benthic analyses

Rocky River

"Barcelona and Rocky River Sediment Chemistry," Federal Water Pollution Control Agency memorandum, June 1970
4 harbor samples
bulk chemistry

"Rocky River, Ohio - Report on the Degree of Pollution of Bottom Sediments - 1975 Harbor Sediment Sampling Program - April 9, 1975," USEPA, Region 5, 1975
6 river, 1 channel samples
bulk chemistry, elutriate, particle size, benthos

"Report on the Analysis of Surface Sediment Grab Samples for the U. S. Army Corps of Engineers' Lake Erie Harbor Dredging Program," Wadsworth Testing Laboratories Inc., November 1981
6 river, 1 disposal, 1 reference samples
bulk chemistry, elutriate, bioassay, particle size

"The Toxicity of Dredged Materials from Rocky River Harbor to Aquatic Organisms," Bionomics Report No. BW-82-3-1129, EG&G Bionomics, March 1982
6 river/harbor sediment samples
bioassay

"Analysis of Sediment from Rocky River, Cuyahoga County, Ohio," Technical Report No. G0159-04, Aqua Tech Environmental Consultants Inc., July 1985
6 sediment samples
bulk chemistry, elutriate, particle size

"Sediment Analyses, Rocky River, Cleveland, Ohio," Technical Report No. G0218-05, Aqua Tech Environmental Consultants Inc., September 1990
5 river/harbor sediment samples
bulk chemistry, elutriate, particle size

"Sediment Sampling for Chemical and Particle Size Analysis - Rocky River, OH", Volumes I & II, Engineering & Environment, Inc., October 1996
6 harbor/river, 2 disposal, 4 reference
chemistry & particle size

Sackets Harbor

"Laboratory Results - Harbor Dredge Samples 6/27/75," (not the title - extract from an unknown report), USEPA, Region 2, July 1975
11 harbor sediment samples
bulk chemistry, bottom characteristics

"Sackets, New York - Report on the Degree of Pollution of Bottom Sediments - Sampled: November 9, 1977," USEPA, Region 5, 1977
10 harbor samples
bulk chemistry, elutriate, particle size, benthos

St. Lawrence River

"Analyses of Sediments Adjacent to Eisenhower and Snell Locks - 1972," Great Lakes Laboratory, September 1972
45 sediment samples
bulk chemistry, sediment characteristics, benthos

"Benthic Organisms, Aquatic Plants, and Substrate Types Found in Three Bottom Samples Collected on 25 May 1976 by Atlantic Testing Laboratories in the Polly's Gut Area of the St. Lawrence River near the Robert Moses Power Dam," Ichthyological Associates Inc., June 1976
benthos and substrate characteristics

"The Analysis of Sediments from the St. Lawrence River," Technical Report No. G0159-09, Aqua Tech Environmental Consultants Inc., September 1985
11 river sediment samples
bulk chemistry, elutriate, bioassay, particle size

"Sediment Quality of the St. Lawrence River, Iroquois Extension," Berk Consultants Limited, March 1991
8 bore holes across river

bulk chemistry, particle size

Sandusky Harbor

"Sandusky Harbor - Sediment Sampling Chemistry," (not the title - extract from an unknown report), USEPA, 1973
7 channel samples
bulk chemistry, benthos

"Sandusky Harbor, Ohio - Report on the Degree of Pollution of Bottom Sediments - 1975 Harbor Sediment Sampling Program," USEPA, Region 5, 1975
14 channel, 5 bay samples
bulk chemistry, elutriate, particle size, bottom characteristics

"Sandusky, Ohio - Report on the Degree of Pollution of Bottom Sediments - Sampled: July 28, 1977," USEPA, Region 5, 1977
11 channel samples
bulk chemistry, elutriate, particle size, benthos

"Sediment Bioassays of Harbors of Lake Erie and Lake Ontario," Technical Report 79200A, Aqua Tech Environmental Consultants Inc., March 1980
11 harbor sediment samples
bioassay

"Leach Testing of Dredged Sediments from Sandusky Harbor, Ohio," Calspan Advanced Technology Center, February 1981
11 harbor, 1 reference, 1 disposal samples
column leach test, bulk chemistry, elutriate, particle size

"Laboratory Column Leach Tests on Sandusky Harbor Sediments, Appendix A," Buffalo District, February 1981
Buffalo District in-house report on the above report

"Sampling of Navigation Channel Sediments at Sandusky and Huron Harbors as Potential Sand Sources for Littoral Nourishment," Buffalo District memorandum, 1984
26 grab, 7 core samples
particle size

"Analysis of Sediment from Sandusky Harbor, Sandusky, Ohio," Technical Report No. G0159-02, Aqua Tech Environmental Consultants Inc., June 1985
11 harbor, 3 disposal, 3 reference samples
bulk chemistry, elutriate, bioassay, particle size

"Sediment Analyses - Sandusky Harbor - Sandusky, Ohio," Technical

Report No. G0218-04, Aqua Tech Environmental Consultants Inc., August 1990
11 harbor/channel, 3 disposal, 3 reference samples
bulk chemistry, elutriate, particle size

"The Analyses of Sediments from Sandusky Harbor," Aqua Tech Environmental Consultants Inc., December 1990
11 harbor/channel, 3 disposal, 3 reference site samples
bioassay

"Sediment Sampling for Chemical and Particle Size Analysis - Sandusky Harbor, OH", Volumes I & II, Engineering & Environment, Inc., October 1996
11 harbor, 2 disposal, 4 reference samples
chemistry & particle size

Sturgeon Point

"The Analyses of Sediments from Sturgeon Point, New York," Technical Report No. G0176-19, Aqua Tech Environmental Consultants Inc., September 1986
3 harbor sediment samples
bulk chemistry, particle size

Toledo Harbor

"Report on the Degree of Pollution of Bottom Sediments - Toledo, Ohio," US EPA, Region 5, September 1975
9 lake channel samples
bulk chemistry, macroinvertebrate study, particle size

"Report on Operational Sampling During Dredging Operations in the Lake Erie Sailing Course of the Detroit River Connecting Channels Project," US Army Engineer District, Detroit, August 1976
4 sediment samples
bulk chemistry, particle size, benthos

"Chemical and Bioassay Analysis, Lake Erie Western Basin, Toledo Harbor," Recra Research Inc., September 1981
32 lake channel sampling stations, 8 composite samples
bulk chemistry, bioassay

"Interpretive Summary, Evaluation of Availability and Plant Uptake of Contaminants from Dredged Material from Buffalo, New York, Toledo, Ohio, and Cleveland, Ohio," WES, January 1982

"Analysis of Sediment from Toledo Harbor - Maumee River, Toledo, Ohio [1983]," Technical Report No. G0130-05, Floyd Browne

Associates Limited, February 1984
7 river, 8 lake channel samples
bulk chemistry, elutriate, bioassay, particle size

"Evaluation of Open Lake Disposal Operations in Lake Erie - 1985," USACE, Buffalo, 1985
analysis of the effects of open lake dumping

"Analyses of Sediment and Water Samples from Toledo Harbor, Toledo, Ohio," Technical Report No. G0159-05, Aqua Tech Environmental Consultants Inc., August 1985
8 river, 7 lake samples, CDF water
bulk chemistry, particle size, settling tests

"Column Leach Testing of Sediments from the Toledo Dike Disposal Facility - Toledo, Ohio [1984]," Technical Report G0159-020B, Aqua Tech Environmental Consultants Inc., August 1985
18 samples, column leach testing
bulk chemistry

"The Analyses of Sediments from Toledo Confined Disposal Facility, Toledo, Ohio," Technical Report No. G0159-12, Aqua Tech Environmental Consultants Inc., October 1985
3 sediment samples
bulk chemistry, particle size

"Evaluation of Open Lake Disposal Operations in Lake Erie - 1986," USACE, Buffalo, 1986
analysis of the effects of open lake dumping

"Use of Spot HRV Data in the Corps of Engineers Dredging Program," CRREL, 1986

"The Analysis of Water samples from the Toledo Confined Disposal Facility," Technical Report No. G0176-04, Aqua Tech Environmental Consultants Inc., January 1986
5 CDF water samples
bulk chemical

"Monitoring of Open-Lake Disposal Program at Toledo Harbor - Toledo, Ohio - July 1986," Aqua Tech Environmental Consultants Inc., August 1986
11 field monitoring events, 13 sample sets, 6 hopper dredge samples
bulk chemistry

"Effects of Open-Lake Disposal of Toledo Harbor Dredged Material on Bioavailable Phosphorus in Lake Erie Western Basin," Joseph DePinto et al, Clarkson University, September 1986
analysis of bioavailability and impact of dredged material

phosphorus

"The Analysis of Water Samples from the Toledo Confined Disposal Facility Overflow - Toledo, Ohio," Technical Report No. G0176-13, Aqua Tech Environmental Consultants Inc., October 1986
CDF overflow, 117 samples for suspended sediment
10 samples & 8 vicinity samples for bulk chemistry

"The Analyses of Sediments from the Proposed Open-Lake Disposal Site at Toledo, Ohio," Technical Report No. G0176-17, Aqua Tech Environmental Consultants, October 1986
9 disposal samples
bulk chemistry, bioassay, particle size, benthos at proposed new disposal site

"Sediment Re-Classification, Toledo Harbor," City of Toledo, October 1986
6 lake samples
bulk chemistry

"The Analyses of Sediments from the Proposed Open-Lake Disposal Site at Toledo, Ohio," Technical Report No. I0175-06A, TP Associates International, December 1987
8 disposal, 1 reference site samples
bulk chemistry, bioassay, benthic study

"The Analyses of Sediments from Toledo Harbor," Technical Report No. I0175-12, T. P. Associates International Inc., June 1988
7 river, 17 lake channel, 4 disposal samples
bulk chemistry, elutriate, bioassay, particle size

"Toxicity of Sediment from Western Lake Erie and the Maumee River at Toledo, Ohio," John Giesy & Robert Hoke, Michigan State University, August 1988
78 river/lake samples
bioassay

"Use of SPOT HRV Data in the Corps of Engineers Dredging Program," Carolyn Merry, Harlem McKin, and Nancy LaPotin (US Army Cold Regions Research and Engineering Laboratory) and John R Adams (USACE, Buffalo), Photogrammetric Engineering and Remote Sensing, Vol. 54, No. 9, September 1988, pp 1295-1299

"Maumee Bay Bottom Characterization Study - 1988," Science Applications International Corporation, March 1989
physical bottom characteristics at 143 points outside Maumee River

"Use of Linear Orthogonal Constraints in Analysis of Environmental Data," Robert Hoke (Michigan State University) and

John R Adams (USACE, Buffalo), Environmental Toxicology and Chemistry, Vol. 9, 1990, pp 815- 819

"Toxicity of Sediments from Western Lake Erie and the Maumee River at Toledo, Ohio, 1987: Implications for Current Dredged Material Disposal Practices," R A Hoke, J P Giesy, G T Ankley, J L Newsted (Michigan State University) and J R Adams (USACE, Buffalo), Journal of Great Lakes Research, Vol. 16, No. 3, 1990, pp 457-470

"Toledo Harbor Dredged Material - Beneficial Reuse Alternatives - Status and Needs Report," Toledo Metropolitan Area Council of Governments, May 1990

"Monitoring Well Design and Placement - Toledo CDF," Buffalo District, January 1991

"ARDL Report No.: 6227, Corps of Engineers - Buffalo District, Toledo Harbor Site," ARDL, Inc., October 1992
6 harbor samples
particle size

"ARDL Report No.: 6256, Corps of Engineers - Buffalo District, Grain Size Analysis Data Package, Toledo Harbor Site," ARDL, Inc., December 1992
3 harbor samples
grain size

"ARDL Repost No. 6255, Corps of Engineers, Buffalo District, Toledo Harbor - Column Settling Test", ARDL Inc., September 1993
3 samples

"Transmittal of Results of Soil Tests, Samples RM1-2, LM0-1, and LM2-3, Toledo Harbor OH", Waterways Experiment Station, October 1993
3 samples

"Application of the *Chironomus tentans* Survival and Growth Bioassay in Evaluating Sediment Quality from Four Great Lakes Harbors," University of Wisconsin-Superior, December 1993
1 river, 7 entrance channel, 2 reference
bioassays

"Toledo Harbor site, ARDL Report Nos. 6351/6352, January 1994
5 CDF core borrow sites
bulk chemistry, particle size

"Evaluation of Proposed EPA Dredged Material Bioassays Using Great Lakes Sediments: the 10-day *Hyalella azteca*, solid-phase bioassay, the 7-day *Pimephales promelas*, elutriate test, and the

21-day *Daphnia magna* elutriate test, " Miscellaneous paper D-94-XX, Waterways Experiment Station, June 1994 (draft)
1 river, 7 entrance channel, 2 reference
bioassays

"Particle Size Summary Report No. 6372 Toledo Harbor Site", ARDL Inc., October 13, 1994
9 river/harbor, 1 reference sample

"Evaluation of Sediments from the Toledo Harbor Area, Lucas County, Ohio", Environmental Science & Engineering, Inc., November 1994
9 river samples
bioassay

"Evaluation of Sediments from the Toledo Harbor Area", Volumes 1 and 2 Environmental Science & Engineering, Inc., January 25, 1995
9 harbor, 1 reference sample
bioassay testing

Touissant River

"Analysis of Sediment from Touissant River, Ottawa County, Ohio," Technical Report No. G0159-01, Aqua Tech Environmental Consultants Inc., November 1984
3 core samples, 2 composite samples of each core
bulk chemistry

"The Analyses of Sediments from Touissant River," Technical Report No. 101788-100, T.P. Associates Inc., October 1988
2 harbor entrance, 4 lake samples
bulk chemistry, elutriate

"The Analyses of Sediments from Touissant River," T. P. Associates International Inc., October 1988
4 samples
bioassay

Vermilion Harbor

"Vermilion Harbor - Sediment Sampling," (not the title - an extract from an unknown report), USEPA, 1973
4 sediment samples
bulk chemistry

"Vermilion, Ohio - Report on the Degree of Pollution of Bottom Sediments - 1975 Harbor Sediment Sampling Program - April 9, 1975," USEPA, Region 5, 1975

8 stream/harbor sediment samples
bulk chemistry, elutriate, particle size, benthos, bottom characterization

"Report on the Analysis of Surface Sediment Grab Samples for the U. S. Army Corps of Engineers' Lake Erie Harbor Dredging Program," Wadsworth Testing Laboratories Inc., November 1981
7 river, 1 harbor, 1 disposal, 1 reference samples
bulk chemistry, elutriate, bioassay, particle size

"The Toxicity of Dredged Materials from Vermilion Harbor, Ohio to Aquatic Organisms," Technical Report No. BW-82-4-1098, EG&G Bionomics, April 1982
8 river/harbor samples
bioassay

"The Analyses of Sediment from Vermilion Harbor," Technical Report No. I0175-03, T.P. Associates International Inc., August 1987
8 river/harbor, 2 disposal, 2 reference samples
bulk chemistry, elutriate, particle size

"Results of 96-Hour Sediment Bioassay Tests of Sediment from Vermilion Harbor, Ohio," Technical Report No. I0175-03, Aqua Tech Environmental Consultants Inc., November 1987
8 river/harbor, 2 disposal, 2 reference
bioassay

"Sediment Sampling and Testing, Vermilion Harbor, OH," Bowser-Morner Inc., September 1992
8 harbor, 1 disposal, 2 reference samples
elutriate, bulk chemical, particle size, bioassay

<u>West Harbor</u>

"Analysis of sediment Port Clinton/West Harbor - Port Clinton, Ohio," Technical Report No. G0130-10, Aqua Tech Environmental Consultants Inc., September 1984
10 harbor, 3 reference site samples
bulk chemistry, elutriate, bioassay, particle size

"The Analyses of Sediments from West Harbor," Technical Report N. G0176-14, Aqua Tech Environmental Consultants Inc., September 1986
4 disposal site samples
bioassay, particle size

"The Analyses of Sediments from the Proposed Open-Lake Disposal Site at West Harbor, Ohio," Technical Report No. G0176-20, Aqua

Tech Environmental Consultants Inc., December 1986
4 disposal site samples
bioassay, benthos

"Sediment and Elutriate Analysis - Open-Lake Disposal and Channel Sites - West Harbor, Ohio," Technical Report No. G0218-12, Aqua Tech Environmental Consultants Inc., July 1991
2 harbor, 9 disposal samples
bulk chemistry, particle size

"ARDL ID No. 6001/6002, Corps of Engineers - Buffalo District, West Harbor, Ohio Site," ARDL Inc., August 1991
15 harbor samples
bulk chemistry, elutriate, particle size

Wilson Harbor

"Wilson Harbor - 1970," (not the title - an extract from an unknown report), Federal Water Quality Administration, 1970
4 harbor samples
bulk chemistry

"Wilson Harbor - 1972," (not the title - an extract from an unknown report), USEPA, 1972
4 harbor samples
bulk chemistry

"Sediment Quality Testing at Olcott Harbor, New York and Wilson Harbor, New York Sampling Sites," Great Lakes Laboratory, State University College at Buffalo, September 1981
5 harbor, 1 reference, 1 disposal samples
bulk chemistry, elutriate, bioassay, particle size

"The Toxicity of Dredged Materials from Wilson Harbor, New York to Aquatic Organisms," Bionomics Report No. BW-82-1-1097, EG & G, Bionomics, January 1982
5 harbor, 2 disposal, 2 reference samples
bioassay

"The Analyses of Sediments from Wilson Harbor," Technical Report No. 10175-14, T. P. Associates International Inc., July 1988
4 harbor, 1 disposal, 1 reference samples
bulk chemistry, elutriate, bioassay, particle size

"Sediment Sampling and Testing, Wilson Harbor, NY," Bowser-Morner Inc., July 1992
5 harbor, 1 disposal, 1 reference site
bulk chemistry, particle size, elutriate, bioassay

Bibliography of Sediment Investigations
at USACE Navigation Projects
within the Chicago District

Burns Waterway Small Boat Harbor

"Summary Report on Sediment Sampling Program at Burns Ditch, Indiana 8 July 1981" USACE Chicago District, August 1981.
bulk chemical & grain size analyses

"Burns Waterway Small Boat Harbor Project Sedimentation Sampling and Analysis Conducted in September 1981" USACE Chicago District, March 1982.
bulk chemical & particle size analyses, elutriate test

Calumet River and Harbor

"Analysis of Sediments from Chicago & Calumet Harbors" by Environmental Research Group, Inc., April/May 1980.
bulk chemical & particle size analyses

"Chicago Sites Sediments Quality Analysis" USACE Chicago District, October 1980.
bulk chemical, organic pollutants, and particle size analyses

"Summary Report on Sediment Sampling Program Chicago River & Harbor & Calumet River & Harbor" USACE Chicago District, August 1981.
bulk chemical analysis and modified elutriate test

"Water Quality Monitoring Chicago Area Confined Disposal Facility Calumet Harbor, Illinois Final Report on Operations April 1989 thru June 1989" by Randolph & Associates, Inc., December 1989.
bulk chemical analysis

"Lake Calumet, Illinois Small Boat Harbor Project Sediment Sampling and Analysis Conducted in September 1985" USACE Chicago District, November 1985.
bulk chemical and particle size analyses

"Lake Calumet Dredged Disposal Area Operation & Monitoring" USACE Chicago District, April 1988.
bulk chemical and physical analyses

Chicago Area CDF

"Water Quality Monitoring Chicago Area Confined Disposal Facility Final Report on Operations October 1984 to December 1984" Daily & Associates, Engineers, Inc., April 1985.

"Water Quality Monitoring of Dredge Equipment Demonstration" Daily & Associates, Engineers, Inc., October 1985.
bulk chemical and physical analyses

"Water Quality Monitoring Chicago Area Confined Disposal Facility Final Report on Operations July 1985 to September 1985" Daily & Associates, Engineers, Inc., February 1986.

"Water Quality Monitoring Chicago Area Confined Disposal Facility Final Report on Operations April 1986 to July 1986" Daily & Associates, Engineers, Inc., October 1986.

"Chicago Area Confined Disposal Facility Monitoring Well Data Report CY 1985" Daily Analytical Laboratories, Inc., 1985 Environmental Instrument Systems, Inc., prior to February 1985.

"Chicago Area Confined Disposal Facility Monitoring Well Data Report CY 1986" Northern Laboratories and Engineering, Inc., 1986.

"Chicago Area Confined Disposal Facility Monitoring Well Data Report CY 1987" Northern Laboratories and Engineering, Inc., 1987.

"Chicago Area Confined Disposal Facility Monitoring Well Data Report CY 1988" Northern Laboratories and Engineering, Inc., 1988.
bulk chemical and organic analyses

"Chicago Area Confined Disposal Facility Monitoring Well Data Report CY 1989" Northern Laboratories and Engineering, Inc., 1989.

Chicago Harbor

"Analysis of Sediments from Chicago & Calumet Harbors" by Environmental Research Group, Inc., April/May 1980.
bulk chemical & particle size analyses

"Chicago Sites Sediments Quality Analysis" USACE Chicago District, October 1980.
bulk chemical and particle size analyses

"Summary Report on Sediment Sampling Program Chicago River & Harbor & Calumet River & Harbor" USACE Chicago District, August 1981.
bulk chemical analysis and modified elutriate test

"Chemical Analysis of Sediment and Biological Samples from Chicago Navigation Project" USACE Chicago District, 1986.
biological tissue and sediment chemical analyses

"Biological Investigations of Chicago Area Navigation Project" USACE Chicago District, 1987.
biological tissue and sediment chemical analysis

Chicago River

"Chicago Sites Sediments Quality Analysis" USACE Chicago District, October 1980.
bulk chemical and particle size analyses

"Summary Report on Sediment Sampling Program Chicago River & Harbor & Calumet River & Harbor" USACE Chicago District, August 1981.
bulk chemical analysis and modified elutriate test

"Chicago River-North Branch Analysis of Sediment Samples Collected in August 1983" USACE Chicago District, December 1983.
PCB analysis

"North Branch and North Branch Canal of the Chicago River Bottom Sediment" by Patrick Engineering, Inc., August 1986.
sample collection only, no analysis

"Sediment and Pore Water Studies of Chicago River-North Branch Sediment" by James M. Brannon, WES, August 1987.
bulk chemical and particle size analyses

"Testing of Chicago River Sediment to Determine the Effects of Cleanup Procedures on Measured PCB Concentrations" by James M. Brannon and Richard Karn, WES, November 1987.
bulk chemical and physical analyses

"Effects of Cleanup Procedures on Measured PCB Concentrations in Chicago River Sediment" by James M. Brannon and Richard Karn, WES, January, 1988.
bulk chemical and physical analyses

"Chemistry of Bottom Sediments from the North Branch of the Chicago River and North Branch Canal, Lake Street to North

Avenue, Chicago, Illinois" by Argonne National Laboratory, January 1989.
metal and organic pollutant analyses

Indiana Harbor and Canal

"Interim Results of Laboratory Settling, Filtering, Leaching, and Coagulation Tests, Indiana Harbor, Indiana" by WES, May 1980.
bulk chemical and physical analyses

"Final Report of Findings, Environmental Tests, Indiana Harbor, Indiana" by WES, May 1980.
bulk chemical analysis and elutriate test

"Sediment Sampling and Chemical Analyses Indiana Harbor Ship Canal East Chicago, Indiana" by Dames & Moore, September 1983.
bulk chemical analysis

"Indiana Harbor and Canal, Analysis of Sediment Samples Collected in August 1983" USACE Chicago District, November 1983.
PCB analysis and EP-Toxicity Tests

"Biological and Chemical Water Quality Survey in Indiana Harbor, the Indiana Harbor Canal, and Southwestern Lake Michigan for the U.S. Army Corps of Engineers Chicago District" by Irwin Polls and Samuel G. Dennison, February 1984.
fish and benthic organism collection survey

"Field Methodology and Results for Indiana Harbor and Two Nearshore Areas of Lake Michigan" by Limo-Tech, Inc., October 1984.
grain size analysis and identification of biological organisms and calculation of their spatial density

"Water Quality Monitoring of Resuspension of Sediment Indiana Harbor Canal 7-8 August 1985, 7 November 1985" Daily & Associates, Engineers, Inc., March 1986.
bulk chemical analysis

"Army Corps of Engineers Analytical Report Indiana Harbor" by Swanson Environmental, Inc., September 1986.
bulk chemical analysis, priority pollutant analysis, and particle size analysis

"Analyses of Impacts of Bottom Sediments from Grand Calumet River and Indiana Harbor Canal on Water Quality" by James M. Brannon et al., WES, October 1986.

"Sediment Survey of the Indiana Harbor Canal, Indiana Harbor, and Adjacent Lake Michigan for the Chicago District, U.S. Army Corps of Engineers" by Irwin Polls, Metropolitan Sanitary District of Greater Chicago, July 1988.
organic chemical analysis and elutriate test

"Chemical Analyses of Sediment and Biological Samples from Indiana Harbor Canal, Indiana Harbor and Adjacent Lake Michigan" J. Bruno Risatti & Suchada Broeren, September 1988.
bulk chemical analysis and PAH & phenol tests

"Wet Air Oxidation Testing of Indiana Harbor Sludge for the U.S. Army Corps of Engineers" by ZIMPRO Inc., October 1988.
bulk chemical and priority pollutant analyses

"Characterization and Treatability of Bottom Sediments from the Indiana Harbor and Canal" by Indiana University Northwest, March 1990.
bulk chemical and particle size analyses

Little Calumet River, Illinois Silt Removal Project

"Little Calumet River, Illinois Sediment Sampling and Chemical Analysis Phase I" USACE Chicago District, January 1981.
bulk chemical analysis

"Little Calumet River, Illinois Sediment Sampling and Chemical Analysis Phase II" USACE Chicago District, April 1982.
bulk chemical and physical analyses, elutriate test

Michigan City Harbor

"Letter Report on Confined Disposal Area for Michigan City Harbor Indiana" USACE Chicago District, May 1976.
elutriate test

"Michigan City Harbor Dredging and Disposal Operations 1978-1979 After-Action Report" USACE Chicago District, July 1979.
bulk chemical analysis

"Sediment Quality Monitoring Michigan City Harbor & Trail Creek Michigan City, Indiana" by Daily & Associates, Engineers, Inc., April 1986.
bulk chemical, particle size analyses

Waukegan Harbor

"Waukegan Outer Harbor Sediment Analysis" USACE Chicago District, June 1981.
grain size analysis & total PCB analysis and elutriate test for total PCB

"Waukegan Harbor, Illinois Analysis of Sediment Samples Collected in October 1981" USACE Chicago District, May 1982.
bulk chemical & particle size analysis and elutriate test

"Waukegan Harbor, Illinois Analysis of Sediment Samples Collected in November 1982" USACE Chicago District, February 1983.
bulk chemical analysis and modified elutriate test

"Waukegan Harbor Sediment Sampling" by Mirza Engineering Inc., October 1987.
depth determination of sediment within the inner harbor

"Waukegan Harbor, Illinois Collection and Analysis of Sediment Samples from the Navigation Project" by Randolph & Associates, Inc., December 1987.
bulk chemical and grain size analysis

"Analysis of Pore Water from Sediment at Waukegan Harbor, Illinois" by James M. Brannon, WES, May 1988.
bulk chemical & grain size analysis and total phenol

"Waukegan Harbor, Illinois Report of Sediment Sampling Activities Approach Channel" by Northern Laboratories, December 1990.
PCB and grain size analyses

Summary of Sediment Investigations
at USACE Navigation Projects
within the Detroit District (as of 11/96)

HARBOR	DATE[1]	CLASS[2]	REMARKS
ALGOMA WISC	1991	PRT	PROPOSED DREDGING FY93
ALPENA MICH	1995	CLN	
ARCADIA MICH	1996	CLN	
ASHLAND WISC	1992	CLN	EPA - MOD POLL
AUSABLE MICH	1991	CLN	WORKING ON EPA CNCR
BAYFIELD WISC	1992	-	
BAY PORT MICH	1991	CLN	FY91-OUT FED CHNL-MFR-IN
BEAVER BAY MINN (SILVER BAY)	1975	PRT	NOT CONSTRUCTED
BELLE RIVER MICH	1978	CLN	
BENTON HARBOR MICH	1983	NC	ONLY PHYS DESC OF CDF SAMPLES
BIG BAY MICH	1994	CLN	
BIG SUAMICO RIVER WISC	1993	CLN	SUITABLE FOR BN
BLACK RIVER (UP) MICH	1994	CLN	SUITABLE FOR BN
BLACK RIVER P.H. MICH	1990	PRT	FY90-5 ADDITIONAL SAMPLES
BOLLES MICH	1994	PRT	FY86-OUTER SECTION ONLY
CASEVILLE MICH	1990	CLN	
CEDAR RIVER MICH	1994	CLN	NOT CONSTRUCTED
CHARLEVOIX MICH	1985	CLN	BOWMAN-EAB WORKING ON EPA
CHEBOYGAN MICH	1992	CLN	'83-ELUTRIATE ANALYSIS ONLY
CHIPPEWA MICH	0	-	NO DREDGING FORECAST
CHNLS LAKE ST CLAIR MICH	1994	CLN	EPA RECOMM ADDNL SAMPLING
CHNLS STRTS OF MACKINAW MICH	0	-	(ONLY WATER Q DATA-1978)
CLINTON RIVER MICH	1989	PRT	
CORNUCOPIA WISC	1995	CLN	SUITABLE BN/PER EAB (CLASS
CROSS VILLAGE MICH	1979	CLN	NOT CONSTRUCTED
DETOUR MICH	0	-	
DETROIT RIVER MICH	1995	CNTM	WORKING ON EPA CNCR FY91
DETROIT RIVER,(PTE. MOUILLEE)	1993	CNTM	ACCESS N&S CHNLS & CELL 1
DULUTH-SUPERIOR MINN-WISC	1995	PRT	FY86,87 PARTIAL;FY90 SUP
EAGLE HARBOR MICH	1986	CLN	
FOX RIVER LOCK&DAMS WISC	1989	-	NO DREDGING FORECAST
FRANKFORT MICH	1992	PRT	
GRAND HAVEN R&H MICH	1996	PRT	FY92-GRAND RIVER(NO
GRAND MARAIS MICH	1994	CLN	
GRAND MARAIS MINN	1987	CNTM	
GRAND TRAVERSE BAY MICH	1990	CLN	
GRAYS REEF PASSAGE MICH	0	-	NO DREDGING FORECAST
GREEN BAY WISC	1994	PRT	FY86 (OUTER END DATA ONLY)
HAMMOND BAY MICH	1991	CLN	
HARBOR BEACH MICH	1991	CLN	(85 MARINA ONLY) CHECK 91
HARRISVILLE MICH	1995	CLN	
HOLLAND MICH	1994	PRT	FY92 ARSENIC & PART
INLAND RTE CHEBOYGAN R. MICH	1992	CLN	
INLAND RTE CROOKED R. MICH	1992	CNTM	
INLAND RTE INDIAN R. MICH	1992	CLN	
KAWKAWLIN MICH	1989	PRT	
KENOSHA WISC	1995	PRT	OUTER CLASS 96

KEWAUNEE WISC	1992	PRT	1986 (OUTER ONLY)
KEWEENAW WATERWAY MICH	1993	PRT	
KNIFE RIVER MINN	1983	CNTM	
LAC LA BELLE MICH	1988	CLN	REC'D PREL CONC
LAPOINTE WISC	1990	CLN	
LELAND MICH	1996	CLN	
LES CHENEAUX MICH	1986	PRT	
LEXINGTON MICH	1996	CLN	SILTY-SAND;DISP PROB OW
LITTLE BAY DENOC MICH	0	-	
LITTLE LAKE MICH	1995	CLN	
LUDINGTON MICH	1991	CLN	
LUTSEN (SCHROEDER) MINN	0	-	NOT CONSTRUCTED
MACKINAW CITY MICH	0	-	
MANISTEE MICH	1991	CLN	
MANISTIQUE MICH	1990	PRT	
MANITOWOC WISC	1995	PRT	WAITING FOR CORRECTED DATA
MARQUETTE MICH	1984	CLN	
MENOMINEE MICH-WISC	1994	PRT	
MILWAUKEE WISC	1993	CNTM	IN 86 - 6 STNS AROUND CDF
MONROE MICH	1993	CNTM	INNER/OUTER DATA
MUSKEGON MICH	1993	CLN	
NEW BUFFALO MICH	1991	CLN	
OCONTO WISC	1988	CLN	REC'D WRITTEN CLASS-UPLAND
ONTONAGON MICH	1995	CLN	WAITING FOR CORRECTED DATA
PENSAUKEE WISC	1991	CLN	NEED STA MAP & PART SIZE
PENTWATER MICH	1992	CLN	
PETOSKEY MICH	0	-	NO FEDERAL CHANNEL
PINE RIVER MICH	1970	CNTM	NO DREDGING FORECAST
POINT LOOKOUT (AU GRES) MICH	1992	CLN	
PORT AUSTIN MICH	1989	CLN	LAST DREDGED 66
PORT SANILAC MICH	1990	CLN	DISP CONCERNS
PORT WASHINGTON WISC	1987	CNTM	
PORT WING WISC	1990	CLN	SUITABLE FOR BN
PORTAGE LAKE MICH	1992	CLN	
PRESQUE ISLE MICH	1992	CLN	
RACINE HARBOR WISC	1976	CLN	NO DREDGING FORECAST
ROUGE RIVER MICH	1993	CNTM	RIVER
SAGINAW RIVER MICH	1996	CNTM	OUTER NOT CLASSIFIED
SAUGATUCK MICH	1986	CLN	
SAXON WISC	1993	CLN	RESTRICTED UNCONFINED UPLAND
SEBEWAING MICH	1991	CLN	
SHEBOYGAN WISC	1993	PRT	OUTER (INNER 84 Y N N, UNDER
SOUTH HAVEN MICH	1992	PRT	
ST CLAIR RIVER MICH	1995	PRT	FY91-8 STA
ST JAMES MICH	1993	-	
ST JOSEPH MICH	1996	PRT	EAB DID DUPLICATE SAMPLING
ST JOSEPH RIVER MICH	0		NO DREDGING FORECAST
ST MARYS RIVER MICH	1992	CLN	
STURGEON BAY WISC	1992	PRT	
TAWAS BAY MICH	0	-	
TRAVERSE CITY MICH	1985	CLN	WORKING ON EPA CNCR
TWO HARBORS MINN	1982	CNTM	
TWO RIVERS WISC	1996	CLN	OUTER HARBOR SUITABLE FOR BN

WASHINGTON ISLE WISC	0	-	
WHITE LAKE MICH	1993	CLN	
WHITEFISH POINT MICH	1988	CLN	REC'D PREL CNCR

[1] Date last sampled. Many of these projects have been sampled every 5 years since the late 1970's.

[2] Classification based on bulk chemistry:
CLN - Clean/uncontaminated
PRT - partially contaminated
CNTM - contaminated

SECTION 3
State Environmental Data

Below is a partial list of reports on ambient water quality conditions in the Great Lakes and tributaries published periodically by State agencies. On the following pages is a list of Great Lakes areas of concerns and remedial action plan (RAP) coordinators at USEPA Regions and State agencies.

Illinois Environmental Protection Agency. 1990. "Illinois Water Quality Report; 1988-1989," Division of Water Pollution Control, Springfield, IL.

Indiana Department of Environmental Management. 1990. "Indiana Water Quality, 1988: Monitor Stations Records," Office of Water Management, Indianapolis, IN.

Michigan Department of Natural Resources. 1990. "Michigan Fixed Station Monitoring; 1989 Annual River Water Quality Report," Surface Water Quality Division, Lansing, MI.

Minnesota Pollution Control Agency. 1989. "Water Quality Sampling Program, Minnesota Lakes and Streams: A Compilation of Analytical Data, October 1984-September 1987," Division of Water Quality, St. Paul, MN.

New York State Department on Environmental Conservation. 1992. "Biennial Report, Rotating Intensive Basin Studies, Water Quality Assessment Program, 1987-1988, Monitoring and Assessment Bureau, Albany, NY.

Wisconsin Department of Natural Resources. 1992. "Background Concentrations of Trace Metals in Wisconsin Surface Waters," prepared by University of Wisconsin, Madison, WI.

Great Lakes Areas of Concern
and Remedial Action Plan (RAP) Coordinators
(updated February 1998)

Area of Concern	USEPA RAP Coordinator	Phone Number	State RAP Coordinator	State Agency	Phone Number
Ashtabula River, OH	Amy Pelka	312-886-0135	Natalie Farber	Ohio EPA	614-644-2143
Black River, OH	Phil Gehring	216-522-7260		Ohio EPA	
Clinton River, MI	Laura Evans		Robert Sweet	Mich DEQ	517-335-4182
Cuyahoga River, OH	Mark Moloney	440-835-5200	Kelvin Rodgers	Ohio EPA	330-963-1117
Deer Lake/Carp River, MI	Mark Messersmith		Sharon Baker	Mich DEQ	517-335-3310
Detroit River, MI	Mark Olender		Robert Sweet	Mich DEQ	517-335-4182
Eighteen Mile Creek, NY	Alice Yeh Barbara Spinweber				
Fox River/Green Bay, WI	Callie Bolattino	312-353-3490	Bob Behrens	Wis DNR	920-448-5133
Grand Calumet River/ Indiana Harbor, IN	Karen Turner	312-886-1437	Scott Ireland	Ind DEM	
Kalamazoo River, MI	Marcia Damato	312-886-6297	Roger Eberhardt	Mich DEQ	517-335-1119
Manistique River, MI	Jim Hahnenberg	312-353-4213	Roger Eberhardt	Mich DEQ	517-335-1119
Maumee River, OH	Dave Barna	440-835-5200	Cherie Blair	Ohio EPA	419-373-3010
Menominee River, MI/WI	Chuck Anderson		Roger Eberhardt Terry Lohr	Mich DEQ Wis DNR	517-335-1119 608-267-2375
Milwaukee Harbor, WI	Steve Jann	312-886-2446	Sharon Gayan	Wis DNR	414-263-8707
Muskegon Lake, MI	Sheri Bianchin		Roger Eberhardt	Mich DEQ	517-335-1119
Niagara River/Buffalo River, NY	Alice Yeh Barbara Spinweber			NY DEC	

Great Lakes Areas of Concern
and Remedial Action Plan (RAP) Coordinators (continued)

Area of Concern	USEPA RAP Coordinator	Phone Number	State RAP Coordinator	State Agency	Phone Number
Oswego River, NY	Alice Yeh Barbara Spinweber		Bob Townsend	NY DEC	518-457-7470
Presque Isle Bay, PA	Chuck Sapp	215-597-9096	Kelly Burch	Penn DEP	814-332-6816
River Raisin, MI	Amy Nerbun		Robert Sweet	Mich DEQ	517-335-4182
Rochester Embayment, NY	Alice Yeh Barbara Spinweber		Margy Peet	NY DEC	518-457-7470
Rouge River, MI	Quintin White		Robert Sweet	Mich DEQ	517-335-4182
Saginaw River/Bay, MI			Robert Sweet	Mich DEQ	517-335-4182
Sheboygan Harbor, WI	Susan Prout		Chip Krohn	Wis DNR	414-229-0862
St. Clair River, MI	Tom Matheson		Robert Sweet	Mich DEQ	517-335-4182
St. Lawrence River, NY	Alice Yeh Barbara Spinweber	212-264-7678	Berton Mead	NY DEC	518-457-7463
St. Louis River/Bay, MN/WI			Brian Frederickson Ted Smith	Minn PCA Wis DNR	218-723-4663 715-635-4071
St. Mary's River, MI	Jennifer Manville		Roger Eberhardt	Mich DEQ	517-335-1119
Torch Lake, MI	Rita Garner		Sharon Baker	Mich DEQ	517-335-3310
Waukegan Harbor, IL	Matt Didier	312-886-6711	Robert Schacht	Ill EPA	
White Lake, MI	Mike Ribardy	312-886-4592	Roger Eberhardt	Mich DEQ	517-335-1119

USEPA Region 5 has RAP Coordinators for areas of concern in Ohio, Michigan, Indiana, Illinois, Wisconsin and Minnesota. Regions 2 and 3 have RAP Coordinators for areas of concern in New York and Pennsylvania, respectively.

SECTION 4
Bibliography of Information Related to STFATE Application

This section contains a partial bibliography of publications containing measurements of water currents and temperature needed to use the STFATE model to determine mixing zones from dredged material disposal operations during Tier 2.

Bennett, J.R. 1971. "Thermally Driven Lake Currents During the Spring and Fall Transition Periods," WIS-SG-72-326, NOAA-72102703, University of Wisconsin, Marine Studies Center, Madison, WI.

Dettman, E.H. 1982. "Transport of Particulate Matter by Shearing Currents in Lake Erie; 1980," GRAI8207 NSA0600, Argonne National Lab, IL, Department of Energy, Washington, DC.

Federal Water Pollution Control Administration. 1967. "Water Quality Investigations, Lake Currents: Lake Michigan Basin," PB-230 819/5, Great Lakes Region, Chicago, IL.

Gedney, R. and W. Lick. 1969 "Numerical Calculations of the Steady-State, Wind-Driven Currents in Lake Erie," NASA-TM-X-52786, National Aeronautics and Space Administration, Lewis Research Center, Cleveland, OH.

Gedney, R.T. and W. Lick. 1971. "Numerical Calculations of the Wind Driven Currents in Lake Erie and Comparison with Measurements," NASA-TM-X-67804, National Aeronautics and Space Administration, Lewis Research Center, Cleveland, OH.

Gottlieb, E.S.; Saylor, J.H.; Miller, G.S. 1989. "Currents and Temperatures Observed in Lake Michigan from June 1982 to July 1983; NOAA-TM-ERL-GLERL-71; NOAA, Great Lakes Environmental Research Lab, Ann Arbor, MI.

Gottlieb, E.S., Saylor, J.H. and G.S. Miller. 1989. "Currents, Temperatures, and Divergences Observed in Eastern Central Lake Michigan during May-October 1984," NOAA-TM-ERL-GLERL-72, NOAA, Great Lakes Environmental Research Lab, Ann Arbor, MI.

Gottlieb, E.S., Saylor, J.H. and G.S. Miller. 1990. "Currents and Water Temperatures Observed in Green Bay, Lake Michigan; Part 1 Winter 1988-1989; Part 2 Summer 1989," NOAA-TM-ERL-GLERL-73, NOAA, Great Lakes Environmental Research Lab, Ann Arbor, MI.

Huang, J.C. 1970. "The Thermal Current in Lake Michigan," AD-725 715, Scripps Institution of Oceanography, La Jolla, CA.

Johnson, R.G. and E.C. Monahan. 1971. "Current Meter Observations of the Circulation in Grand Traverse Bay of Lake Michigan; Mooring Methods and Initial Results," TR-18, NOAA-72020202, University of Michigan, Dept of Meteorology and Oceanography, Ann Arbor, Michigan.

Katz, P.L. and G.M. Schwab. 1976. "Currents and Pollutant Dispersion in Lake Michigan, Modeled with Emphasis on the Calumet Region," UILU-WRC-76-0111, W77-03732, Illinois University at Urbana-Champaign, Water Resources Center, Office of Water Research and Technology, Washington, DC.

Lick, W. 1976. "Numerical Models of Lake Currents," GRAI7615, Case Western Reserve University, Ohio Dept. of Earth Sciences, Cleveland, OH.

Monahan, E.C. and C.R. Zietlow. 1968. "A Study on the Onset of Whitecapping with Increased Surface Wind Speeds," Northern Michigan University.

Monahan, E.C. and P.C. Pilgrim. 1975. "Coastwise Currents in the Vicinity of Chicago, and Currents Elsewhere in Southern Lake Michigan," NOAA-75082103, NOAA, Office of Sea Grant, Rockville, MD.

Ragotzkie, R.A. 1966. "The Keweenaw Current, A Regular Feature of the Summer Circulation of Lake Superior," TR-29, University of Wisconsin, Dept of Meteorology, Madison, WI.

Saunders, K.D. and L.S. Van Loon. 1976. "Water Resources Research Program; Nearshore Currents and Water Temperatures in Southwestern Lake Michigan, Progress Report, June-December 1975," GRAI7712, NSA0200, Energy Research and Development Administration, Argonne National Lab., IL.

Saunders, K.D. and J.D. Ditmars. 1981. "Nearshore Currents in Lake Michigan Between Milwaukee and Chicago, 1977-78: Implications for Transport," IINR-81/31, Argonne National Lab., IL Energy and Environmental Systems Div. Corp, Illinois Inst. of Natural Resources, Chicago, IL.

Saylor, J.H and G.S. Miller. 1976. "Winter Currents in Lake Huron," NOAA-TM-ERL-GLERL-15, NOAA-77112304; EPA/905/4-75-004, NOAA, Great Lakes Environmental Research Lab, Ann Arbor, MI.

Saylor, J. H. and G.S. Miller. 1984. "Investigation of the Currents and Density Structure of Lake Erie; 1983," NOAA-TM-ERL-GLERL-49, NOAA-84010302, NOAA, Great Lakes Environmental Research Lab, Ann Arbor, MI.

Sheng, Y.P. 1975. "Lake Erie International Jetport Model Feasibility Investigation," WES-CR-H-75-1, Case Western Reserve University, Ohio Dept of Earth Sciences, Cleveland, OH.

Sloss, P.W. and J.H. Saylor. 1976. "Measurements of Current Flow During Summer in Lake Huron," NOAA, Great Lakes Environmental Research Lab, Boulder, CO.

Sloss, P.W. and J.H. Saylor. 1976. "Large-Scale Current Measurements in Lake Superior," GLERL-8, GLERL-CONTRIB-61, NOAA-76060104, NOAA-TR-ERL-363, NOAA, Great Lakes Environmental Research Lab, Ann Arbor, MI.

U.S. Public Health Service. 1963. "Lake Michigan Studies, Currents in the Southern Basin," LM 12, GRAI7707, Great Lakes-Illinois Rivers Basins Project, Chicago, IL.

U.S. Public Health Service. 1963. "Lake Michigan Studies, Introduction to Lake Current Studies," LM 7, Great Lakes-Illinois River Basins Project, Chicago, IL.

SECTION 5
List of Great Lakes Critical Pollutants

The attached is a consolidated list of critical contaminants identified in Lakewide Management Plans (LaMPs) and bioaccumulative chemicals of concern (BCCs) defined in the proposed Great Lakes water quality criteria. The attached may be used in Tier 1 to aid in the development of a contaminant of concern list and identifying bioaccumulative contaminants.

SECTION 6
Lipid Levels of Selected Aquatic Organisms

The following pages contain tables of lipid levels in selected fish species of the Great Lakes as a reference for applying the TBP procedures in Tier 2. These tables were from the following references:

Clarke, J.U., Whitman, P.L. and J. Dorkin. 1992. "Trends in PCB contamination in fishes from the Wisconsin waters of Lake Michigan," Miscellaneous Paper D-92-3, USACE Waterways Experiment Station, Vicksburg, MS.

USEPA. 1992. "National study of chemical residues in fish," EPA 823-R-92-008 A&B,

Average Percent Lipid For Selected Species and Sample Types in Fishes From the Wisconsin Waters of Lake Michigan, 1978-1986 (after Clarke et al. 1992)

Fish species	% Lipid (N)			
	Whole Fish	Edible Portion	Fillet	Skin-on Fillet
Brook trout	7.0 (1)	3.8 (2)	4.7 (89)	n/a
Brown trout	14.2 (3)	4.0 (2)	11.0 (251)	n/a
Bullhead sp.	2.6 (8)	n/a	2.3 (2)	1.3 (10)
Carp	11.3 (60)	n/a	13.4 (46)	n/a
Channel catfish	15.0 (1)	n/a	n/a	9.4 (14)
Chinook salmon	6.6 (12)	n/a	3.8 (417)	2.53 (23)
Coho salmon	3.6 (15)	n/a	3.9 (145)	2.5 (23)
Lake trout	12.9 (7)	2.7 (1)	13.4 (269)	n/a
Northern pike	3.1 (6)	n/a	1.3 (25)	n/a
Rainbow trout	3.0 (3)	3.9 (2)	6.9 (125)	n/a
Smallmouth bass	6.6 (2)	n/a	1.2 (10)	n/a
Walleye	11.1 (7)	n/a	4.1 (23)	n/a
Yellow perch	5.1 (8)	n/a	1.0 (26)	n/a
36 fish species combined (N) [Standard Error]	9.4 (235) [0.44]	3.4 (16) [0.26]	7.6 (1606) [0.15]	4.2 (50) [0.61]

Legend: n/a = no analyses performed
N = number of samples analyzed.

NOTE: Species, size and age, sex, season, lake or sub-basin, and tissue collection type are all important variables that are sometimes correlated with percent lipid. Averages from larger data sets are more appropriate for use in TBP algorithm.

Average Percent Lipid For Selected Species and Sample Types in Fishes From the Waters of Great Lakesother than Lake Michigan, 1983-1989 (from USEPA 1992)

Fish species	% Lipid (N)			
	Whole Fish	Edible Portion	Fillet	Skin-on Fillet
LAKE ERIE Carp	8.1 (4)	n/a	n/a	n/a
LAKE ERIE Coho salmon	n/a	n/a	1.8 (2)	n/a
LAKE ERIE Smallmouth bass	3.1 (1)	n/a	3.1 (1)	n/a
LAKE HURON Brown trout	n/a	n/a	5.6 (1)	n/a
LAKE HURON Carp	7.3 (1)	n/a	4.5 (29)	n/a
LAKE HURON Channel catfish	n/a	n/a	7.7 (4)	n/a
LAKE HURON Coho salmon	n/a	n/a	2.4 (1)	n/a
LAKE HURON Smallmouth bass	n/a	n/a	1.0 (1)	n/a
LAKE HURON Walleye	2.1 (30)	n/a	n/a	n/a
LAKE HURON Yellow perch	n/a	n/a	0.5 (2)	n/a
LAKE ONTARIO Coho salmon	n/a	n/a	1.6 (3)	n/a
LAKE SUPERIOR Coho salmon	n/a	n/a	2.8 (2)	n/a
LAKE SUPERIOR Northern Pike	n/a	n/a	1.4 (1)	n/a

Legend: n/a = no analyses performed
N = Number of samples analyzed. Some may be composites of multiple fish.

NOTE: Species, size and age, sex, season, lake or sub-basin, and tissue collection type are all important variables that are sometimes correlated with percent lipid. Averages from larger data sets are more appropriate for use in TBP algorithm.

SECTION 7
State Fish Consumption Advisories for the Great Lakes

The Great Lakes States and Canadian provinces have an agreement to work together to develop common fish consumption advice for Great Lakes waters. A technical committee established by the Great Lakes Governors and Premiers Conference meets each year to compile all available data and determine the advice for the coming fishing season. This information is usually published by the Health Departments of each State in April of each year, and is distributed with fishing licenses, posted in appropriate locations, and announced in press releases. Typically, the statewide advisory is prepared by a committee including toxicologists from the human health field, fisheries biologists, and analytical personnel from State laboratories.

Fish consumption advisories are data driven, and subject to change, especially if important new data become available after publication of the year's advisory. They are sometimes supplemented by press releases during the year.

The most recent State advisories for the Great Lakes have been stable for two years. A tabular summary of these advisories for 1991 or 1992 is provided on the following pages. It should be noted that the State advisory documents themselves contain specific information on the species and sizes of fish affected, specific advice on maximum consumption rates for sensitive population groups such as women of child bearing age, and advice on cleaning and preparation of fish prior to consumption.

A list of State points-of-contact for further information on fish advisories is also provided in a table.

Summary of Public Health Fish Consumption Advisories
for the Great Lakes

	POLLUTANT(S)
LAKE ERIE BASIN	
Lake Erie (Applies to **Michigan, Ohio, and Pennsylvania** waters)	PCBs, Chlordane
Michigan	
Clinton River (Downstream from Yates Dam, Oakland County)	PCBs, Mercury
Detroit River	PCBs, Mercury
Lake St. Clair	PCBs
River Raison (Downstream from Winchester Bridge, Monroe)	PCBs
Rouge River (Middle Branch downstream from Phoenix Lake and Main Branch downstream from M-153/Ford Road)	PCBs
Rouge River, Lower Branch (Wayne County)	PCBs
St. Clair River	PCBs, Mercury
Ohio	
Ottawa River - Toledo, State Route 23, Route 475 to Lake Erie - 19 miles	PCBs
Black River - 31st Street Bridge (Lorain) to Harbor 6.2 miles (includes Continued Disposal Facility)	PAHs
Ashtabula River - 24th Street Bridge to Ashtabula River Mouth (includes Harbor area within breakwater - 2.3 miles	PCBs, Hexachlorobenzene Pentachlorobenzene Tetrachlorobenzene
LAKE HURON BASIN - MICHIGAN	
Lake Huron	PCBs
Au Sable River at Oscoda (Losco County)	PCBs
Cass River (Downstream from Bridgeport)	Dioxin
Cheboygening Creek (Saginaw County)	PCBs
Kawkawlin River (Bay County)	PCBs
Pine River (Downstream from St. Louis, Gratiot, and Midland Counties)	PCBs
Saginaw Bay	PCBs
Saginaw River (Entire Length)	PCBs, Mercury
St. Mary's River	Mercury
Shiawassee River (Byron Road to Owosso)	PCBs
Shiawassee River, South Branch (M-59 to Byron Road)	PCBs

Thompson Lake (Livingston Co.)	PCBs, Mercury
Thunder Bay (including Thunder Bay River to first dam.)	PCBs
Tittabawasee River (Downstream from Midland)	PCBs, Dioxin
LAKE MICHIGAN BASIN	**POLLUTANTS**
Lake Michigan: Wisconsin, Michigan, Illinois, & Indiana Waters - Tributaries	PCBs, Mercury, Chlordane
Michigan	
Bear Lake (Muskegon County)	PCBs, Mercury
Black River downstream from South Branch and South Branch downstream from Breedsville Dam (Van Buren Cty)	PCBs
Escanaba River (Between Dam 1 and Dam 2, Delta County)	Dioxin
Glen Lake (Leslanau County)	Mercury, Chlordane
Grand River (Clinton County)	PCBs
Green Bay (South of Cedar River, applies to Michigan and Wisconsin Waters including Menominee, Oconto, and Peshtigo Rivers from mouth to first dam)	PCBs
Hersey River (Downstream from Read City)	PAHs
Kalamazoo River (Downstream from City of Battle Creek to Morrow Pond Dam, Kalamazoo County)	PCBs
Kalamazoo River (Downstream from Morrow Pond Dam to Allegan Dam) and Portage Creek (Downstream from Monarch Mill Pond, Kalamazoo County)	PCBs
Kalamazoo River (Downstream from Allepan Dam to Lake Michigan, Allegan County)	PCBs
Lake Macatawa (Ottawa County)	PCBs, Mercury
Lake Michigamme, Michigamme Reservoir, Peavy Pond, Paint River Pond, and the Michigamme River System to its junction with the Menominee River	Mercury
Little Bay de Noc (Lake Michigan)	PCBs, Mercury
Manistique River (Schoolcraft County Downstream from M-94/Old U.S.2)	PCBs
Menominee River	Mercury
Mona Lake (Muskegon County)	Mercury, Dioxin
Net River (Iron County)	PCBs, Mercury
Round Lake (Marquette County)	Mercury
St. Joseph River (Downstream from Barrian Springs Dam)	PCBs

White Lake (Muskegon County)	PCBs, Mercury, Chlordane
Wisconsin	
Menominee River from Pier's Gorge through Sturgeon Falls Flowage Menominee River at Lower Scott Flowage	Mercury
Peshtigo River at Peshtigo Flowage from its mouth at Green Bay up to the Peshtigo Dam	Mercury PCBs, Pesticides
Lake Michigan Basin Tributaries (Continued)	**Pollutants**
Lower Fox River from its mouth at Green Bay up to the Peshtigo Dam	PCBs, Pesticides
Lower Fox River from the DePere Dam up to the Neenah-Menasha Dam	PCBs, Pesticides
East and West Twin Rivers from their mouths up to the first dam **NOTE:** Follow Lake Michigan advisory above for trout and salmon.	PCBs, Pesticides
Manitowoc River from its mouth up to the first dam.	PCBs, Pesticides
Sheboygan River in Sheboygan County from the dam at Sheboygan Falls to the Coast Guard station in the City of Sheboygan, including **Greendale and Weedens Creek**	PCBs
Milwaukee River in Milwaukee County (includes Milwaukee Harbor) from its mouth up to the North Avenue dam, including the Kinnickinnic and Menomonee Rivers **NOTE:** Follow Lake Michigan advisory.	PCBs
Milwaukee River from the North Avenue dam in Milwaukee County upstream to the Lime Kiln Dam at Grafton (Ozaukee County)	PCBs
Cedar Creek from the Milwaukee River up to bridge Road in the Village of Cedarburg including Zeunert Pond	PCBs
Root River in Racine County from its mouth upstream to the Horlick Dam in the City of Racine	PCBs
Pike River in Kenosha County from its mouth up to Carthage College in the City of Kenosha	PCBs
Indiana	
Grand Calumet River, East & West Branches and Indiana Harbor Ship Canal	PCBs, Dioxin
LAKE SUPERIOR BASIN	
Lake Superior, Minnesota, Wisconsin, & Michigan Waters	PCBs, Mercury, Chlordane, Toxaphene
Michigan: Dear Lake, Carp R., & Carp Cr. (Marquette County)	Mercury
Torch Lake (Houghton County)	Mercury, Fish Tumors, Course Unknown

Minnesota: St. Louis River, Fond du Lac to the mouth, including Superior Harbor	Mercury, PCBs, Dioxin
LAKE ONTARIO BASIN (New York State)	
Lake Ontario and Niagara River	PCBs, Mirex, Dioxin
Buffalo River and Harbor (Erie County)	PCBs
Oswego River (Power dam in Oswego to Fulton dam)	PCBs
Salmon River (Mouth to Salmon Reservoir, Oswego County)	PCBs
St. Lawrence River (entire river)	PCBs, Mirex, Dioxin

State Points-of-Contact for Information on Fish Consumption Advisories

Agency	Point of Contact	Phone Number
Illinois Dept of Health Illinois EPA	Tom Long Tom Hornshaw	217-782-5830 217-785-0832
Indiana Dept of Health Indiana DEM	Greg Steele C. Lee Bridges	317-633-8554 317-243-5030
Michigan Dept of Public Health Michigan DNR	John Hesse Chris Waggoner	517-335-8350 517-335-4189
Minnesota Dept of Health Minnesota PCA	Pam Shubat Marvin Hora	612-627-5059 612-296-7250
New York Dept of Health New York DEC	Tony Fort Larry Skinner	518-458-6409 518-457-1769
Ohio Dept of Health Ohio EPA	Tracey Shelly John Estenik	614-466-1060 614-644-2866
Pennsylvania DER Pennsylvania Fish Commis	Robert Frey Dave Wolf	717-787-1783 717-657-4518
Wisconsin Dept of Health Wisconsin DNR	Henry Anderson Jim Amrheim	608-266-1253 608-266-1253

GREAT LAKES
DREDGED MATERIAL TESTING AND EVALUATION MANUAL

APPENDIX D
SEDIMENT SAMPLING & HANDLING GUIDANCE

prepared by:

Jan A. Miller
USACE, Great Lakes & Ohio River Division

John L. Dorkin
USEPA, Region 5

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ATTACHMENTS

1. APPLICABILITY

This appendix provides recommended procedures for the collection and handling of bottom sediments for chemical, physical and biological testing. Bottom sediments may be sampled and tested for a variety of purposes. The guidance provided here is directed toward a contaminant determination as part of the evaluation conducted in relation to Section 404(b)(1) of the Clean Water Act. These guidance and procedures may not be fully applicable to other study or project purposes. In addition, the sampling methods discussed here are appropriate for Great Lakes tributaries and nearshore areas. Procedures for deep-water sediment sampling are not discussed because these areas are not usually sampled as part of a 404(b)(1) evaluation.

A number of references are available which discuss sediment sampling procedures, including: USEPA (1977); International Joint Commission (1987 and 1988); Lowe and Zaccheo (1991); Mudroch and MacKnight (1991), and; ASTM (1991). These references should be consulted if the guidance provided here is not suitable for study or project purposes.

This appendix will first discuss the planning and design of a sediment sampling program. Sampling equipment, supporting equipment, and handling procedures will then be discussed.

2. PLANNING AND DESIGN

Prior to any field activities, sound planning is necessary to determine the type, number, location, and size of samples to be collected, and to assure that the samples are not altered, biased or contaminated in a way which would invalidate their use. The planning of a sediment sampling program should logically flow from the results of the tiered evaluation outlined in the regional guidance manual. Using the tiered testing approach, sediment and water samples may need to be collected on more than one occasion. The results of historic data compiled during Tier 1 may serve as the foundation for the design of a sampling program for Tier 2 testing. The results of Tiers 1 and 2 would direct the focus of sampling for Tier 3 tests, if necessary.

2.1 Objectives

The first, and perhaps most important step in developing a sediment sampling plan is to define the objectives, which are a function of the types of information needed and the decisions to be made with that information.

The information obtained from a sampling plan for a 404(b)(1) evaluation is used to evaluate potential contaminant impacts from the discharge of dredged material. However, the type of information needed for this evaluation may differ from tier to tier. In many cases, these differences result in different number of samples, sampling locations and sampling methods for each tier. For example, a few composited grab samples may be suitable during Tier 1 to confirm the applicability of an exclusion or to help develop a contaminant of concern list, but might not be appropriate for a Tier 2 or 3 sampling plan.

Data quality objectives (DQOs) are qualitative and quantitative statements of the overall uncertainty a decision maker is willing to accept in results or decisions derived from environmental data. A qualitative statement of the DQOs for contaminant determinations as part of a Great Lakes 404(b)(1) dredged material evaluation is provided in Appendix E.

In summary, the objectives of a sediment sampling plan should address the type of information to be obtained, the decisions that will be made with that information, and level of uncertainty that is acceptable for those decisions. These objectives should be elaborated in the written sampling plan.

2.2 Information Gathering

The types of data that should be compiled prior to initiating the sampling plan includes:

- proposed dredging depths and locations,
- water depths and level fluctuations,
- obstructions (bridges, pipelines, ships, etc),
- access sites for mobilizing equipment,
- sources of contaminants (point and non-point),
- navigation use (commercial and recreational),
- hydraulic/other factors affecting sediment distribution,
- historic sediment quality data,
- survey benchmarks for referencing elevations at sampling locations,
- landmarks for referencing sample locations, and
- emergency assistance (Coast Guard, hospitals, etc.).

Most of the above information should have been compiled as part of the Tier 1 evaluation. Many site-specific factors will affect where and how sediment samples need to be collected. The complexity of the sampling plan will mirror the complexity of the anticipated sediment contaminant distribution. If the dredging area has few sources of sediment contamination or a very predictable contaminant distribution, the sampling layout may be

relatively uncomplicated with focus at a single or a few reaches or zones. However, if there is a complex set of sediment contaminant concerns throughout a dredging area, the sampling layout may be complex as well.

2.3 Management Units

In an ideal situation, all types of information would be available on every grain of dredged material. Due to to costs of sampling and testing, this is impractical. We must therefore attempt to make the best use of finite resources in evaluating the contaminant potential of dredged material. The recommended method is to "focus" the sampling and analysis in a series of steps, consistent with the tiered approach. This method begins by characterizing the dredged material at a large number of locations, using "coarse" (and inexpensive) analyses. Successive steps employ more sophisticated (and expensive) analyses at fewer locations.

Every sediment sample will represent some larger area or volume in the evaluation. It is recommended that the area or volume represented by a sample be defined as part of the planning process, prior to field activities, where practical. This should enhance the objectivity of the evaluation and interpretation of data.

A **management unit** is defined in the Inland Testing Manual (USEPA/USACE, 1998) as a "manageable, dredgeable unit of sediment which can be differentiated by sampling and which can be separately dredged and disposed within a larger dredging area". It is a spatially-defined volume of sediment located in a proposed dredging area for which the test results from a single sample (or composite) will be used to make a management decision about dredging or disposal. The management unit is therefore a decision unit.

In the case of a 404(b)(1) contaminant determination, the decision that needs to be made is whether or not open-water disposal of the volume of sediments within the management unit would be in compliance with the Guidelines with respect to sediment contamination. Two factors will be used to delineate management units; constructability and homogeneity.

A management unit must be constructible. That is to say that it must be practicable for normal dredging and disposal operations. A management unit could be as large as the entire dredging project area, or it could be a small portion of that area. A management unit should not be so small that it could not be dredged separately from other units. A management unit must also be constructible under a negative determination. That is,

it must be possible to dredge other units and leave the one behind.

The homogeneity of the proposed dredged material, both physically and chemically, is the other factor to be used to delineate management units. Within a limited geographic area of a single waterway, it is reasonable to assume that sediments having similar physical and chemical characteristics would have similar potential for contaminant impacts. Although no predictable correlation between sediment chemistry and benthic or water column toxicity has been scientifically proven, the homogeneity of sediment physical and chemical properties is recommended as a reasonable basis for delineating management units and distributing sample locations.

2.4 Management Unit Delineation

Only a few generalizations about the appropriate size, number and distribution of management units can be made. The delineation of management units is very site specific, and should consider all available information. Ultimately, the decision relies on best professional judgment.

A subset of the information gathered about the dredging site should be considered in laying out the management units:

- proposed dredging depths and locations,
- water depths and seiche/tidal fluctuations,
- sources of contaminants (point and non-point),
- hydraulic/other factors affecting sediment distribution,
- and historic sediment quality data.

The first step is to map out the proposed dredging area. This is often not one contiguous area, but a number of shoals with varying surface areas and thickness. An example is shown on figure D-1. For a 404(b)(1) evaluation, sampling should be limited to the area to be dredged.

Information about the locations of known or suspected sources of contamination, factors affecting the movement of sediments and contaminants, and any historical sediment quality data can be used to estimate patterns of contaminant distribution in the proposed dredged material. The distribution of sediment contaminants in a riverine setting is generally more predictable than in the harbors and marinas at the mouths of Great Lakes tributaries. In the former case, sediment contaminants tend to be more spatially linked to specific sources of pollution. In the later, the contaminant distributions are complicated by the natural mixing of fluvial sediments from the river with littoral drift sediments moving along the near shore lake.

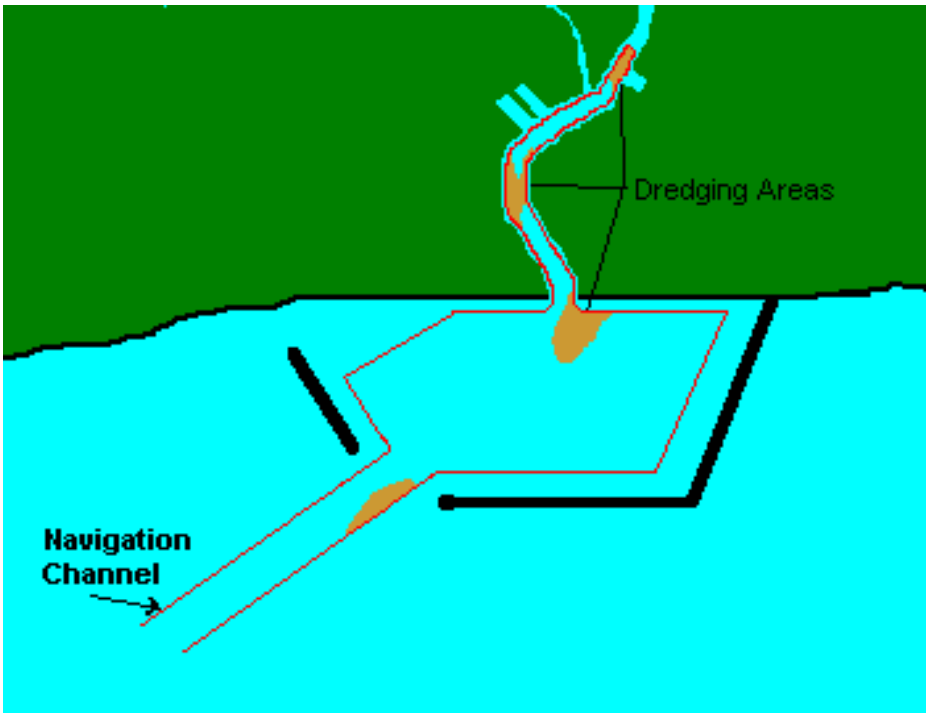


Figure D-1. Hypothetical Dredging Areas

The physical and chemical characterization of sediments is closely interrelated, and the distribution of many contaminants often parallel the distribution of sediment physical characteristics. All other factors being equal, the most likely place to find elevated levels of contaminants are at locations having fine-grained sediments with higher levels of organic matter. A knowledge of the principles of sediment transport combined with information about the hydraulics of a waterway can help identify portions of a proposed dredging area with sediments most likely to have the highest levels of contamination.

In cases where there is little or no existing physical or chemical data on a proposed dredged material or disposal site sediment, a visual survey of sediments from the area, collected with a grab sampler can yield highly valuable and inexpensive information. Field observations of sample odor and visual characteristics (see section 4.3), together with laboratory analysis of sediment grain size distribution (sieve analysis) and organic content (total volatile solids or total organic carbon) are quick and reliable indicators of the distribution of sediment contamination within a given area.

If the sediments are believed to be relatively homogeneous, management units should be delineated in a fashion that divides the dredging area into units of approximately equal volumes. If there is less historic data in one portion of the dredging area than others, or if existing information suggests that there is a

greater probability for contamination in one portion of the dredging area than others, it is appropriate to delineate smaller management units in these areas. If there are known or suspected patterns of physical or chemical characteristics of sediments in the dredging area, it is appropriate to delineate management units in line with these patterns.

In some areas, the physical and chemical characteristics of sediments may change with depth. This is common in navigation projects that have not been dredged in many years or in areas of a waterway which took many years for a deposit to accumulate. In these cases, the sources of contamination may have changed or been eliminated, and less contaminated sediment deposits overlay older sediments with higher levels of contamination. In some projects involving "new work" dredging, a different pattern of vertical stratification can be found where surficial deposits of "recent", more contaminated sediments overlay uncontaminated deposits of sand or clay laid down in preindustrial times.

Sediment deposits which accumulate rapidly are less likely to have significant vertical stratification of contaminants. Areas that are routinely dredged every few years should, in most cases, need not be vertically divided into more than one management unit. In cases where there are suspected vertical patterns of sediment physical or chemical properties, it may be practical to consider different disposal alternatives for different layers or strata. Such sediment layering may form the basis of management unit delineation. For example, if the area to be dredged had a deposit of unconsolidated silty sediments overlying an older deposit of compacted sand, management units could be divided vertically at the interface of the deposits.

The size and delineation of management units should finally be checked for constructability. A management unit should not be less than 2 feet in thickness, which is the practical limit of accuracy for many dredges in open water. It should be remembered that if a management unit is determined to be unsuitable for disposal at the proposed disposal site, it may then represent a volume or area that is left undredged, or perhaps dredged and disposed at a different time. The delineation of management units should therefore consider the consequences of a negative or mixed determination as well as a positive one.

2.5 Sampling Plan for the Dredging Site

The type, number, and location of sediment samples are determined concurrently with the management unit delineation. In most cases a management unit is represented by a single sample. This may be a discrete sample collected at one location within the management unit or a composite of samples collected at

several locations or depths. Because the results of analyses are intended to characterize the entire management unit, compositing is recommended, where practicable, especially for large management units. Sampling equipment, handling and compositing procedures are discussed in more detail later in section 3.

There are two general types of sediment samples; grab and core. Grab samples are collected from the sediment surface and core samples may be collected from depths within the sediment deposit. The collection of grab samples requires less supporting equipment, and generally costs much less than core samples.

Sediment deposits that are a foot or two in thickness can usually be sampled using grab sampling equipment. Thicker deposits that have accumulated rapidly, or have existing information suggesting that they are vertically homogeneous may also be sampled as surface grabs. Core sampling should be used in deposits where there are known or suspected vertical trends in sediment physical or chemical properties or if there is more than one management unit vertically.

It is recommended that grab samples comprise a composite of two or more grabs (of approximately equal volume) collected at locations within the management unit. Care must be exercised in collecting grab samples to avoid the loss of fine-grained sediments and the introduction of sampling bias. Large grab samplers (winch operated) and small clamshell dredge buckets (crane operated) will often obtain better sample recovery and deeper sample penetration than small, hand-held grab samplers.

Core samples should be collected for the entire thickness of the management unit, where practicable, and this length composited. Compositing of multiple cores within a management unit is desirable, but is often prohibitively expensive.

Dredging site water samples must be collected in order to prepare the elutriate as part of Tier 2 testing. Unless there is a significant difference in the quality of water at sediment sampling stations within the dredging area, in particular, differences in pH and redox potential, a single sampling location may be used for water collection. Alternatively, water may be collected from several sites and composited.

2.6 Sampling Plan for the Disposal Site

The sediments that are to be dredged occupy a three-dimensional space, and the management units used to divide the dredging area are therefore three-dimensional. The disposal site, on the other hand, is only two-dimensional from a 404(b)(1) evaluation point-of-view. When we compare the sediments from the

disposal site to the dredged material, we are comparing the existing sediment surface of the disposal site to a proposed future sediment surface, covered by the dredged material. For this reason, the disposal site should always be sampled with a grab sampler.

The homogeneity of sediments at the disposal site may be less predictable than those at the dredging area. It is therefore advisable that a number of grabs be collected for visual inspection before collecting the samples to be kept for analyses. As stated above, care must be exercised in collecting grab samples to avoid the loss of fine-grained sediments and the introduction of sampling bias. In many areas of the nearshore lake, sediments may be more compacted and consolidated than the sediments in the dredging area. As a result, sample recovery may be poor. The use of different sampling equipment at the dredging area and disposal site may be considered to overcome this type of problem.

It is not appropriate to divide the disposal site into management units, since the term has no meaning here. The number and distribution of samples collected at the disposal site is dependent on the homogeneity of the sediments and the importance of accounting for the natural variability of sediments at the disposal site by the evaluator. For most contaminant determinations, the disposal site is represented a single, composite sample. Where there is considerable heterogeneity in surficial sediments at a disposal site, a single composite sample may not reflect this variability. In order to capture this variability, at least three samples would be needed. If a multiple disposal-site sediment is to be utilized, the evaluator should coordinate with the appropriate agencies in advance as to how the disposal site sediment results will be treated statistically.

The location of sediment sample(s) within the disposal site need not be highly systematic, but care should be taken to avoid non-representative samples. In most cases, the disposal site will be in the nearshore lake. In these areas, the positioning of the sampling vessel may be approximate and maintaining the vessel at a fixed location for extended periods difficult. Using a marker buoy to designate the center of the reference site, samples can be collected at distances and bearings from the marker.

2.7 Sampling Plan Documentation

A written plan for sediment sampling should be prepared and should include the following information:

- map of area to be dredged showing the delineation of management units, proposed sampling locations, and bathymetry,
- rationale for management unit delineation,
- map of disposal site showing proposed sampling locations,
- proposed sampling methods and equipment,
- proposed supporting equipment, vessels, and methods for positioning laterally and vertically,
- proposed logistics for access/mobilization,
- proposed sample compositing, handling and transport,
- identify personnel and contractors who will implement sampling and/or provide equipment,
- QC/QA provisions, and
- health and safety provisions.

This sampling plan can be used for a number of project purposes, including:

- interagency coordination,
- scope of work for contract or in-house,
- part of the Quality Assurance Project Plan (QAPP), and
- part of final report on sediment sampling and analysis.

The sampling plan should be one of the first documents used to initiate interagency coordination for a proposed dredging/disposal project. Without a comprehensive sampling plan, other agencies would not be able to provide comments prior to sampling or adequately evaluate the results upon completion.

The sampling plan will be the scope of work for those who are to implement the sampling, whether through contract or with in-house labor. The sampling plan may prepared as part of a larger document which covers both sampling and laboratory analysis. This is advantageous if the entire effort is to be performed by a single contractor.

The sampling plan is an integral part of the Quality Assurance Project Plan (QAPP), as discussed in Appendix F. The field manager who directs the execution of the sampling program must have read, understood, and signed the QAPP before initiating sampling.

Finally, any sampling program must be flexible to allow for changes based on field conditions. Sampling locations are often changed in the field, and any modifications to the plan should be documented. The plan, along with all field notes or logs should become a part of the final report of sampling and testing.

2.8 Coordination

Interagency coordination on sediment sampling, testing and evaluation should begin as early in the planning process as possible. Coordination during Tier 1 can help identify additional historic sediment data, sources of contamination, and contaminants of concern. Coordination during the development of sampling plans will assure that all agencies understand the reasoning behind the plan, promote cooperative sampling efforts, and reduce the potential for disagreements over the interpretation.

Project proponents are encouraged to use the written sampling plan, discussed in section 2.7, as a basis for coordination with Federal and state agencies prior to field sampling. Given the potential costs of having to repeat sampling and analysis, and the consequences of delays to project schedules, proponents of dredging projects take a serious risk in proceeding with sediment sampling without adequate interagency coordination.

3. SAMPLING EQUIPMENT

There are three types of equipment generally needed to collect sediment samples: a sampler, a mechanism for holding, driving or lifting the sampler, and a floating platform to work from. Sediment sampling can be as simple as scooping a shovel into a shallow creek by hand or as complex as driving Teflon lined Shelby tubes from a truck-mounted drill rig on a spud barge in 25 feet of water. The size of a sampling operation can be one person or a crew of four or five. Equipment costs can range from nothing to $10,000 per day. This section will discuss the available equipment for sediment sampling and provide guidance on where it may be appropriate.

3.1 Sediment Samplers

There are two basic types of sediment samplers; grab samplers and core samplers. Both types of sampling devices can vary considerably in size and degree of difficulty in deployment. The selection of which type and size of sampling device is, like other aspects of the sampling plan, project-specific. The features of sediment samplers commonly used in the Great Lakes are summarized on table D-1.

Table D-1. Features of Sediment Sampling Equipment

Sampler Type	Applicability	Penetration and Recovery	Sample Volume[1]	Supporting Equipment	Cost (new)
Hand held grab	Surface grabs in shallow depths, all sediments	Penetration controllable, recovery usually good	<2 liters	None, except w/ divers	
Drag line	Surface grabs in shallow depths, hard or compacted sediments	Shallow penetration	<1 liter	Small boat	
Small dredge	Surface grabs in all depths, all sediments	Penetration and recovery vary with sediment	1-2 liters	Small boat, winch	$200-700
Clamshell bucket	Surface grabs in all depths, all sediments	Penetration of 1' or more, even in compacted sediment	>100 liters	Floating plant, crane	
Hand held corer	Cores in shallow depths, soft sediments	Penetration controllable, recovery variable	1-2 liters	Pontoon boat or barge	
Gravity corer	Cores in all depths, soft sediments	Penetration and recovery vary with sediment	1-2 liters	Small boat with winch	
Box core	Short cores in all depths, all sediments	Shallow penetration, recovery usually good	variable	Boat with winch	
Vibracore	Cores is depths less than 30', soft sediment	Penetration controllable, recovery usually good	variable	Floating plant & drill rig	
Split spoon	Cores in depths less than 30', all sediments	Penetration controllable, recovery variable	1-2 liters	Floating plant & drill rig	
Piston tube	Cores in depths less than 30', all sediments	Penetration controllable, recovery good.	1-3 liters	Floating plant & drill rig	

[1]Volume of sampler with good recovery.

3.1.1 Sampler selection

In comparing the different types of grab and core samplers, and selecting the one most appropriate for a particular application, the primary factors to consider are:

- supporting equipment requirements,
- physical restrictions,
- depth of sample (penetration)
- sample recovery,
- sample bias,
- sampler material, and
- sample volume.

Supporting equipment requirements: The type and size of supporting equipment needed for sampler operation may determine the feasibility of operation, and will greatly affect sampling costs. Supporting equipment are described further in section 3.2.

Physical restrictions: Physical restrictions which might limit the operation of a sampler (and supporting equipment) include the water depths, currents/tidal/wave conditions, and sediment characteristics.

Sample penetration: The depth from which the sample is collected is determined by the depth of sampler penetration. For some samplers, this depth may be controlled. With other samplers, this depth is dependent on the type and size of sampler used, water depth, and consistency (soft/hard) of the bottom sediments.

Sample recovery: Recovery is an indication of how much sample is present in the sampler, and is usually estimated as a percentage, with a full sampler being 100% recovery. Poor recovery can result from the sampler failing to close properly or sample loss during lifting.

Sample bias: Sample bias is a significant concern, especially for samples that have poor recovery. As the sampler is pulled up, sample may be lost to the water column through the sampler screen or if the sampler is not fully closed. Fine sediment particles are most susceptible to loss, and this preferential loss may bias the sample.

Sampler materials: Consideration must be given to the contaminating properties of the sampling devices themselves. Often there will be conflicting requirements for different test parameters. The general rule is that for metals analysis, samples should not contact metal samplers or containers and for trace organic analyses, samples should not contact any plastics. These general rules are not always practical to apply and may not

be necessary for the data quality objectives of 404(b)(1) evaluations.

Since the 404(b)(1) contaminant determination does not rely on the mere presence of contaminants (which may have come from sampler materials) for final decisionmaking, samplers made of (or lined with) stainless steel, aluminum, Teflon, and high density polyethylene (HDPE) plastics should be acceptable for use. Samplers made of other material may also be suitable if the sample not in contact with the device can be selectively removed. All samples should be collected with the same sampler materials where possible. The use of different samplers for different analysis might complicate the interpretation of results.

Sample volume: The volume of sediment needed will vary with the test requirements. A summary of the sample volumes required for the tests described in the Great Lakes Dredged Material Testing and Evaluation Manual is provided on table D-2. The approximate volume of sample provided by full (100% recovery) samplers are listed on table D-1.

Table D-2. Sample Volumes Required for Analyses

Analyses	Sample Volume	
	Sediment	Water[1]
Sieve analysis	0.5 liter	
Hydrometer analysis	0.5 liter	
Bulk chemistry[2]	0.5 liter	
Elutriate	1 liter	4 liters
Column settling test	40 liters	
Water column toxicity		
Whole sediment toxicity		
Bioaccumulation		

[1] Site water required for elutriate test. Other tests can use laboratory water.
[2] Volume shown for analysis of metals, nutrients, PCBs and PAHs. Larger volumes may be needed for analysis of other parameters or lower detection limits.

3.1.2 Grab samplers

A grab sampler is any type of device that collects a disturbed sample at the sediment-water interface. A "disturbed" sample is one that has lost its vertical and lateral integrity and can't be subdivided into meaningful layers or fractions (as can some core samples). Grab samples are collected at the sediment surface, and represent the depth of sediment penetrated by the sampler.

Hand-held samplers: Shovels, trowels, and buckets can be used to collect sediment samples by hand in shallow streams. Sediment sampling in deeper waters by divers using hand-held samplers is becoming a fairly common practice. Hand-held grab samplers are inexpensive, require little or no supporting equipment, can control sample penetration to a limited extent, and generally have good recovery.

Drag-line samplers: Samplers have been developed which are operated by dragging along the bottom. These type of samplers include bottom dredges equipped with nets for collecting biological materials. A pipe dredge is a metal tube, about 6" in diameter and 18" long, which is used to collect surface samples from hard, rocky surfaces. This type of sampler may be more suitable for the disposal site than the dredged material.

Small dredge samplers: There are a number of small, light-weight dredge samplers available from commercial sources. Comparisons of these types of grab samplers are provided by Mudrock and MacKnight (1991), ASTM (1984), Elliott and Drake (1981) and Sly (1969). Some of these samplers come in several sizes. For example, the Ponar petite sampler (6" x 6") weighs about 25 pounds and can be operated by hand from a small boat. The Ponar (9" x 9"), shown on figure D-2, weighs about 50 pounds and needs a boat with a winch and cable for operation.

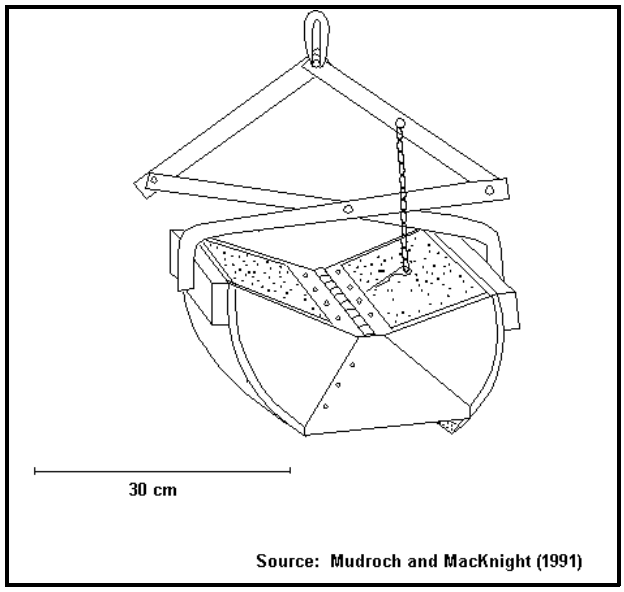


Figure D-2. Ponar Dredge Sampler

Most small dredge samplers will only penetrate 1-3 inches in sandy sediments. The same sampler might penetrate 6-12 inches in fine-grained sediments that are soft and unconsolidated. Sediments that have a hard, consolidated surface often give poor

recovery and may be subject to sample bias. Soft sediments will often yield 100% recovery with grab samplers. Dredge samplers typically cost $200-700.

Clamshell dredge bucket: Although not designed for sampling, commercial clamshell dredges (0.5-3 cubic yard bucket) can also be used for collecting sediment samples. Clamshell buckets are operated by a crane, and require a sizable floating plant (figure D-3). The bucket is typically lowered onto the deck of the floating plant, and sample(s) removed with shovels or trowels. A crane operated clamshell bucket can penetrate several feet, even into compacted sand. Recovery is usually good. Sample bias can be avoided by compositing several subsamples from different areas within the bucket grab.

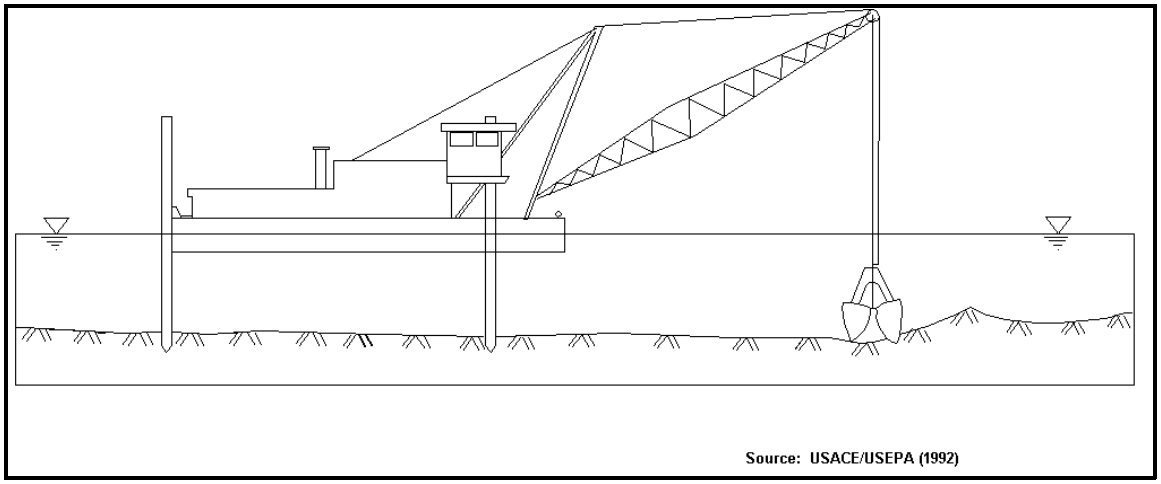


Figure D-3. Clamshell Dredge on Spud Barge

The clamshell dredge bucket will provide far more sample than is necessary for sediment contaminant testing for 404(b)(1) evaluations. Multi-purpose sediment sampling may require large volumes. Several hundred gallons of sediment have been collected with clamshell dredges for testing and evaluation for confined disposal and treatability studies on contaminated sediments.

3.1.3 Core samplers

A core sampler is a device that extracts a vertical cylinder of sediments of some length. The core sample may or may not fully retain its integrity. Some types of core samplers are designed to assure the least loss of vertical integrity. For others, some loss of integrity is acceptable. Core sampling equipment that may be used include equipment designed for geotechnical exploration and well construction. In addition, there are several pieces of equipment developed specifically for sampling bottom sediments. The features of the most commonly

used core samplers are summarized on table D-1.

Hand-held samplers: Hand-held core samplers are available from commercial sources. Many laboratories and contractors have fabricated core sampling equipment from lengths of pipe. For some applications, this type of improvised sampler is quite acceptable. The pipe material used should be selected to avoid sample contamination (see discussion in section 3.1.1). Tubes or sleeves of noncontaminating materials are available for commercial corers.

The hand-held core sampler is pushed into the sediments to the desired depth, withdrawn, and the sediments pushed out with a rod, or the pipe or tube cut to expose the sample for removal. Hand-held samplers can by used by wading in shallow streams or by divers. Hand-held samplers should not be operated from a boat, since it is generally necessary that the operator stand to drive and withdraw the sampler. Vessels with a flat deck, such as a small barge, pontoon boat or floating plant are needed to safely support the sampler.

The operation of a hand-held core sampler is limited by the depth of water, sediment characteristics, and sediment thickness. For total depths (water + sediment) greater than 10-15 feet, the length of the sampler becomes unwieldy for hand operation. Hand-held cores can be easily pushed through soft sediments, but are not recommended for consolidated materials. Recovery with hand-held core samplers is variable. A catcher is typically used at the front end of the core to hold the sediments in-place as the sampler is withdrawn. Samplers with an open end can also be "capped" by driving them through the soft sediments and a few inches into hard clay or sand.

The bias of a core sample is related to its recovery. A sample with poor recovery may have preferentially lost sediment from the leading (deeper) end. Hand-held cores may loose some vertical integrity, as sediments may be compressed in the core. A 3-foot drive may yield only 2 feet of sample, even with good recovery. Consequently, hand-held cores are acceptable for vertically composited samples, but vertical subdivision may not reflect the true elevation of sub-samples.

Gravity core samplers: There are a number of commercially available core samplers that are deployed on a cable and penetrate the bottom sediments with only the force of gravity. A summary of available corers is provided by Mudroch and MacKnight (1991) and Sly (1969). Most corers have small diameters (1-2") with variable lengths, and come equipped with additional weights and a catcher. Some have vanes or stabilizing fins. A typical gravity corer is shown on figure D-4.

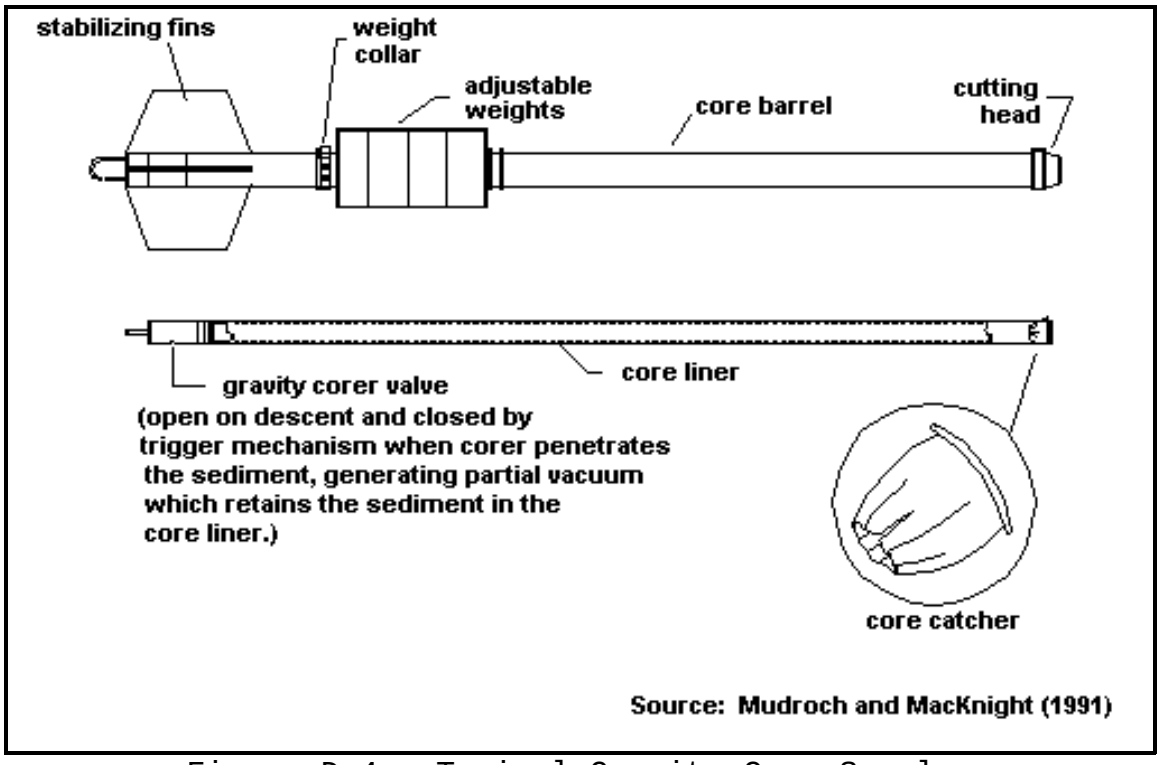


Figure D-4. Typical Gravity Core Sampler

Small gravity corers can be operated from small and medium sized boats by hand or with a winch. Best performance is found where the corer is allowed to freefall between 2-3 meters (Mudroch and MacKnight 1991). Gravity corers can collect up to 2 meters of soft sediments, and are not suitable for hard or consolidated sediments. Sample recovery and vertical integrity are variable.

Box core sampler: Box corers are gravity corers designed for collecting large rectangular sediment cores of the upper 50 cm sediment layer (Mudroch and MacKnight 1991). A typical box corer is shown on figure D-5. Small box corers, weighing about 30 pounds, are equipped with additional weights (up to 100 pounds) to improve penetration. Much larger box corers, up to 2m x 2m and weighing 800 Kg, have been

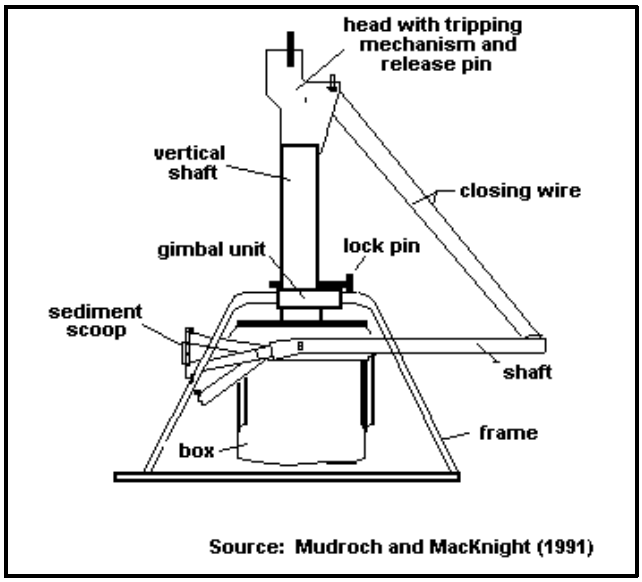


Figure D-5. Box Core Sampler

fabricated (Mudroch and MacKnight 1991). The corer is lowered to the bottom by acable with little freefall, and the triggered with a messenger. Small box corers may be operated from a boat with a winch.

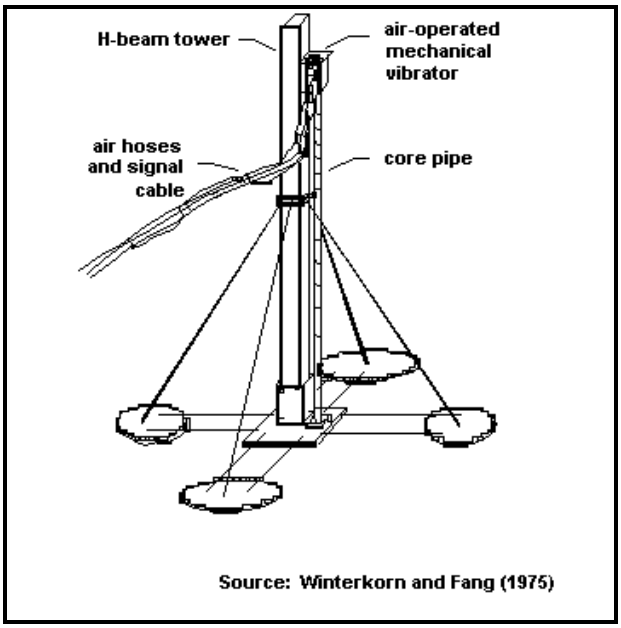


Figure D-6. Vibracore

Vibracore: The vibracore is a long continuous tube that is driven into the sediment using vibrating action, typically of a pneumatic impactor, as shown on figure D-6. The entire core is withdrawn, at which point the entire sample can be extruded and subdivided, or the tube may be cut into segments for sample extraction later. Guidance on the fabrication of a vibracore from readily available components is available in Smith and Clausner (1993). The vibracore can be operated from a small floating plant or barge with a tripod or small derrick and winch to assist in raising and lowering. Vibracores are typically 2-4 inches in diameter. Sample lengths up to 20 feet have been sucessfully removed from sites in Great Lakes tributaries.

The vibracore is only suitable for unconsolidated sediments, particularly sandy sediments. They can not penetrate most consolidated or coarse materials. Cores can be equipped with a catcher or the tube driven into a layer of compacted material, which forms a "cap" at the bottom. The vibration of the tube has been known to consolidate the sample. The vertical integrity of vibracore samples may be disturbed. Vibracores are well suited for the collection of samples to be vertically composited.

Split-spoon: The split-spoon sampler is basic equipment for geotechnical exploration of unconsolidated soils. The sampler is a metal cylinder which is divided in half, lengthwise, as shown on figure D-7. The two halves of the spoon are held together by small pieces of threaded pipe at each end. An open cap, with a catcher is screwed on the tip. The sampler is attached to lengths of steel rod and driven into the sediments with a hammer or weight. After the sampler is withdrawn, the front and rear end pieces are unscrewed, the sampler opened, and the sample removed with a spoon.

Split-spoon samplers can be used for most types of

sediments, including consolidated sand and clay. Recovery is variable, sometimes poorer with soft, fine-grained sediments. Split-spoon samplers are typically 2-3 inches in diameter, and available in lengths from 2-5 feet. Successive vertical samples can be taken by driving casing (typically a 5-inch pipe) and cleaning out the drill hole between samples, as shown in figure D-7. The vertical integrity with of an individual split-spoon sample is variable, but a vertically composited sample can be obtained between two elevations with accuracy.

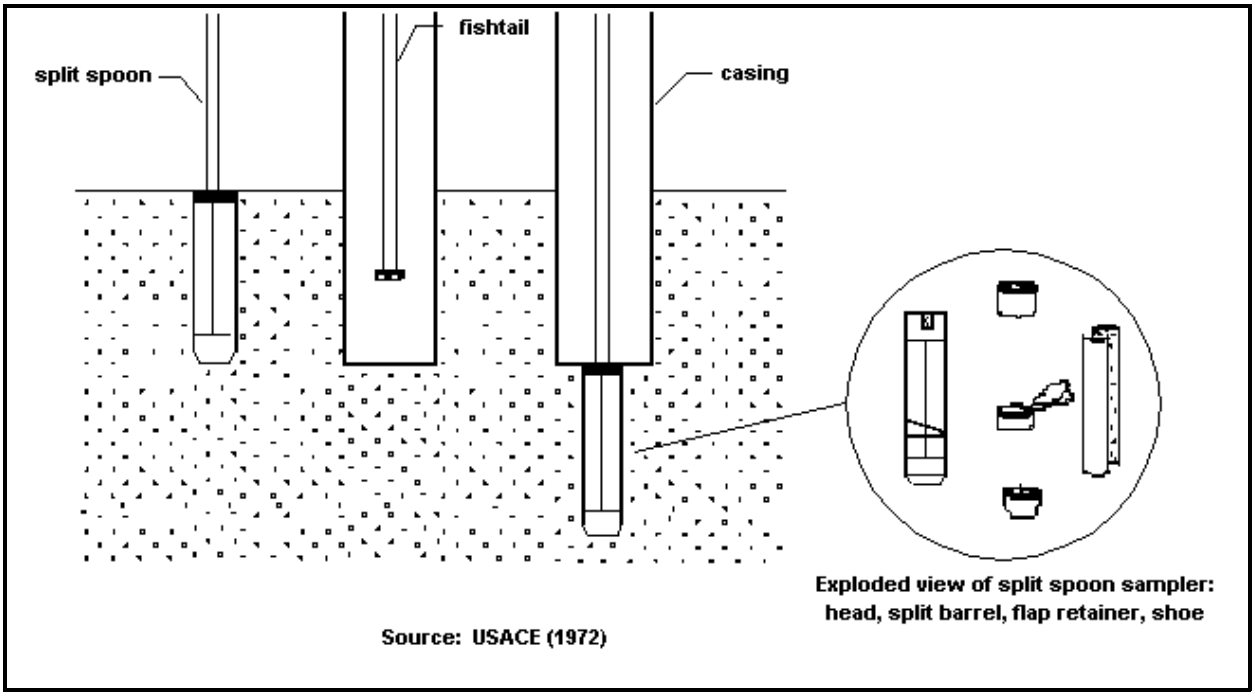


Figure D-7. Split-Spoon Sampler

Piston samplers: There are a number of samplers that use a thin metal tube that is extended forward under hydraulic force. These include the thin-wall stationary piston sampler, Lowe-Acker stationary piston sampler, and the Osterberg (as shown on figure D-8) and McClelland piston samplers (Winterkorn and Fang 1975). Piston samplers can be operated from a variety of drill rigs on small floating plants. The sampler, with tube retracted, is attached to a steel rod and pushed into the sediments to the desired starting depth. The hydraulic force is applied (water pump) and the tube extended. The sediments in the tube are held in a partial vacuum, and the assembly withdrawn. The tube is removed and the sediments extracted.

Piston samplers are suitable for soft, unconsolidated sediments. The sampler can penetrate some consolidated fine-grained sediments, but not coarse materials. Recovery with soft, fine-grained sediments is excellent. Sampler tubes are

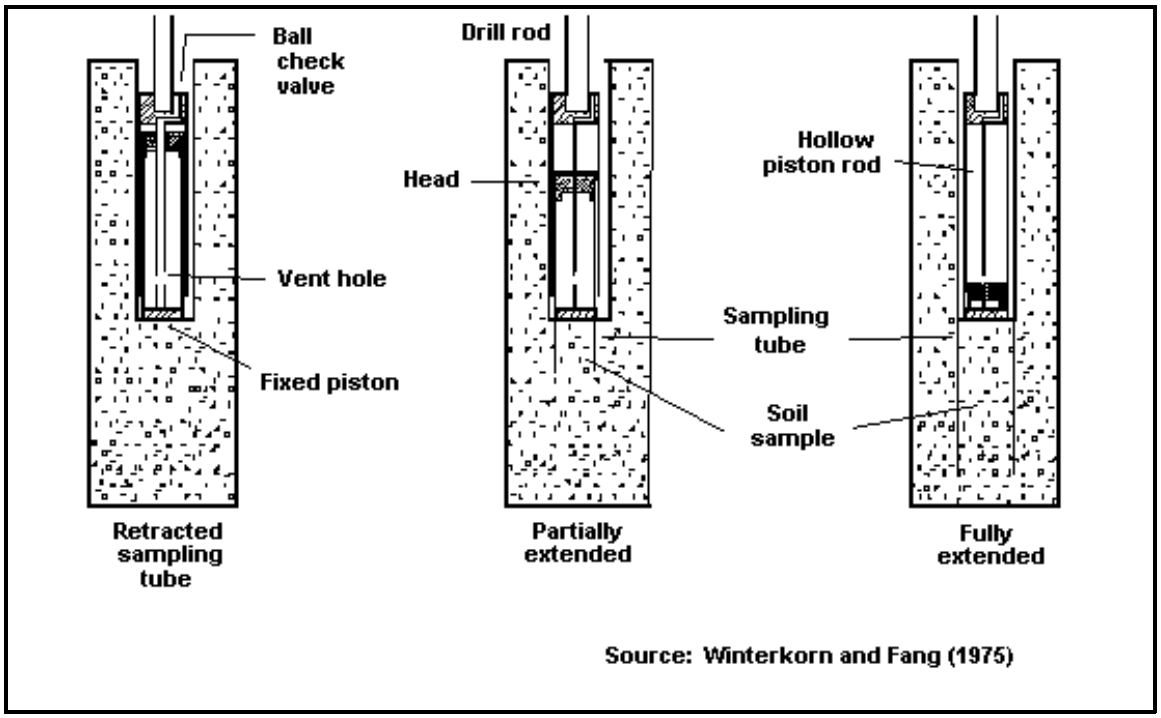


Figure D-8. Piston Sampler

typically 3-4 inches in diameter and 3 feet long. The vertical integrity of individual samples is variable, but a vertically composited sample can be obtained between two elevations with accuracy, and without the need for casing.

3.2 Supporting Equipment

The size and complexity of supporting equipment varies for the different samplers. Most supporting equipment requires experienced operators. Some require a crew of several persons. Information will be provided on marine equipment, cranes and drilling rigs. Information on marine equipment, cranes and drilling rigs needed to support sediment sampling activities is summarized on table D-3.

3.2.1 Marine equipment

Almost every size and type of boat, barge, and floating platform has been used for sediment sampling at one time or another. The suitability of a boat or floating platform is determined by the size and operating requirements of the sampler and the physical restrictions of the sampling site(s). These site restrictions include:

- water depth,
- wave/tidal/current conditions, and
- accessibility.

Table D-3. Supporting Equipment for Sediment Sampling

Equipment	Applicability	Crew[1]	Cost[2] ($/day)
Small boat (<16 ft)	For use with handheld or winch-operated dredge, box, or gravity core samplers. Suitable for shallow conditions.	Operator (1)	$200-500
Large boat	For use with handheld or winch-operated dredge, box or gravity core samplers. Suitable for near-shore conditions.	Operator and mate (1-2)	$400-1,000
Pontoon boat	For use with handheld core, dredge, box and gravity core samplers. Suitable for calm waters only.	Operator (1)	$400-800
Collapsible drill rig	For use with split spoon or piston samplers on small barge.	Driller and helper (2)	$1,000-2,000
Truck-mounted drill rig	For use with split spoon or piton samplers on larger barge.	Driller and helper (2)	$1,000-2,000
Skid-mounted drill rig	For use with split spoon or piston samplers. Suitable for calm waters and moderate depths.	Driller and helper (2)	$1,000-2,000
Crane, 20-ton	For use with clamshell dredge bucket, to mobilize sectional barge, or to lift spuds.	Operator (1)	$500-1,500
Small sectional barge	For use with Collapsible drill rig. May require supporting vessel for propulsion. Suitable for calm waters and moderate depths.	Operator (1)	$400-1,000
Spud barge	For use with truck-mounted drill rig or crane. May require supporting vessel. Suitable for near-shore and depths to 30 feet.	Operator and mate (2)	$1,000-3,000

[1] Crew size of combined equipment may be reduced if crew perform multiple duties. For example, if barge operator also operates crane.
[2] Costs are for planning purposes only.

Equipment availability and cost may also be important factors in the selection of supporting equipment. For example, if a marine construction contractor is already mobilized near the sampling site, it may be more cost efficient to rent the contractor's equipment, even though it is larger than needed for sampler operation. In all cases, safety must be the overriding consideration in the selection of supporting marine equipment.

Boats: Small boats with outboard motors may be suitable for supporting some small clamshell dredge samplers and drag-line samplers in small tributaries and nearshore waters. Larger boats, with an electric or hand-crank winch are suitable for supporting larger clamshell dredge samplers, small gravity core samplers, and small box corers. Pontoon boats are suitable for supporting all grab samplers (except crane-operated clamshell buckets), hand-held cores and gravity and small box cores.

Sampling boats should generally be anchored at stations as best possible for safety and sample collection proficiency reasons. Some grab samplers will not function properly when drift causes them to strike the bottom on an angle less than perpendicular. Anchoring is an especially important safety consideration when divers are operating the sampling devices.

A qualified boat operator and sampler operator are the minimal crew for most small boats. Larger boats, suitable for work in the lake or large tributaries, may require additional crew members.

Floating platforms: A variety of barges, skiffs and marine floating plants can be used for supporting larger sampling equipment. The selection must consider the size and weight of other supporting equipment (crane or drill rig) and the need to be stationary. Some barges and skiffs are self propelled, others require boats or small tugboats for propulsion. Crew sizes range from two to four.

If core sampling equipment is used, it is necessary to keep the sampler position laterally stationary. There are only a few methods of holding a barge, skiff, or floating plant in place at the sampling site. Anchoring is not always reliable in keeping a large vessel in place, except under very calm water conditions. If the sampling location is immediately next to land, the vessel can be tied to available structures.

The most reliable method of stabilizing a barge, skiff, or floating plant is the use of spuds, as shown on figure D-3. Spuds are long steel posts which are lowered into the sediments, typically at each end of the vessel. Some vessels have spuds which are hydraulically lifted, while others have them lifted

with a crane on deck. On some small, sectional, spud barges, the spuds are lifted by hand.

Most small barges and skiffs are transported overland by trailer and moved into and out of the water by a winch or using a crane. Larger barges and floating plants are usually transported to the site by water.

3.2.2 Cranes and drill rigs

Cranes are used to operate clamshell dredge buckets and/or to lift spuds on floating plants. A crane may also be used to place a barge or floating plant into the water.

There are many types of drill rigs used in geotechnical explorations (Winterkorn and Fang 1975), but only the smaller, collapsible varieties shown on figure D-9 have been routinely used for dredged material sampling at sites on the Great Lakes. A drill rig is basically a vertical frame or scaffold used to hold long lengths of pipe or core steady as they are lowered, raised, connected and disassembled.

A-frame and tripod drill rig assemblies are small, collapsible, and can be assembled on very small barges. A truck-mounted drill rig, commonly used in drilling potable water supply wells and installing monitoring wells, has a collapsible rig mounted on a truck. Truck-mounted drill rigs can be driven onto barges or floating plants and chained to the deck. Drill rigs can operate through a hole in the barge or floating plant (if available), or over the side. Small drill rigs are typically operated by a driller and a helper (crew of two).

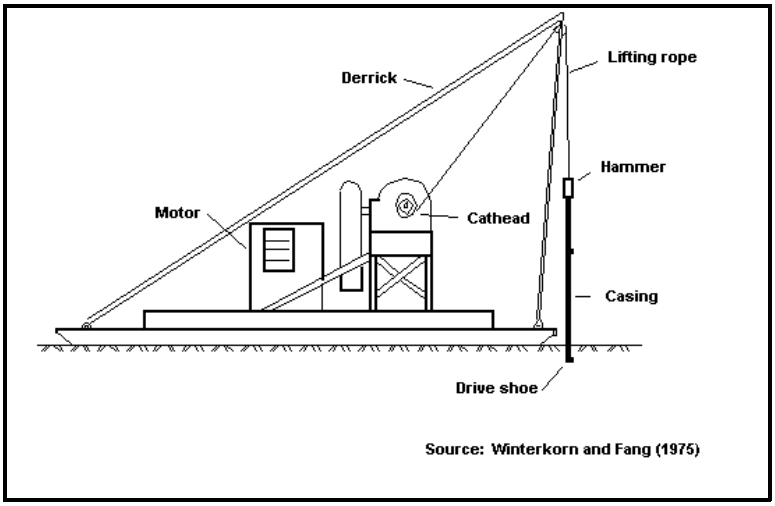


Figure D-9. Collapsible Drill Rig

3.3 Sampling Costs

The primary costs of a sediment sampling program are primarily for labor and supporting equipment. These tend to increase together, as larger equipment generally requires a larger, and more skilled crew. Estimates of the daily rates for

supporting equipment (including crew) are shown on table D-3. These rates should only be used for the initial planning of a sampling project, and will vary with the locale and equipment availability.

A significant amount of the cost involved in sediment sampling is the mobilization and demobilization (setup and breakdown) of supporting equipment. The mobilization cost is determined by how far the contractor has to come with his/her equipment. It is not uncommon to have a mobilization cost equal to 1-2 days effort, and demobilization equal to a one day effort.

The rate at which sample collection takes place is project and equipment specific. If sampling locations are far apart, significant time may be spent in relocating equipment. The positioning of sampling locations may also require additional time (see section 4.1). After the site has been reached and positioned properly, grab samples can usually be collected in 5-15 minutes. Core samples of a continuous length can be collected in 15-60 minutes. Core samples collected at three depth intervals might take 1-3 hours. After the sample(s) have been collected, additional time is required to visually inspect and characterize them, and prepare them for storage. It may or may not be feasible to do this on the way to the next station.

For most projects, once the equipment has been mobilized, the cost of collecting additional samples can be relatively inexpensive. The collection of extra samples at planned sampling locations, or at different sampling locations can provide a valuable contingency in the event of sample loss or anomalous sample results. On practice is to collect extra samples and make provisions with the laboratory to store them for a limited time and dispose of them if not needed.

4. GUIDANCE ON FIELD ACTIVITIES

4.1 Location Stationing

4.1.1 Horizontal positioning

The location of a sampling station needs to be determined both horizontally and vertically. The precision of location stationing will vary with the requirements of the sampling plan and site conditions. Depending on requirements and conditions, it may be adequate to position sampling stations visually, without instrumentation, using available landmarks for reference. Generally, this is only appropriate in small rivers or in harbor locations near (<50 feet) land, piers, or breakwaters.

Positioning of stations in larger rivers, open harbors or the nearshore lake should be done using some type of instrumentation. A variety of instruments may be used, including land survey equipment, Loran, and global positioning systems (GPS). Most commercial navigation vessels and larger recreational craft are equipped with Loran, while GPS is becoming more commonly available. If stations are positioned by a dedicated survey team, it will be more cost efficient to have all stations located, and marked with buoys on one day rather than to have the survey team stay for the duration of sediment sampling.

4.1.2 Vertical positioning

Location positioning should include the elevations of the water surface and sediment-water interface. This is especially important for dredging projects. Since sediments are typically dredged to a fixed elevation, a sample collected below this elevation would not be part of the material to be dredged. Water depth can be determined using a lead line, sounding basket, or bathymetric instrumentation. Because all water surfaces fluctuate over time, the water surface elevation must be referenced to an fixed datum.

The accepted elevation datum for the Great Lakes is the International Great Lakes Datum (IGLD), which is referenced to the zero point at Rimouski, Quebec. This datum was adjusted in 1955 and again in 1985 to account for movements of the earth's crust (Coordinating Committee on Great Lakes Basin Hydraulic and Hydrologic Data 1992). Conversions between IGLD 1955 and other elevation datum are summarized on table D-4.

Table D-4. Elevation Conversion Chart

Given	To Find				
	CCD[1]	IGLD 55	MTNY[2]	MSL[3] 1912	MSL 1929
CCD		+578.18	+579.88	+579.91	+579.48
IGLD 55	-578.18		+ 1.70	+ 1.74	+ 1.30
MTNY	-579.88	- 1.70		+ 0.04	- 0.40
MSL 1912	-579.91	- 1.74	- 0.04		- 0.44
MSL 1929	-579.48	- 1.30	+ 0.40	+ 0.44	

[1] Chicago City Datum
[2] Mean Tide New York
[3] Mean Sea Level

Low Water Datum (LWD) are the planes of reference to which most Great Lakes navigation charts are referenced. The LWD elevation reflects the average low water elevation of the individual lakes. The Low Water Datum elevations of each of the Great Lakes referenced to IGLD 55 and IGLD 85 are shown on table D-5.

Table D-5. Low Water Datum for IGLD 85

Lake	IGLD 55 (feet)	IGLD 55 (meters)	IGLD 85 (feet)	IGLD 85 (meters)
Lake Superior	600.0	182.9	601.1	183.2
Lake Michigan	576.8	175.8	577.5	176.0
Lake Huron	576.8	175.8	577.5	176.0
Lake St. Clair	571.7	174.2	572.3	174.4
Lake Erie	568.6	173.3	569.2	173.5
Lake Ontario	242.8	74.0	243.3	74.2

Water surface elevations may be referenced from survey markers installed by the USACE or other agencies, or from fixed structures that have been surveyed and elevations recorded. USACE survey markers are small (3 1/2" diameter) brass plates, placed at locations around authorized navigation projects. Their locations and elevations can be obtained from the appropriate USACE district office. Water levels can be obtained from recording gages maintained at selected sites on the Great Lakes (NOAA 1992a), although these are not as representative as "on-site" measurements and should be used only as a last resort.

Bridges are often used to reference water level elevations. The elevation of low steel, the lowest point of the bridge span, is available for many bridges from city or state highway departments, railroads, port authorities, and the USACE. Bridge clearances, to the nearest foot above LWD, are also published in the "Coast Pilot" (NOAA 1992b).

4.2 Logistics

Pre-planning is necessary to assure a successful sediment sampling project. This planning should address several logistical features, including access locations, sampling sequence, contingency, and overall scheduling.

Access to sampling locations is not usually a problem in and around authorized navigation channels. Launch ramps for boats

and small floating plants are available at most lakefront and riverside marinas and local, State and Federal parks. Larger boats and floating plants may need a secure docking area for mobilizing equipment. At waterways away from authorized navigation channels, access may be a problem. Bridges, pipeline crossings and other obstructions may necessitate that sampling vessels be remobilized several times. In some cases, easements may have to be obtained from landowners to gain access. Access locations should be identified and inspected during the planning of a sediment sampling project to assure they will be available and feasible.

In many rivers and harbors around the Great Lakes, the levels of sediment contamination increase as one proceeds upstream from the nearshore lake. One way to help minimize cross-contamination of sampling gear is by scheduling the sampling stations so that the most contaminated areas are sampled last. While this approach will not eliminate the need for decontaminating equipment between samples, it will reduce the potential for cross contamination.

Almost all sampling plans will be subject to unforeseen complications. Many of these problems can be avoided by assuming the plan is imperfect from the start and preparing contingencies. The most common problems are equipment failure and bad weather. If possible, it is advisable to carry a spare for any equipment subject to failure. The location and phone numbers of sources for parts or equipment repair should readily available.

Storm events can delay or interrupt sampling, so contingencies need to be arranged with both the sampling team, delivery service, and the laboratories. All contingent field changes should either be identified in the sampling plan or at the very least, a chain of command should be defined by which clear responsibility is assigned for each such decision.

Scheduling requires understanding of the operational capability of the laboratories. Sampling tends to occur on weekdays, and delivery or sample holding over a weekend can be problematic, especially since the material needs to be refrigerated. The delivery of samples at a rate that overwhelms the laboratory is also not desirable, because holding times are extended. However, these types of timing problems may not always be avoidable, depending upon the cost of sampling equipment mobilization and other such factors.

4.3 Sample Collection

There are many reasons why slow methodical collection protocols are best, not the least of which is safety. Taking

extra time to be sure that the vessel is on station, the proper sampling device is outfitted with the correct attachments, the correct jars and labels are being used, the proper methods of sample splitting and mixing are being deployed, and all activity and conditions are fully documented in the sampling log can save having to repeat these activities.

Because of the complexity associated with sediment sampling, it is always good practice to assign all team members specialized responsibilities. Further, a single lead team member should work with the vessel operator and oversee all sampling and handling activities. This team leader is usually also responsible for documenting the field work in the sampling log.

A field log should always be prepared to describe the conditions and events of the sediment sampling project. The field log used by the USACE for geotechnical borings (ENG Form 1836) is provided as Attachment D-1. An example of a field log which is more appropriate to grab sampling is provided at Attachment D-2. Field logs should document conditions of the sampling location, elevations of the water and sediment surfaces, information about the sampling equipment and sample recovery. The logs should also record the physical appearance of the sediment sampled. Categories of sediment characteristics are listed on table D-6. Photographs of the sample are another way to document physical appearance.

All sampling and field measurement equipment should be checked and tested before leaving shore. Sampling equipment (the parts which contact the sediment sample) should be cleaned before the sampling project and in-between project samples. Recommended pre-project cleaning procedures are as follows:

- wash with non-phosphate detergent,
- triple rinse with distilled water,
- rinse with acetone,
- rinse with reagent grade hexane, and
- air dry.

In the field, sampling equipment should be cleaned between samples to avoid cross-contamination. Although the above cleaning procedures are appropriate, the use of acetone and hexane on some sampling vessels or with some sampling equipment may be infeasible or present safety problems. The following are minimum cleaning procedures between samples:

- brush wash with site water, and
- rinse with distilled water.

Table D-6. Categories of Sediment Characteristics

Type	Size or Characteristic
Inorganic components[1]	
Cobbles	75 to 300 mm (3 to 12")
Gravel	4.75 to 75 mm (3/16 to 3")
Sand	0.075 to 4.75 mm
Silt	0.005 to 0.075 mm in diameter
Clay	< 0.005 mm; smooth, slick feeling when rubbed between fingers
Organic Components[2]	
Detritus	accumulated wood, sticks, and other undecayed coarse plant materials
Fibrous peat	partially decomposed plant remains; parts of plants readily distinguishable
Pulpy peat	very finely divided plant remains; parts of plants not distinguishable; varies in color from green to brown; varies greatly in consistency-often semi-fluid
Muck	black, finely divided organic matter; completely decomposed

[1] Unified Soil Classification System
[2] USEPA (1973)

4.4 Sample Handling and Containers

Sediments should be removed from samplers and handled using non-contaminating equipment. In most cases, stainless steel spoons and bowls which have been cleaned in the same manner as the sampler are appropriate. One very common mistake made during sediment sample handling is pouring off "excess water". This water, and the fine particulates in suspension, are part of the sample. Discarding it may bias the sample.

The homogenization, or mixing of a sediment sample in the field is not necessary for most circumstances. If the sediment sample is to be analyzed by a single laboratory, homogenization can be conducted at the lab under more controlled conditions. In cases where the sample is to be divided into two or more containers for shipment to different laboratories, sample homogenization can be conducted in the field, or the entire sample can be shipped to one laboratory, where the sample is homogenized and aliquots are shipped to other labs. Laboratories should be given specific instructions about sample homogenization and notified that water in sample containers should not be discarded, but homogenized with the sample.

Homogenization in the field may be appropriate where the volume of sample collected is far greater than the volume to be transported, and the intent is to have the sample placed in the containers representative of the whole sample collected. In this case, slow and smooth mixing techniques should be used. Overmixing may cause spillage and the aeration of the sediment sample which may alter the sediment chemistry. The larger the volume of a sediment sample, the more difficult it will be to mix the sample in the field. Samples must be protected from external sources of contamination, such as boat splash and fuel and lubricants, during handling.

Sediment samples should be placed into containers and stored at 4° C as rapidly after collection as possible. Containers should be filled to the top with the sample, leaving no head space.

Containers for sediment samples should be made of clean, non-contaminating materials. If the sediment sample were solely for a specific type of chemical analyses, it might be appropriate to chose the container materials which avoids contamination or bias. Since most sediment samples for 404(b)(1) determinations are intended for a variety of analyses, and because of the difficulty in assuring that sub-samples prepared in the field are homogeneous, it is recommended that samples be contained in one type of container for transport to the laboratory, where the sample can be homogenized and sub-divided.

Recommended container materials include wide-mouth glass jars with Teflon-lined caps and high density polyethylene (HDPE) plastic buckets with lids. Each has its advantages and disadvantages. Glass jars are available in a variety of sizes, and are most suitable for smaller sample volumes (1-4 liters) needed for bulk chemistry, grain size analysis and elutriate tests. Glass jars require considerable care in packing and transport, and can break despite the best precautions. HDPE plastic containers are available in small and large sizes, including 5-gallon tubs which are well suited to the large volume samples needed for toxicity, bioaccumulation and column settling tests. Sample containers and lids should be cleaned as follows:

- wash with acid (chromic or HCl),
- rinse with distilled water,
- wash with non-phosphate detergent,
- triple rinse with distilled water,
- rinse with acetone,
- rinse with reagent grade hexane, and
- air dry.

New containers from laboratory supply companies are generally

cleaned to the above or better specifications. New containers from bulk supply companies may need to be cleaned before use.

Sample containers must be identified unambiguously, and a precise sample labeling and coding system should be developed prior to field work to avoid costly mistakes. It is recommended that sampling jars and vessels be pre-labelled, as field conditions are often wet and bumpy, and labels can become scribed indelibly. Labels must be able to withstand the conditions of transport and storage without deterioration or becoming loose. Labels on glass jars stored in wet ice have been known to become "unglued" in transport. To prevent such problems, glass jars may be placed into plastic bags and sealed (this also helps control the mess if a jar breaks in transport). Plastic containers can be marked with indelible pens or markers in addition to regular labels, as a safeguard. Examples of labels used by the USACE for soil samples and by USACE division laboratories are provided at Attachment D-3.

4.5 Sample Transport

Sample containers should be packaged for transport in a manner that maintains them at 4° C and protects them from breakage or spillage. A variety of packing materials and containers are available specifically for the transport of environmental samples. Considering the costs of sample collection and analyses, these materials are a sound investment.

There are a number of transport modes for sediment and water samples. Most overnight transport carriers will accept environmental samples providing they are in secure containers. Some carriers will not accept wet ice, and most will not accept dry ice. Packaged refrigerants ("blue ice") are accepted by most carriers. When large samples are collected (several 5-gallon tubs), it may be more cost effective to lease a refrigerated truck or contract a specialized carrier. The temperature of samples should be measured and recorded at the time of arrival in the laboratory.

Procedures for documenting chain-of-custody for dredged material samples are recommended. An example of a USACE chain-of-custody record for potentially hazardous samples is provided at Attachment D-4.

4.6 Health and Safety

Worker health and safety must be a paramount consideration during all sediment sampling activities. USACE contractors are required to follow the procedures of the Safety and Health Requirements Manual (USACE 1987). Corps districts require that

its boat operators have completed certified training from the U.S. Coast Guard and that its divers be certified and follow the procedures in the U.S. Navy Diving Manuals (U.S. Navy 1988).

Most rivers have numerous crossings by utilities buried beneath the river bottom, and in some cases exposed on the sediment surface. These include water and sewer pipelines, gas and petrochemical pipelines, electrical and telecommunucations cables. Navigation charts typically show utility crossings, but are not always complete or up-to-date. Developers of sediment sampling plans should contact the appropriate utilities to confirm the presence and locations of crossings, especially when any drilling is planned.

Each crew member should be fully outfitted with appropriate safety equipment and properly fitted clothing. Provisions should also be made for staff to clean-up during and after sampling. Soaps, brushes, sponges, water and change of clothing should be available when appropriate. Rain and weather protective clothing and life vests are always appropriate for on-board stowage.

In general, sediments that are being considered for open-water disposal will not contain sufficient levels of contamination to require sophisticated personnel protective equipment (PPE). Workers should avoid any dermal contact with sediments and all sampling equipment should be handled with protective gloves. With more contaminated sediments, disposable Tyvec© clothing should be worn.

Pre-planning for sediment sampling should identify the location and telephone numbers of emergency assistance, including police, Coast Guard, marine assistance, and hospital emergency service. This information should be readily available to the entire field crew.

4.7 Environmental compliance

The USACE has issued nationwide permits under Section 10 of the Rivers and Harbors Act and Section 404 of the Clean Water Act for minor dredging and discharge of quantities less than 25 cubic yards (Federal Register, November 21, 1991). In most instances, sediment sampling for testing purposes will be covered by these nationwide permits, and no separate permits required under these authorities. In cases where sampling is conducted in waterways adjacent to private property, the rights of riparian owners should be considered.

The disposal of field generated waste, other than excess sediment samples, are regulated by Federal, State and local laws and regulations.

5. QUALITY ASSURANCE/QUALITY CONTROL

Most of the specific quality control procedures that are appropriate for sediment sampling and handling have been described in this appendix. These included recommended sampling protocols, methods for cleaning sampling equipment and containers, sample handling, and transport. The written sampling plan should identify the specific methods to be employed and the rationale for variances from the guidance provided here.

Detailed written prototols, or standard operating procedures (SOPs) should be developed and used for field collection activities. Equipment used for making field measurements (e.g. bathymetric survey equipment) should have a quality assurance plan which includes schedules and procedures for calibration, maintenance and repair. Operators should be familiar with these plans and trained in equipment use and operation.

The data quality objectives for a contaminant determination made as part of a 404(b)(1) evaluation are outlined in Appendix E. Because these evaluations are comparative (dredged material are compared to disposal site), all sediment samples must be handled in a similar manner. Field blanks are not generally necessary for dredged material evaluations, and field replicates are not considered useful indicators of QC for sediments.

6. REFERENCES

American Society for Testing and Materials (ASTM). 1984. "Standard guide for selecting grab sampling devices for collecting benthic macroinvertebrates," ASTM D 4387-84, November 1984.

ASTM. 1989. "Standard guide for core sampling submerged, unconsolidated sediments," ASTM D 4823-88, April 1989.

ASTM. 1991. "Standard guide for collection, storage, characterization, and manipulation of sediments for toxicological testing," ASTM E 1391-90, January 1991.

Coordinating Committee on Great Lakes Basin Hydraulic and Hydrologic Data. 1992. "IGLD 1985: Brochure on the International Great Lakes Datum 1985," Washington, DC.

Elliott, J.M. and C.M. Drake. 1981. "A comparative study of seven grabs used for sampling benthic macroinvertebrates in rivers," Freshwater Biology 11, 99-120.

Hummel, P.L. and R.J Krizek. 1974. "Sampling of maintenance dredgings," in Journal of Testing and Evaluation, 2(3), ASTM, Philadelphia, PA.

International Joint Commission (IJC). 1987. "Guidance on characterization of toxic substances problems in areas of concern in the Great Lakes basin: A report from the Surveillance Work Group based on the recommendations from the Monitoring in Areas of Concern Workshop held at Canada Centre for Inland Waters, October 3-4, 1985," a report to the Great Lakes Water Quality Board, Windsor, Ontario.

IJC. 1988. "Procedures for the assessment of contaminated sediment problems in the Great Lakes," by the Sediment Subcommittee to the IJC, Windsor, Ontario.

Lowe, J. and P.F. Zaccheo. 1991. "Subsurface explorations and sampling", in Foundation Engineering Handbook, H. Fand (Ed), Van Nostrand Reinhold, New York, NY.

Mudroch, A. and S.D MacKnight. 1991. CRC Handbook of Techniques for Aquatic Sediments Sampling, CRC Press, Boca Raton, FL.

National Oceanic and Atmospheric Administration (NOAA). 1992. "United State Coast Pilot, 6, Great Lakes," National Ocean Service, Washington, DC.

National Oceanic and Atmospheric Administration (NOAA). 1993. "Great Lakes Water Levels; 1992," Washington, DC.

Palmer, M. 1984. "Methods manual for bottom sediment sample collection", EPA-905-4-85-004, USEPA Great Lakes National Program Office, Chicago, IL.

Plumb, R.H. 1981. "Procedures for handling and chemical analysis of sediment and water samples," Technical Report EPA/CE-81-1, Environmental Protection Agency/Corps of Engineers Technical Committee on Criteria for Dredged and Fill Material, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Sly, P.G. 1969. "Bottom sediment sampling," in Proceedings of 12th Conference on Great Lakes Research, International Association of Great Lakes Research,

Smith, J.B. and J.E. Clausner. 1993. "An inexpensive method for vibracoring sands and fine-grained sediments in shallow water," Dredging Rersearch Technical Note DRP-2-06, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

U.S. Army Corps of Engineers (USACE). 1972. "Soil sampling," Engineer Manual EM 1110-2-1907, Office of the Chief of Engineers, Washington, D.C.

USACE. 1983. "Dredging and dredged material disposal," Engineer Manual EM 1110-2-5025, Office of the Chief of Engineers, Washington, D.C.

USACE. 1987. "Safety and health requirements manual," EM 385-1-1, Office of the Chief of Engineers, Washington, DC.

U.S. Environmental Protection Agency (USEPA). 1977. "Methods manual for bottom sediment sample collection", Region V, Great Lakes Surveillance Branch, Chicago, IL.

USEPA. 1985. "Sediment sampling quality assuirance user's guide," EPA-600/4-85-048, Environmental Monitoring Systems Laboratory, Las Vegas, Nevada.

USEPA/USACE. 1992. "Ecological evaluation of proposed discharge of dredged material into ocean waters," Chapter 8, Collection and preservation of samples, USEPA Office of Marine and Estuarine Protection, Washington DC.

USEPA/USACE. 1998. "Evaluation of dredged material proposed for discharge to waters of the United States: Testing Manual (Inland Testing Manal)," EPA-823-B-98-004, Office of Water, Washington DC.

U.S. Geological Survey. 1978. "National handbook of recommended methods for water-data acquisition," Chapter 3, Sediments, USGS Office of Water Data Coordination, Reston, VA.

U.S. Navy. 1988. "U.S. Navy Diving Manuals," NAVSEA 0994-LP-001-9010 and NAVSEA 0094-LP-001-9020, Revision 2, Naval Sea Systems Command, Washington, DC.

Winterkorn, H.F. and H. Fang. 1975. Foundation Engineering Handbook. Van Nostrand Reinhold, New York, New York.

Hole No.

DRILLING LOG	DIVISION	INSTALLATION	SHEET OF SHEETS
1. PROJECT		10. SIZE AND TYPE OF BIT	
2. LOCATION (Coordinates or Station)		11. DATUM FOR ELEVATION SHOWN (TBM or MSL)	
3. DRILLING AGENCY		12. MANUFACTURER'S DESIGNATION OF DRILL	
4. HOLE NO. (As shown on drawing title and file number)		13. TOTAL NO. OF OVER-BURDEN SAMPLES TAKEN	DISTURBED / UNDISTURBED
5. NAME OF DRILLER		14. TOTAL NUMBER CORE BOXES	
6. DIRECTION OF HOLE [ ] VERTICAL [ ] INCLINED _____ DEG. FROM VERT.		15. ELEVATION GROUND WATER	
7. THICKNESS OF OVERBURDEN		16. DATE HOLE	STARTED / COMPLETED
8. DEPTH DRILLED INTO ROCK		17. ELEVATION TOP OF HOLE	
9. TOTAL DEPTH OF HOLE		18. TOTAL CORE RECOVERY FOR BORING %	
		19. SIGNATURE OF INSPECTOR	

ELEVATION a	DEPTH b	LEGEND c	CLASSIFICATION OF MATERIALS (Description) d	% CORE RECOVERY e	BOX OR SAMPLE NO. f	REMARKS (Drilling time, water loss, depth of weathering, etc., if significant) g

ENG FORM 1836 MAR 71 PREVIOUS EDITIONS ARE OBSOLETE.

(TRANSLUCENT)

PROJECT

HOLE NO.

Attachment D-1

SEDIMENT SAMPLING FIELD LOG

Project: Date:

Station Information	Site Conditions
Station ID:	Temperature:
Description:	Precipitation:
	Wind:
Positioning method:	Waves/current:

Elevation

Water surface:	Water depth:
Measurement equipment:	Measurement equipment:
Datum:	Sediment surface:

Sampling Information

Sampler type:	Sample volume required:
Recovery:	Number of sub-samples taken:
Sample ID:	Sample homogonized:
Decontamination:	Container(s):

Visual Inspection

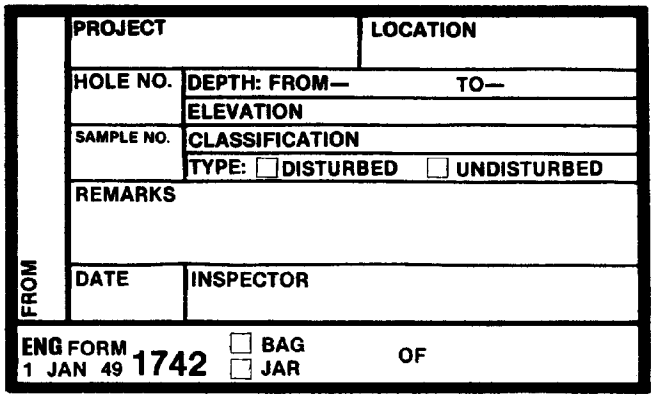
Odor:

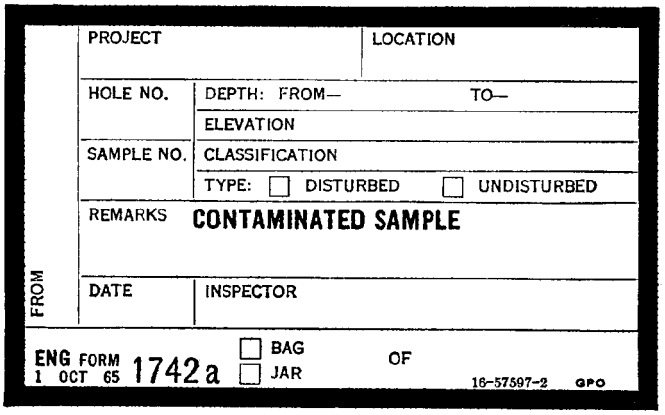
Color:

Texture/grain size:

Remarks:

Log prepared by: _____


PROJECT
LOCATION
HOLE NO.
DEPTH: FROM— TO—
ELEVATION
SAMPLE NO.
CLASSIFICATION
TYPE: DISTURBED UNDISTURBED
REMARKS
FROM
DATE
INSPECTOR
ENG FORM 1 JAN 49 1742
BAG
JAR
OF


PROJECT
LOCATION
HOLE NO.
DEPTH: FROM— TO—
ELEVATION
SAMPLE NO.
CLASSIFICATION
TYPE: DISTURBED UNDISTURBED
REMARKS
CONTAMINATED SAMPLE
FROM
DATE
INSPECTOR
ENG FORM 1 OCT 65 1742a
BAG
JAR
OF
16-57597-2 GPO

U.S. Army Corps of Engineers

Chain of Custody Record

(ER 1110-1-263)

Proj. No.	Project Name					Number of Containers								
Sampler : *(Signature)*														
Date	Time	Pres.	Grab	Comp	Site Code/Sample Number									Remarks:

Sampler Relinquished by:	Date/Time	Received by: (Sig.)	Date/Time	Hazards Associated with Samples
Relinquished by: (Sig.)	Date/Time	Received by: (Sig.)	Date/Time	
Relinquished by: (Sig)	Date/Time	Received for Laboratory by: (Sig.)	Date/Time	Remarks at time of receipt:
Custody Seal No.		Lab case No.:		

ENG Form 5021-R, Oct 90

Proponent: CEMP-RT

Attachment D-4

Attachment D-4

GREAT LAKES
DREDGED MATERIAL TESTING AND EVALUATION MANUAL

APPENDIX E
QUALITY ASSURANCE GUIDANCE

prepared by:

Patricia M. Boone
USEPA, Region 5

Jan A. Miller
USACE, Great Lakes & Ohio River Division

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ATTACHMENTS

1. PURPOSE AND APPLICABILITY

This appendix provides guidance on the quality assurance program for the testing and evaluation of dredged material proposed for discharge into the Great Lakes. Section 2 of this appendix defines and discusses the principal components of a "quality system" for an organization. Section 3 summarizes the quality assurance program for Great Lakes dredged material testing and evaluation.

This quality assurance guidance is intended for use by the USACE in contracts for dredged material data collection. This guidance is also intended for use by Section 404 permit applicants as the minimum quality assurance requirements for data which the USACE will accept for a permit determination regarding the discharge of dredged material to waters of the U.S.

Specific protocols for project design, sample collection, handling and storage, sample and data custody, field and laboratory analysis and reporting, and data assessment and interpretation are described in the Great Lakes Dredged Materials Testing and Evaluation Manual (GLTEM) and Appendices D, F and G.

2. QUALITY SYSTEM COMPONENTS

The complexity of environmental data collection demands that a systematic process and structure be established to provide decision makers with the necessary confidence in the quality of data produced for decisions as well as the means to determine when the data are not fully usable. This section will define the components of such a systematic process and the structure for an organization, termed a quality system.

2.1 Quality Systems

A **quality system** provides the framework for planning, implementing and assessing work performed by and/or for an organization. A quality system consists of the policies, principles, authority, objectives, responsibilities, accountability, and implementation plan for ensuring quality in work processes, products, and services. The principal components of a quality system include:

- quality assurance management plans (Section 2.2),
- quality assurance program plans (Section 2.3),
- data quality objectives planning process (Section 2.4),
- quality assurance project plans (Section 2.5),
- standard operating procedures (Section 2.6),
- data quality assessments (Section 2.7), and
- QA program assessments (Section 2.8).

Quality assurance (QA) is an integrated system of management activities involving planning, implementation, assessment, reporting, and quality improvements to ensure that a process, item, or service is of the type and quality needed.

Quality control (QC) is the overall system of technical activities that measures the attributes and performance of a process, item, or service against defined standards to verify that they meet the stated requirements.

QC for environmental data collection projects can be divided into two basic types: sample performance QC and method performance QC. Sample performance QC provided quantitative information on the quality of the sample. Method performance QC provides quantitative information on the quality of the method during implementation for a given sample.

2.2 Quality Assurance Management Plan

As a first step to establishing a quality system, each organization documents their quality assurance policy and management structure in a **quality assurance management plan (QAMP)**. The QAMP provides the blueprint for how an individual agency will plan, implement and assess the quality of the environmental work performed by or on behalf of an organization. The QAMP consists of the following ten elements:

- quality management and organization,
- quality system,
- personnel qualification and training,
- procurement of items and services,
- quality documentation and records,
- use of computer hardware and software,
- quality planning,
- quality implementation of work processed,
- quality assessment and response, and
- quality improvement.

Relevant QAMPs applicable to Great Lakes dredged material testing and evaluation are discussed in Section 3.1.

2.3 Quality Assurance Program Plans

Quality assurance program plans are written to further define the management structure and applicable QA requirements for individual programs (e.g., NPDES, Superfund, TSCA) within the organization, according to the regulations and policies for each environmental program. The quality assurance program plan institutes processes, recommends procedures, sets minimum standards, and documents how and when QA and QC are applied at

the technical/project level during planning, implementation, and assessment.

Section 3 of this appendix presents the quality assurance program plan for Great Lakes dredged material testing and evaluation.

2.4 Data Quality Objective Process

The data quality objective process is used to establish data collection requirements for environmental programs and projects within an organization. The iterative 7-step data quality objective process provides the framework for planners to focus their planning efforts (USEPA 1993d). It is almost always necessary to revisit previous steps.

The data quality objective process differs from historical planning approaches in that acceptable probabilities of making false negative and false positive decisions are set prior to the project, and the study is designed such that data collected can verify that these probabilities were achieved. Decision error is a product of the uncertainty in results. Uncertainty is determined by data quality and quantity. Some of the common sources of uncertainty are listed in table E-1.

Measuring and allocating overall uncertainty typically requires pilot studies to estimate environmental heterogeneity to design an effective sampling program, and sufficient data to render sampling/analytical bias and imprecision less than environmental heterogeneities (i.e. define the magnitude of uncertainty and the confidence level in the magnitude of uncertainty observed).

Neither pilot studies nor statistical project designs are possible, or arguably, appropriate for individual dredged material evaluations. For dredged material testing, the quantification of uncertainty is still in the realm of research and development. Therefore, decisions will continue to be based on "best professional judgement" rather than "statistical uncertainty". This does not mean the data quality objective process cannot be used. The probability of discharging contaminated dredged material to waters of the U.S. (i.e., a false negative decision) is difficult to determine, but an attempt to control uncertainty has been made by setting minimum specifications and controlling protocols for collecting environmental data for dredged material evaluations.

2.4.1 Data quality objectives

Data quality objectives (DQOs) are qualitative and

quantitative statements derived from the outputs of the steps of the data quality objective process which specify the program/study objectives, domain, limitations, the most appropriate type of data to collect, use of the data (the decision), decision criteria (action levels), and the levels of decision error that will be acceptable. The general DQOs for Great Lakes dredged material testing and evaluation are presented throughout the GLTEM and appendices, and are summarized in Section 3.

Table E-1 Common Sources of Error

Sources of Overall Error (in decreasing order of importance)
- Pollutant distribution
- Sample design and collection (varies w/ analyte and matrix)
- Sample procedures and handling
- Laboratory sample preparation
- Laboratory sample analysis
- Data handling

Sample Design and Collection Errors

- Not homogeneously distributed
- Unrepresentative number of samples
- Unrepresentative spots sampled
- Migration not accounted for
- Wrong type of sampling (e.g. random)

Common Sampling Procedure Errors
- Inappropriate equipment
- Cross contamination
- Disturbs composition

Laboratory Preparation and Analytical Errors
- Subsampling errors
- Lose sample (all or part)
- Contamination
- Wrong protocol
- Acceptance limits determined for different matrix
- Wrong calibrate or reference used

2.4.2 Data quality indicators

Data quality indicators (DQIs) are quantitative statistics and qualitative descriptors that are used to define "the most appropriate data to collect" and to assess the degree of acceptability or utility of the data collected to the user. Project DQIs are set as part of Step 3 of the data quality objective process. Historically, DQIs include sensitivity,

precision, accuracy, completeness, representativeness, and comparability. A detailed discussion of these indicators is provided in Attachment E-1.

DQIs apply to sample designs, all types of field and laboratory measurements, as well as "secondary" data produced by modeling or manipulation of field and laboratory measurements. It is critical for the quantitative DQIs (i.e. sensitivity, precision, accuracy, and completeness) that appropriate means/processes be used to measure/estimate the DQIs and that acceptance criteria for DQIs be determined using the means/processes that will be used in the project.

The DQIs for Great Lakes dredged material testing and evaluation are presented in Section 3.4.

2.5 Standard Operating Procedures

Standard operating procedures (SOPs) are written documents that detail the method of operation, analysis, or action with prescribed techniques and steps. Consistency and thoroughness are best maintained by following written SOPs. Documentation ensures all requirements were met and provides proof that the procedure was conducted properly if questions arise later.

SOPs are officially approved as the method for performing certain routine or repetitive tasks. SOPs should be periodically reviewed and updated as necessary, and may be modified to fit the individual sampling and analysis activities of specific projects. Guidance on preparing SOPs is provided as Attachment E-2.

The "Inland Testing Manual" (USEPA/USACE 1998) contains a number of technical appendices which will function as SOPs for procedures and analyses required for making a 404(b)(1) contaminant determination:

Appendix B: Guidance for evaluation of effluent discharges from confined disposal facilities
Appendix C: Evaluation of mixing (STFATE model)
Appendix D: Statistical methods

This, and other appendices to the GLTEM provide guidance on sediment sampling and handling (Appendix D), physical and chemical analyses (Appendix F), and biological effects-based tests (Appendix G). The GLTEM is intended to serve as SOPs for the majority of dredged material testing and evaluation. Guidance on SOPs for modified or new procedures for Great Lakes dredged material evaluations is provided in Section 3.5.

2.6 Quality Assurance Project Plans

A **quality assurance project plans** (QAPP) is the principal product of the project planning process inasmuch as it integrates all technical and quality aspects for the life-cycle of the project, including planning, implementation and assessment.

During project planning, the QAPP documents the outputs of the data quality objective process and is used for project coordination and oversight. During project implementation, the QAPP serves as a blueprint for project personnel. The following are the 16 traditional elements of a QAPP:

1) Title and signature page(s).
2) Table of contents.
3) Project description.
4) Organization and responsibility.
5) Quality assurance objectives.
6) Sampling procedures.
7) Sample and data documentation and custody.
8) Calibration.
9) Methods.
10) Internal quality controls.
11) Data reduction, validation, and reporting.
12) Performance and systems audits.
13) Preventive maintenance.
14) Data quality assessment and usability.
15) Corrective action.
16) Quality assurance reports to management.

The QAPP is the primary resource for assessing the usability of and interpreting project results. The QAPP may be supplemented by previously prepared planning documents or concurrently prepared procurement documents. A modified QAPP format for Great Lakes dredged material evaluations is discussed in Section 3.6. Additional guidance on preparing QAPPs is in USEPA (1991c; 1993a).

2.7 Data Quality Assessment

Assessment is the evaluation process used to measure the performance or effectiveness of a system and its elements. Assessment is an all-inclusive term used to denote any of the following: audit, performance evaluation, management systems review, peer review, inspection or surveillance.

Once the DQO process has been completed, the planning team will have the information needed to choose the sampling design that best meets the needs of their study. The needs of the planning team have not been fully met, however, until the

sampling data are analyzed to ensure that any decision made from the data will meet project specifications. This analysis is part of a related process called **data quality assessment (DQA)**.

The DQA process is used to assess the scientific and statistical quality of data for a specified purpose. During the DQA process, data is analyzed scientifically for technical anomalies and to judge if the context of the data is correct. At the same time, the data may be evaluated statistically. The outcome of DQA analysis will determine whether a decision can be made using the existing data or whether additional sampling data must be collected. The DQA process is also useful for determining whether a sampling design is appropriate for similar studies.

DQA guidance for Great Lakes dredged material testing and evaluation is provided in Section 3.7.

2.8 Quality Assurance Program Assessments

There are three types of assessments of a QA program: reviews, inspections and audits. **Reviews** and **inspections** are assessments of the conformance of systems to qualitative requirements or specifications. **Audits** are assessments of the conformance of systems to quantitative specifications.

Management systems reviews (MSRs) assess the effectiveness of the implementation of the approved QA program. These reviews consider linkages across organizational lines and can be used to discern areas requiring improved guidance. The effectiveness of the management system is generally measured using judgement based on non-technical information assembled and analyzed. Management systems reviews should be performed on at least an annual basis and should be conducted according to the goals and procedures stated in the organization's QAMP. Guidance on preparing and conducting MSRs is provided in USEPA (1994a). Refer to the QAMPs listed in Section 3.1 for more information on management system reviews that are part of the QA program for Great Lakes dredged material testing and evaluation.

Systems inspections assess project QC activities and environmental data collection systems. A systems audit qualitatively evaluates all components of the measurement system to determine proper selection, maintenance, and use. This audit includes a careful evaluation of both field and laboratory quality control procedures and records. General guidance for planning and conducting technical systems audits is provided in USEPA (1993f).

Performance audits quantitatively evaluate the field and/or laboratory personnel's performance and the instrumentation or analytical systems used. Performance audits evaluate the accuracy and precision of the total measurement system with samples of known composition or behavior.

Audits of data quality (ADQ) are a qualitative evaluation of the documentation and procedures associated with environmental measurements to verify that the resulting data are of acceptable quality. ADQs address whether or not sufficient information exists for the data sets to support data quality assessment.

Quality assurance program assessments for Great Lakes dredged material testing and evaluation are discussed in Section 3.8.

3. QUALITY ASSURANCE PROGRAM FOR GREAT LAKES DREDGED MATERIAL TESTING AND EVALUATION

The program for regulating the discharge of dredged material into the U.S. waters of the Great Lakes basin is managed by the USACE in cooperation with the USEPA and Great Lakes States. USACE district offices in Buffalo, Chicago, Detroit and St. Paul administer the Section 404 permit program. The USACE districts at Buffalo, Chicago and Detroit also conduct the maintenance dredging of Federal navigation projects in the Great Lakes. The USACE District Engineer is ultimately responsible for making determinations of compliance with Section 404. State regulatory agencies are responsible for issuing water quality certifications for dredged material discharges under Section 401.

Environmental data is collected as part of a 404(b)(1) evaluation to make a contaminant determination. The "Inland Testing Manual" and the GLTEM utilize a tiered testing approach to identify the data needed to determine compliance. Great Lakes dredged material testing requirements are consistent with the "Inland Testing Manual," but have been tailored to the needs of the Great Lakes. The GLTEM provides more specific testing requirements based on physical, chemical and biological conditions representative of the Great Lakes. For example, laboratory methods for chemical analysis of sediments were selected based, in part, on their ability to achieve target detection limits representative of background levels in the Great Lakes sediments.

The USEPA and USACE, in developing the GLTEM and Appendices have formulated a quality assurance program for Great Lakes dredged material testing and evaluation. An overview of this program is shown on figure E-1.

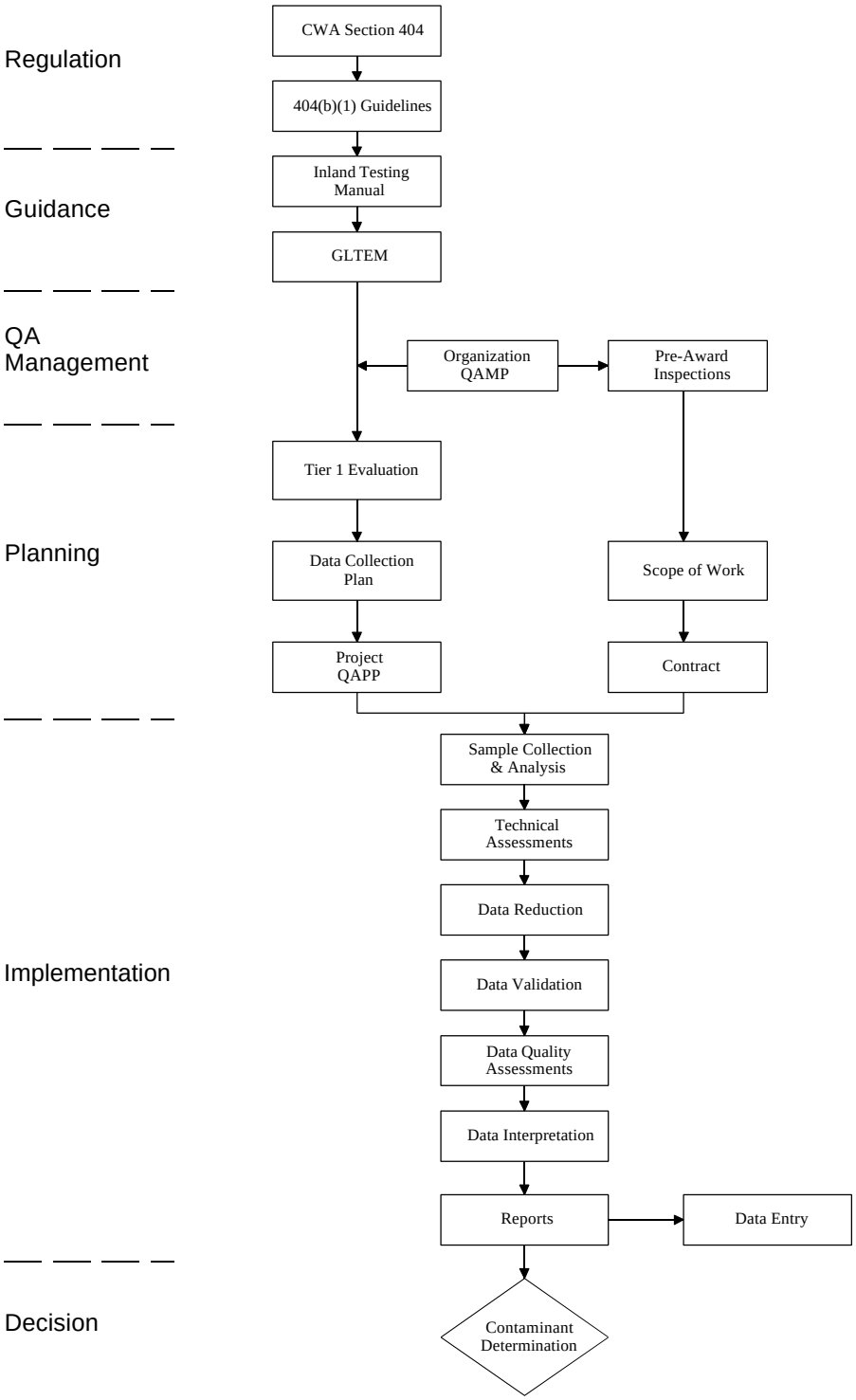


Figure E-1. Overview of Quality Assurance Program for Great Lakes Dredged Material Evaluations

The structure and objectives of the program are described in this section, including:

- relevant quality assurance management documents,
- project coordination,
- project decisions and decision criteria,
- data quality indicators,
- special project needs,
- quality assurance project plans, and
- program and data quality assessments.

3.1 Quality Assurance Management

All organizations involved in collecting data for a 404(b)(1) contaminant determination should have a quality management system. The USACE North Central Division (NCD) "Quality Assurance Management Program (QAMP) for Environmental Data Collection" (NCD 1994) describes the fundamental QA requirements for environmental data collection activities conducted by or on behalf of the USACE districts. This QAMP requires that all districts have a district-specific QAMP and a District Quality Assurance Coordinator.

Applicants for Section 404 permits collecting environmental data should have an established QA management system and a QA Officer. The permittee QA management system should be documented through a plan that describes corporate QA policies and general requirements for all environmental data collection activities.

Each field or laboratory contractor should have an established QA management system and a QA Officer. The contractor QA management system should be documented through a plan that describes corporate QA policies and requirements for all environmental data collection activities as well as standard operating procedures for both QA management and data collection activities. The QA program of subcontractors should be included in the contract and should meet the same requirements expected of the prime contractor.

USACE contractors may be requested to develop contract-specific QA management plans that are presented for USACE review or approval as delineated in the contract bid. Specific recommendations for contractor QA management systems are defined in the USACE QAMPs, and USACE contract guidance documents.

USACE districts, permit applicants and contractors should continually monitor the effectiveness of their QA management through reviews and assessments, as defined in Section 2.8. For contractors, project staff should also review performance to ensure compliance with contractual requirements. Contractors

should review the performance of subcontractors.

3.2 Project Coordination

Coordination of proposed dredged material disposal projects is discussed in various sections of the GLTEM. The purpose of coordination is to solicit input from agencies which will take part in the decision making process prior to any field activities. Coordination should occur during the planning and review of data collection activities. Coordination mechanisms may include scoping meetings, review of planning documents, and review of project reports.

For USACE dredging projects, the responsibility for coordination with other agencies rests with the Project Manager. For Section 404 permit applications involving dredged material discharge, it is strongly recommended that applicants coordinate with the USACE prior to contracting or initiation of field work. The USACE will facilitate coordination of permit evaluations with other agencies.

Several documents produced during a 404(b)(1) evaluation are critical to project coordination. These include the Tier 1 evaluation and the data collection plan. The recommended contents of the Tier 1 evaluation report are discussed in the GLTEM. A data collection plan (DCP) is a document which describes, in detail, the proposed sampling and analysis. The DCP serves as the primary document for project coordination in advance of the proposed sampling and testing. It will also provide much of the information needed for the QAPP and may serve as a scope(s) of work (SOW) for contractors who will implement all or part of the plan.

The DCP should clearly define the goals of the project, define performance criteria for sample design and analytical data quality, establish QA guidelines consistent with project goals, and develop technical strategies to minimize project costs and maintain timelines. The DCP should clearly describe all field and laboratory activities, describe procedures, define performance criteria, and establish QA and QC consistent with the goals in the GLTEM. The plan should discuss organization and responsibilities for implementation and oversight of field and laboratory activities as well as reduction, review, and reporting of results.

The plan should balance the need for an appropriate level of detail with timeliness and cost considerations. Accepted methods and procedures detailed in the GLTEM and appendices can be included by reference. More extensive documentation would be necessary for work to be done by modified/new methods.

Documentation for modified and new methods is discussed in Section 3.5.

Project coordination should continue during implementation of sampling and analysis as problems or changing conditions arise. The relatively short time period for dredged material evaluations will normally limit communication to informal contacts, such as telephone calls and on-site visits. Procurement and contracting personnel should be notified of any contractor problems.

3.3 Project Decisions and Decision Criteria

Dredged material testing and evaluation is ultimately directed toward a single project decision; whether or not the dredged material will have unacceptable contaminant-related impacts on the aquatic environment. The path to this "contaminant determination" involves numerous other decisions in the tiered testing framework. At the end of each of the first three tiers, one of the following conclusions can be made:

- the information available is sufficient for a decision of compliance,
- the information available is sufficient for a decision of non-compliance, or
- the information available is not sufficient for a decision and further testing is necessary at a higher tier.

Testing is conducted in this tiered structure only to the tier at which a decision of compliance or non-compliance can be made. Decisions of compliance can be made independently for each "management unit" of dredged material delineated. Management units are discussed in Sections 2.3 and 2.4 of the GLTEM and Appendix D.

The major decision criteria for dredged material evaluations were promulgated by the USEPA and USACE in the 404(b)(1) Guidelines, published as final in 1986. The water quality standards adopted by States are also decision criteria for a dredged material evaluation. Other decision criteria were established by the USEPA and USACE as part of guidance published in the "Inland Testing Manual" and GLTEM. For some projects, additional decision criteria will be developed by the USACE in consultation with the USEPA during the planning phase.

Most of the decision criteria are relative, based on a comparison of the proposed dredged material with the sediment at the disposal site. The physical and chemical characteristics of dredged material and disposal site sediments, and results of biological effect-based tests with these materials are compared

to make decisions about compliance and the need for further testing. Some of these comparisons are quantitative (statistical significance) while others are more qualitative (weight of evidence). The disposal site is considered as a single unit (i.e., one value with a known uncertainty for each parameter) and serves as the source of comparison for all management units.

Absolute decision criteria for dredged material evaluations have been developed for water column toxicity tests results and compliance with State water quality standards. The following sections discuss the intended use of each type of data collected for the four tiers.

3.3.1 Historical data and records

Historical data and records are compiled during Tier 1 in order to determine if any additional data collection is necessary for a determination of compliance. Sources of these records are discussed in the GLTEM and Appendix C. Historical data can be used as decision points in Tier 1 to determine the applicability of the exclusions from testing. In one decision point, historic data is used to determine the absence of contamination in the proposed dredged material. In the other decision point, historic data on the physical, chemical and biological characteristics of sediments from adjacent dredging and disposal sites are compared, as follows:

> IF the physical, chemical and biological characteristics of sediments at the proposed dredging and disposal sites are not substantially different and the geochemical environments at the sites are similar, THEN no further testing should be necessary to make a contaminant determination.

Tier 1 decision points are based on a "weight-of-evidence" approach. Historical datasets can also be used as a decision point in Tier 1 where there is adequate information of previous Tier 2 and/or 3 testing to make a determination.

3.3.2 Field measurements

Field observations and measurements are conducted as part of every sampling event, and may be used in dredged material evaluations for a number of purposes, including:

- establish positions of sampling locations,
- assess disposal site or management unit homogeneity,
- characterize site conditions at the time of sampling,
- identify and/or characterize the samples collected, and
- as input parameters for the mixing model.

Field observations and measurements may be used as decision points in Tier 1 evaluations, in conjunction with historic information.

3.3.3 Physical characterization of sediment

Physical characteristics of sediments are used as a decision point in Tier 1 to determine the applicability of exclusions from testing, as discussed above. Sediment physical measurements are also used in conjunction with other information as follows:

- indicators of sediment heterogeneity for use in sampling design,
- identify appropriate control and disposal site sediments,
- input parameters to the mixing model, and
- adjust and/or evaluate contaminant concentrations measured (e.g., adjust wet weight to dry weight concentrations).

3.3.4 Chemical analysis of sediment

Sediment bulk chemical concentrations can be used as a decision point in Tier 1 to determine the applicability of exclusions from testing, as discussed above. In some cases, new physical and chemical data are collected to verify a decision in Tier 1.

Sediment chemical data is also used as part of two decision points in Tier 2. The data is used as input to the mixing model to determine the **potential** for exceeding State water quality standards:

> IF the calculated water column concentrations of all contaminants of concern at the edge of the mixing zone are within applicable State water quality standards and IF no interactive effects are suspected, THEN the proposed dredged material discharge should not adversely affect the water column.
>
> IF the calculated water column concentrations exceed applicable State water quality standards, THEN the model must be re-run using elutriate concentrations.

Chemical concentrations of bioaccumulative contaminants of concern and total organic carbon (TOC) in the dredged material and disposal site sediment are used as input to the TBP model to determine the **potential** for benthic bioaccumulation in the dredged material, relative to the disposal site:

IF the calculated TBP from the proposed dredged material is not greater than that of the disposal site material, THEN benthic bioaccumulation testing for the specific contaminant is not required.

IF the calculated TBP from the proposed dredged material exceeds that of the disposal site material, THEN a benthic bioaccumulation test is required.

The TBP decision point is limited to non-polar organic contaminants and sediments having TOC greater than 0.4 percent.

Sediment bulk chemical data is also used in conjunction with other information as follows:

- to develop or modify the contaminants of concern list,
- to indicate distribution of sediment contaminants for the delineation of management units for subsequent sampling, and
- identify appropriate control and disposal site sediments.

3.3.5 Chemical analysis of water and elutriate

The results of the standard elutriate tests serve as the input to the mixing model for determining if the dredged material discharge will exceed applicable State water quality standards, after allowing for mixing:

IF the calculated water column concentrations of all contaminants of concern at the edge of the mixing zone are within applicable State water quality standards and IF no interactive effects are suspected, THEN the proposed dredged material discharge should not adversely affect the water column.

IF the calculated water column concentrations exceed applicable State water quality standards outside the mixing zone, THEN the discharge would not be in compliance UNLESS the State waived 401 certification.

IF State water quality standards do not exist for all contaminants of concern, or IF interactive effects are suspected among parameters, THEN water column impacts must be evaluated by the bioassays in Tier 3.

Chemical data for elutriates can also be used to identify potential non-contaminant impacts to biological test conditions (i.e., ammonia toxicity). Elutriates prepared for biological testing are routinely monitored to assure that test conditions are maintained within acceptable limits.

3.3.6 Water column toxicity tests

If a contaminant determination is not reached in Tier 1, and there are potential interactive effects of dredged material contaminants, the impacts of the dredged material discharge on the water column will have to be assessed in Tier 3. Appendix G describes protocols for water column toxicity tests for three organisms and two exposure periods. The GLTEM recommends that only one test organism need be utilized for a Tier 3 assessment, either *Daphnia magna* or *Ceriodaphnia sp.* and that only the acute (short-term) exposures and survival end-point be used at this time.

The GLTEM suggests that the water column toxicity tests be first run only with the 100-percent elutriate, and interpreted as follows:

> IF the survival in the 100-percent elutriate treatment is not statistically different from the dilution water using a two-sample t-test, THEN the elutriate is predicted not to be acutely toxic to water column organisms.
>
> IF the survival in the 100-percent elutriate treatment is greater than 50 percent, AND the calculated elutriate concentration at the edge of the mixing zone is less than 0.01 of the 100-percent elutriate, the dredged material discharge is predicted not to be acutely toxic to water column organisms outside the mixing zone.

If the survival in the 100-percent elutriate treatment is less than 50 percent, the water column tests must be rerun using a dilution series in order to calculate the LC_{50}. The mixing model is then used to calculate the concentration at the edge of the mixing zone:

> IF the concentration at the edge of the mixing zone is less than 0.01 of the LC_{50}, the dredged material discharge is predicted not to be acutely toxic to water column organisms outside the mixing zone.
>
> IF the concentration at the edge of the mixing zone is greater than 0.01 of the LC_{50}, the dredged material discharge is not in compliance.

3.3.7 Benthic bioassays

If a contaminant determination is not reached in Tier 1, and there are potential interactive effects of dredged material contaminants, the impacts of the dredged material discharge on benthic organisms will have to be assessed in Tier 3. Appendix G

describes protocols for two benthic organisms; *Chironomus tentans* and *Hyalella azteca*. The GLTEM recommends that both test organisms should be utilized for a Tier 3 assessment, and that survival (both organisms) and growth (*C. tentans* only) end-points be measured.

The results of benthic bioassays with the proposed dredged material are statistically compared to those of the disposal site material. Evaluations are made using Fisher's Least Significant Difference (LSD) when the response of two samples means is being compared. The LSD is usually performed following with analysis of variance (ANOVA). When parametric tests are not appropriate for multiple comparisons because the normality assumption is violated, nonparametric procedures should be employed.

The results for survival and growth are evaluated independently:

IF the mean survival of either test organisms exposed to the proposed dredged material is not less than that with the disposal site material by more than 10-percent (20-percent for *C. tentans*), OR the survival of either test organisms exposed to the proposed dredged material is not statistically less that with the disposal site material, THEN the dredged material should not adversely affect the benthos.

IF the mean survival of either test organisms exposed to the proposed dredged material is less than that with the disposal site material by more than 10-percent (20-percent for *C. tentans*), AND the survival of either test organisms exposed to the proposed dredged material is statistically less that with the disposal site material, THEN the dredged material would have unacceptable adverse impacts on benthos.

IF the mean weight of *C. tentans* exposed to the proposed dredged material is equal to or greater than 0.6 mg/organism, OR is not less than that with the disposal site material by more than 10 percent, OR is not statistically less than that with the disposal site material, THEN the dredged material should not adversely affect the benthos.

IF the mean weight of *C. tentans* exposed to the proposed dredged material is less than 0.6 mg/organism, AND is less than that with the disposal site material by more than 10 percent, AND is statistically less than that with the disposal site material, THEN the dredged material would have unacceptable adverse impacts on benthos.

Unacceptable survival for either test organism or for *C. tentans* growth will produce a negative determination.

3.3.8 Bioaccumulation tests

If a contaminant determination is not reached in Tier 1, and there are bioaccumulative contaminants of concern, and if the results of TBP model analysis in Tier 2 indicates the potential for unacceptable bioaccumulation, the impacts of the dredged material discharge on benthic bioaccumulation will have to be assessed in Tier 3. Appendix G describes a test protocol for benthic bioaccumulation in *Lumbriculus variegatus*.

The concentrations of bioaccumulative contaminants of concern in the tissues of the organisms exposed to the dredged material are compared to those in organisms exposed to the disposal site material:

> IF the contaminant concentrations in the tissue exposed to the dredged material does not statistically exceed that of tissue exposed to disposal site material, THEN the dredged material should not have unacceptable bioaccumulation impacts.
>
> IF the contaminant concentrations in the tissue exposed to the dredged material is statistically greater than that of tissue exposed to disposal site material, THEN the dredged material would have unacceptable adverse impacts on benthos.

3.3.9 Tier 4 site specific testing

Testing procedures and decision criteria for Tier 4 will be developed jointly by the USACE and USEPA for project specific applications. In most cases, the decision criteria will be similar to those used in Tier 3, based on a comparison of biological effects of organisms exposed to dredged material and disposal site material or the responses of organisms exposed to dredged material elutriate preparations.

3.4 Data Quality Indicators

Data quality indicators (DQIs) are measurable attributes that are used to assess if the necessary quality of data was attained. Indicators include sensitivity, accuracy, precision, completeness, representativeness and comparability. Acceptance limits for the DQIs for each measurement represent a minimum standard of performance required of project design, equipment, or methods.

Acceptance criteria for project DQIs should be specified in project planning documents as well as associated contractual

documents. When performance does not meet these acceptance criteria, corrective actions should be initiated immediately. Corrective action should also be initiated when seven or more results within acceptance criteria form a trend. If acceptable performance cannot be obtained, the samples and/or measurements may be qualified or invalidated during internal verification or external validation. Only valid data can be interpreted and assessed prior to making decisions. A detailed discussion of data quality indicators is provided in Attachment E-1.

For the GLTEM, the minimum acceptance limits for DQIs correspond to the QC acceptance criteria stated in the protocols in Appendices F and G. These protocols are summarized in table E-2. Tables E-3 through E-6 summarize the sensitivity or method detection limit, precision, and accuracy for the measurements in Appendices D, F and G. These DQIs should be suitable for most dredged material evaluations. However, DQIs may have to be modified or established for specific measurement needs. For project measurements which have more than one intended use, the stricter DQI requirements should generally apply.

Table E-2 Standardized Methods in Appendices F and G

Parameter	Water/ Elutriate	Sediment
Total solids	-	+
Particle size	N/A	+
Total volatile solids	+	+
Specific gravity	-	+
Total dissolved solids	+	-
Total suspended solids	+	-
Ammonia-nitrogen	+	+
Cyanide, Total	+	+
Arsenic, Total	+	+
Cadmium, Total	+	+
Chromium, Total	+	+
Copper, Total	+	+

Table E-2 Standardized Methods in Appendices F and G (continued)

Mercury, Total	+	+
Nickel, Total	+	+
Lead, Total	+	+
Zinc, Total	+	+
Parameter	Water/ Elutriate	Sediment
Total organic carbon	+	+
Total phenols	+	+
Total petroleum hydrocarbons	+	+
Total PCBs and pesticides	+	+
Polynuclear aromatic hydrocarbons	+	+
Ceriodaphnia dubia	+	-
Chironomus tentans	-	+
Daphnia magna	+	-
Hyalella azteca	-	+
Pimephales promelas	+	-
Lumbriculus variegatus	-	+

N/A = not applicable

Guidance for setting DQIs for non-typical measurements is discussed in Section 3.5. Additional DQI guidance is provided in USEPA (1993d) and Sturgis (1990).

3.4.1 Field measurements

General guidance on field measurements associated with sediment sample collection is provided in Appendix D. No specific DQIs have been developed for field measurements associated with Great Lakes dredged material evaluations. USACE districts may establish DQIs for field measurements as part of SOPs for sediment sampling.

Table E-3 Data Quality Indicators for Physical Characterization of Sediment

Measurement	Intended Data Uses	MDL[a]	Precision	Accuracy
Particle size	• determine exclusion from testing • input variable to mixing model • compare dredging and disposal sites • choose control sediment for bioassays	0.001g	RPD ≤10% each fraction	N/A
Specific gravity	• input variable to mixing model • compare dredging and disposal sites	0.001g	≤ 10%	N/A
Total volatile solids (%, dry)	• determine exclusion from testing • input variable to mixing model • compare dredging and disposal sites	0.001g	≤ 10%	N/A
Total solids (%)	• input variable to mixing model • for calculating dry weight results	0.001g	≤ 10%	N/A

[a] method detection limit determined by sensitivity of balance (1 mg)

Legend: N/A=not applicable
RPD=relative percent difference between duplicates

Table E-4 Data Quality Indicators for Physical Characterizations of Water/Elutriate

Measurement	Intended Data Uses	Sensitivity	Precision	Accuracy
Total dissolved solids (mg/l)	• input parameter to mixing model • monitor biological test conditions	0.001g[a]	≤ 10%	N/A
Total suspended solids (mg/l)	• input parameter to mixing model • monitor biological test conditions	0.001g[a]	≤ 10%	N/A
Total volatile solids		0.001g[a]	≤ 10%	N/A
Hardness (mg/l $CaCO_3$)	• adjust chemical elutriate concentrations of Cd, Cu, Cr^{+3}, Pb, Ni, Zn (criteria @ 100 mg/l, std tables and regression equations exist) • monitor biological test conditions			
pH	• adjust chemical elutriate concentrations of ammonia and phenols • monitor biological test conditions			
Dissolved oxygen	• monitor biological test conditions			
Temperature	• monitor biological test conditions			

[a] method detection limit determined by sensitivity of balance (1 mg)

Legend: N/A=not applicable

Table E-5 Data Quality Indicators for Chemical Composition of Sediments

Measurement	Intended Data Uses	MDL(dry weight)	Precision	Accuracy[a]
Ammonia-N	• determine exclusion from further testing • input variable to water quality screening model • compare dredging and disposal sites	0.1 mg/kg	≤ 20%	± 15%
Arsenic (total)	• same as ammonia-N	1 mg/kg	≤ 20%	± 15%
Cadmium (total)	• same as ammonia-N	1 mg/kg	≤ 20%	± 15%
Chromium (total)	• same as ammonia-N	20 mg/kg	≤ 20%	± 15%
Copper (total)	• same as ammonia-N	5 mg/kg	≤ 20%	± 15%
Lead (total)	• same as ammonia-N	10 mg/kg	≤ 20%	± 15%
Nickel (total)	• same as ammonia-N	15 mg/kg	≤ 20%	± 15%
Mercury (total)	• same as ammonia-N	2 μg/kg	≤ 20%	± 15%
Zinc (total)	• same as ammonia-N	30 mg/kg	≤ 20%	± 15%
Total cyanide	• same as ammonia-N	2 mg/kg	≤ 20%	± 15%
Total organic carbon	• same as ammonia-N • input parameter to TBP model	0.1%	≤ 20%	± 15%
Total petroleum hydrocarbons	• same as ammonia-N • indicator parameter for presence of PAHs	5 mg/kg	≤ 20%	± 15%[b]
Total phenols	• same as ammonia-N • input parameter to TBP model	0.1 mg/kg	≤ 20%	± 15%[b]
Total polychlorinated biphenyls	• same as ammonia-N • input parameter to TBP model	10 μg/kg[c] 1 μg/kg	≤ 25%	± 30%[b]
Polynuclear aromatic hydrocarbons	• same as ammonia-N • input parameter to TBP model	50 μg/kg	≤ 25%	± 30%[b]

[a] Accuracy within (±) of known or certified value, whichever is larger.

[b] Lab control sample recommended be developed for accuracy check with acceptance limit of ± 3 standard deviations from mean value.

[c] MDL for pesticides.

Table E-6 Data Quality Indicators for Chemical Composition of Water/Elutriates

Measurement	Intended Data Uses	MDL[a]	Precision	Accuracy
Ammonia-N	• input variable to mixing model • compare to State water quality standard • monitor biological test conditions	30 μg/L	≤ 20%	± 15%
Arsenic (total)	• input variable to mixing model • compare to State water quality standard	75 μg/L[b]	≤ 20%	± 15%[b]
Cadmium (total)	• same as Arsenic	1 μg/L 4 μg/L[c]	≤ 20%	± 15%[b]
Chromium (total)	• same as Arsenic	1 μg/L 7 μg/L[c]	≤ 20%	± 15%[b]
Copper (total)	• same as Arsenic	1 μg/L 6 μg/L[c]	≤ 20%	± 15%[b]
Lead (total)	• same as Arsenic	50 μg/L	≤ 20%	± 15%[b]
Nickel (total)	• same as Arsenic	25 μg/L	≤ 20%	± 15%
Mercury (total)	• same as Arsenic	0.2 μg/L	≤ 20%	± 15%[b]
Zinc (total)	• same as Arsenic	20 μg/L	≤ 20%	± 15%
Total cyanide	• same as Arsenic	5000 μg/L	≤ 20%	± 15%
Total petroleum hydrocarbons	• indicator parameter for PAHs	100 μg/L	≤ 20%	± 15%
Total phenols	• same as Arsenic	50 μg/L	≤ 20%	± 15%
Total polychlorinated biphenyls	• same as Arsenic	.01 μg/L	≤ 25%	± 30%
Polynuclear aromatic hydrocarbons	• same as Arsenic	10 μg/L	≤ 25%	± 30%

[a] Single values shown represent MDL for metal by ICP.
[b] Same limits for both ICP and GFAA.
[c] MDL for ICP, which is acceptable if value is < criteria.
[d] Detection limit for individual congeners.

3.4.2 Sediment sample collection

Field blanks and duplicate samples are commonly used to assess sampling precision and accuracy for many environmental media, but neither are recommended for routine dredged material sampling because of the difficulty in interpreting results and the non-homogeneity of sediments. Representativeness is the primary DQI for sediment sampling, and rationale behind most of the procedures for management unit delineation, collection, and sample homogenization recommended in Appendix D.

3.4.3 Physical and chemical analyses

Minimum acceptable levels of sensitivity, precision and accuracy for physical and chemical analyses of sediment, water, elutriates and tissues as part of Great Lakes dredged material evaluations are listed for each method in Appendix F and summarized on tables E-3 through E-6. The chemical analytical procedures were selected, in part, because of their ability to reliably measure chemical concentrations at background levels representative of the Great Lakes waters and sediments.

3.4.5 Toxicity and bioaccumulation tests

Procedures and acceptance criteria for sensitivity (reference toxicants), precision (minimum number of replicates) and accuracy (organism verification and test conditions) are listed in Appendix G.

3.4.6 Model evaluations

The "Inland Testing Manual" and GLTEM utilize two models to predict water column impacts and bioaccumulation potential. The sensitivity and accuracy of model calculations cannot be evaluated in the traditional sense since the sensitivity of the output to changes in the input(s) will vary with the function of the input variable(s) in the algorithm. The sensitivity of a particular output will depend on the dominant input variable(s) for a project, and has to be evaluated on a parameter-specific basis.

Precision of model outputs should be calculated by using each replicate data point rather than the average of the replicates. A minimum acceptable level of precision for the two models does not exist. However, if a sufficient number of replicates were tested, minimum acceptable levels of precision can be determined using a statistical test for outliers. This is beyond the scope of most dredged material evaluations.

3.5 Special Project Needs and Alternate Procedures

During the planning of a project, it may become evident that modified or new procedures will be required. Reasons for requiring new or modified procedures include:

- sediment sampling procedures recommended in Appendix D are not feasible or will not satisfy project DQOs,
- contaminant of concern list includes parameter(s) for which an approved analytical method is not provided in Appendix F,
- matrix effects have limited the usability of results generated using the approved methods in Appendix F, and
- any Tier 4 testing.

For projects requiring new or modified procedures, additional lead-time will be needed for planning, documentation and coordination. The data quality objective process (discussed in Section 2.4) should be completed to ensure appropriate procedures and associated QA/QC are chosen.

Standard methods are easier to incorporate into a project than method modification or new method development. "Standard methods" are published methods which have been approved by a recognized authority and may generally be incorporated directly into project documents. Modified and new method performance must be evaluated prior to QAPP preparation. Method modification and development typically require special contract-SOWs.

It is important to distinguish method modifications from options stated in the method. Modifications are changes to specific instructions in the method and may affect the validity or quality of results. Options are variations, allowed at the user's discretion, which should not affect the validity of results if appropriate performance is maintained.

Permittees or USACE contractors may propose alternative standard procedures to those in Appendices D, F, and G of the GLTEM. Detailed descriptions of the alternative methods and demonstration of their ability to meet project DQOs should be submitted to the USACE for review and approval prior to their use. The USACE may consult with the USEPA on alternate method acceptance and can dismiss data not obtained by accepted procedures.

3.5.1 Setting decision criteria

The decision criteria for data utilized in Tiers 1, 2, and 3, as discussed in Section 3.3, are not changed for data collected using alternate methods. For Tier 4 evaluations there

are no specific protocols recommended, and project-specific decision criteria will have to be developed for all tests utilized. In most cases, Tier 4 decision criteria will be based on comparisons of results with dredged material and disposal site material.

3.5.2 Selection of methods and setting DQIs

The selection of DQIs and methods are inherently related. Very often, the available method(s) is the determinant for sensitivity/method detection limit, comparability and representativeness as well as to a lesser extent, precision, accuracy, completeness.

Sample collection and handling: The primary DQI considered in selecting sampling equipment and procedures is sample representativeness. Refer to section 4 of Appendix D for guidance in choosing appropriate sample handling equipment and techniques.

Physical and chemical analytical methods: Parameters which are not included in Appendix F should be analyzed using a "standard method", if available. The "Inland Testing Manual", which has a more extensive list of parameters than the GLTEM, should be consulted for method recommendations. For parameters not discussed in the "Inland Testing Manual", methods approved for the Clean Water Act (Federal Register Volume 49, Number 136, October 26, 1984) or the Resource Conservation and Recovery Act (Federal Register Volume 58, Number 167, August 31, 1993) may be appropriate, depending on the constituent and matrix. Other possible method references are USEPA (1979; 1983; 1991b; 1993e; 1993h), Plumb (1981), and APHA/AWWA (1993).

Modifications of "standard procedures" may be needed to achieve a lower MDL, measure a new analyte, remove interferences, and validate a method for a new sample matrix. Lower MDLs can be attained by increasing sample size and concentrating the sample into a smaller volume. Interferences can be physically removed from the sample prior to analysis, or by manipulations during or after analysis. Physical removal of interferences typically requires additional "clean-up" steps and associated QC be performed. A new analyte may be measured in a sediment matrix using a modification of procedures used for water and wastewater analysis if sediment preparation and appropriate clean-up procedures are included.

Biological effects-based tests: Modifications to the toxicity and bioaccumulation tests described in Appendix G and new tests for Tier 4 application should not be pursued without USACE and USEPA coordination. The "Inland Testing Manual" has a

listing of alternate test organisms which may be considered, although not all are appropriate for application to Great Lakes dredged material evaluations. Other possible method references include USEPA (1993i; in prep) and ASTM (1993).

3.5.3 Review and approval of new or alternate methods

Standard operating procedures: Modified standard methods and new methods developed should be documented as an SOP. Guidance for preparing SOPs is provided in Attachment E-2. Protocol format should be similar to those in the Appendices D, F and G. The procedure to be used to validate the method should be described in detail. Criteria for "acceptable method performance" should be included in the procedure. Both the type and amount of data, and the acceptance criteria should be set by reviewing project data quality objectives.

For alternate standard methods not in Appendices D, F, and G, laboratories may prefer to substitute the SOP with a reference to the method manual and procedure number(s) and an addendum page specifying any options listed in the method.

Method verification and validation: Modified and new sampling procedures should be tested prior to collection of samples, if reasonably possible. The verification of performance is not as rigorous as the validation procedure for laboratory tests. Performance of the sampler is typically assessed in terms of percent sample recovery and reproducibility. Bias should be determined by comparing samples collected with two or more different types of samplers.

For modified standard methods, a single laboratory evaluation should be performed which include the following:

1) Identifies the limits of reliable measurement. Two concentrations should be selected, one near the lower and one near the upper end of the response range. Four to ten replicates of each concentration should be analyzed to verify that sensitivity, precision and accuracy do not deteriorate at either extreme.

2) Identifies method precision and accuracy using a single concentration of a standard reference material. Four to ten successive analyses (i.e., a series that yields valid responses by following the method protocol) are typically conducted for each step. The determination of method precision, for example, requires that ten successive independent analyses be conducted on the same sample material. Multistage calculations to determine the required number of analyses might be conducted as more information becomes available on the expected variance. However,

10 analyses will allow the test laboratory to estimate the standard deviation to within 45% of its true value (at a 95% confidence interval). Each value must represent a valid test response and, therefore, includes whatever QC analyses (e.g. blanks, replicates. etc.) are required in the original method protocol to ensure a valid test response.

3) Have performance-based matrix-specific QC data to evaluate data quality parameters such as precision, accuracy, uncertainty, completeness, representativeness, and comparability. This includes, as a minimum:

- MDL or reference toxicant study,
- method blanks or negative control,
- matrix spike or analysis of test materials and associated mean/percent recovery data for at least three representative types of materials,
- standard deviation data from replicate analyses ($n \geq 3$),
- calibration or response range, and
- method interferences and limitations.

Full validation of new methods requires:

1) Evaluating performance during single-laboratory testing.

2) Identification of procedural variables that must be carefully controlled (ruggedness testing).

3) Evaluating method sensitivity by sequential analysis.

4) Evaluating systematic error (bias). Tested materials should include certified reference materials or reference materials, or synthetic samples based upon availability of each material for the specific test.

5) Using performance-based matrix-specific QC data to calculate false positive and false negative rates as a function of concentration and uncertainty as a function of concentration.

6) Multi-laboratory (minimum of 3 labs) confirmation testing.

Review and approval: The results of method verification/validation should be documented and submitted with the proposed SOP to the USACE for review and approval. The USACE will coordinate the review with the USEPA and other experts, as necessary.

3.6 Quality Assurance Project Plans

As stated in Section 2.5, the purpose of Quality Assurance Project Plans (QAPPs) is to document how QA/QC activities are planned, implemented, and assessed during the life cycle of a project. Since 1980, the USEPA has required a QAPP format that follows the 16 essential elements. Use of a standard format promoted consistency between projects and expedited preparation and review of the documents. However, the development and review of a QAPP does represent a significant effort.

QAPPs have not been routinely prepared for dredged material evaluations, and the time and effort required for developing and coordinating traditional QAPPs are beyond the resources of typical dredging projects and would cause unacceptable delays in Section 404 permit decisions. However, the complexity and cost of testing procedures required by the GLTEM necessitate that quality assurance procedures be documented in some form.

3.6.1 Modified QAPP format

A modified QAPP format has been adopted for Great Lakes dredged material evaluations which provides the same information as the traditional 16-sectioned QAPP, but gives project managers flexibility in how and where this information is documented. The project manager always has the option of generating a traditional 16-sectioned QAPP.

The modified QAPP format was developed to minimize the duplication of information by allowing the GLTEM and other project documents containing the relevant information to be cited. Several project documents are developed which may contain the information about the proposed dredging and disposal, data collection implementation, and quality assurance, including:

- Tier 1 evaluation reports,
- data collection plans (DCPs),
- project coordination documents, and
- scopes of work (SOWs) for contracts.

For many projects, the majority of the QAPP can be developed simply by cross-referencing the 16 critical elements with existing project documents. The elements of the modified QAPP and possible information sources are summarized on table E-7. A more detailed discussion of the QAPP contents is provided in Attachment E-3.

3.6.2 Applicability

This modified QAPP format is applicable to the majority of proposed dredged material discharge projects, where the DQOs,

Table E.7 QAPP Element Content and Sources

Element	Description	Contents	Potential Sources
1	Title and signature page	• signatures of project manager, QA coordinators, field and lab managers	• original
2	Table of contents	• self evident	• original
3	Project description	• description of proposed dredging and disposal actions • background information (see Section 3.7 of GLTEM) • objectives of dredged material evaluation • project decisions and decision criteria • sampling plan	• Tier 1 evaluation report • DCP
4	Project organization and responsibility	• organization plan which identifies key personnel and assigns responsibilities for implementation	• QAMP • DCP
5	Sampling and measurement quality objectives	• DQIs	• Appendices D, F & G • DCP or SOW (for modified or new procedures)
6	Sample collection and handling procedures	• sampling equipment and procedures • sample containers • sample handling and storage	• Appendix D • DCP • SOW (contract)
7	Sample documentation, custody and tracking	• sample labeling and documentation • chain-of-custody procedures • bulk sample transfer/distribution	• Appendix D • DCP • SOW (contract)
8	Calibration procedures and frequency	• identify analytical equipment or instruments • describe calibration procedures	• Appendices D, F & G • DCP or SOW (for modified or new procedures)
9	Field and laboratory measurement procedures	• SOPs for analytical methods	• Appendices D, F & G • DCP or SOW (for modified or new procedures)

10	Internal quality control checks	• identify stages where QC checks are made to calculate DQIs • identify all QC samples and checks	• Appendices D, F & G • DCP or SOW (for modified or new procedures)
11	Data reduction, verification, deliverables and data validation and reporting	• describe reduction of raw data to final units • describe verification • describe validation procedures • specify reporting requirements	• Appendices D, F & G • DCP • SOW (contract)
12	Performance audits and systems inspections	• describe pre-award laboratory inspections and criteria • describe internal and external audits • reporting requirements and formats	• QAMP • DCP • SOW (contract)
13	Equipment/instrument maintenance and consumables inspection	• identify equipment or instruments requiring maintenance • describe maintenance protocols • verify availability of critical spare parts • discuss how repairs will be made • discuss how supplies and consumables are inspected and acceptance criteria	• Appendices D, F & G • DCP • SOW (contract)
14	Procedures to assess data usability	• describe procedures to assess data usability for project decision • describe procedures to assess data acceptability for contract payment	• QAMP • DCP • SOW (contract)
15	Corrective action	• list activities potentially requiring corrective action • describe mechanism to implement corrective actions • format for reporting	• Appendices D, F & G • DCP • SOW (contract)
16	Quality assurance reports	• describe QA reports to management	• QAMP

DQIs, and procedures of the GLTEM and appendices are utilized without significant modification. This approach may also be applicable for projects using other "standard methods", if the method SOP contains all of QAPP-required method and QC information.

For projects involving substantial modifications to approved methods, or new methods requiring extensive outside review or compilation of information (i.e., non-typical parameters, site-specific or Tier 4 testing), a traditional 16-sectioned QAPP may be efficacious.

3.7 Data Quality Assessments

A DQA is a quantitative process that focuses on whether the data can be used to make project decisions and, if not, what the use limitations are. DQA applies to all types of validated environmental data, including field measurements and model results. How DQA is performed and by whom, should be specified in each project QAPP.

Validated data should be assessed for compliance with project DQOs. Special emphasis should be placed on how overall DQIs (e.g., sensitivity, precision, accuracy, completeness, representativeness, comparability) were derived from the data. The data assessor should compare the precision and accuracy achieved with that required to verify that the measurement system was in control and met the project objectives. The degree of precision and accuracy serve as an estimate of the uncertainty, and influence the level of confidence with which decisions are made. Audit findings and corrective actions should be reviewed since they may affect the reported error estimations and place limits on the uses of certain sample values.

Data completeness can be assessed for two purposes; compliance with a contract scope of work, and compliance with the amount of data required for decision making. The first assessment is made to determine if the terms of a contract have been fulfilled prior to payment. The completeness of the final valid data set is assessed to determine if sufficient information is available to make a determination with the required degree of confidence.

The data assessor must verify that the field design, sample collection and handling, laboratory subsampling and analysis were performed according to criteria and procedures identified in the QAPP. In addition, each type of measurement should be compared with previous information and correlated with other project data to check the reasonableness and validity of results. Statistical and graphical methods may be used for such comparisons.

One common test is the "outlier test" which verifies that all values of the set statistically "belong". Depending on the importance of the data and project requirements, outliers may be accepted and identified or rejected and selectively removed. If the reason for an outlier can be explained, it can generally be removed from a data set. Outliers removed from a data set must be reported and the reasons for their removal justified. Data may be analyzed with and without outliers.

The DQA should be documented as part of the final report on project data and interpretation.

3.8 Quality Assurance Program Assessments

Performance audits and system inspections of field and laboratory activities should be conducted to verify that work is in accordance with specified requirements. The type and frequency of audits conducted by personnel internal and external to the organization should be specified in project QAPP. These types of audits and inspections may be used by:

- contracting personnel to assess contractor capability and performance prior to contract award,
- project management and QA personnel to evaluate the quality of generated data and monitor the effectiveness of the project QA plan, as designated in the project QAPP, and
- contract personnel to monitor compliance with the organization's QA plan, contract SOWs, or project QAPPs.

Performance audits and system inspections should be conducted by individuals not directly involved in the process. Internal audits should be conducted by management and QA personnel in the organization responsible for performing the work. External audits may be conducted by the USACE or USEPA.

3.8.1 Pre-award laboratory inspections

Because dredged material testing for a project is typically conducted at one time, and because of the limited holding times for sediments, problems with laboratory performance discovered after testing has begun may not be correctable. If laboratory performance is not acceptable, sample collection and analysis may have to be repeated entirely. For these reasons, it is imperative that laboratory qualifications and performance be assessed before analysis is started.

Inspections and audits should be used to assess laboratory capability and performance prior to contract award. USACE regulations require that all laboratories performing work for the USACE be inspected prior to contract award. USACE districts will

also inspect contract laboratories for permit applicants, upon request. USACE guidance on laboratory contracting and inspections is provided in USACE (1988) and Sturgis (1990). USACE district QAMPs may include more specific requirements for laboratory inspections. General guidance on laboratory inspections is also found in ISO/IEC (1990) and USEPA (1991a).

Laboratories should be required to have documented records of performance for all methods to be employed. If the laboratory has proposed to conduct a method it has not previously used, or has insufficient performance records, an initial performance study should be conducted for each method prior to analysis of samples. The initial performance study should be repeated any time there is a major change in equipment or in the method.

For analytical procedures, the initial performance study typically consists of assessing precision and accuracy for 4-7 replicates for samples spiked at 10x the MDL. The procedure should be written in the SOP along with initial acceptance criteria and triggers for repeating the study.

For toxicity tests, intralaboratory precision of the range for the test should be determined by performing five or more tests with different batches of test organisms, using the same reference toxicant, at the same concentrations, with the same test conditions, and same data analysis methods. A reference toxicant concentration series (0.5 or higher) should be selected that will consistently provide partial mortalities at two or more concentration of the test chemical.

For biological evaluations, the laboratory should also demonstrate its competence by conducting five control exposures. It is recommended that these five exposures be conducted concurrently with five reference tests. For whole sediment tests, laboratories should also demonstrate their personnel are able to recover an average of at least 90% of the organisms from whole sediment.

Blind performance samples (discussed below) should be used to evaluate laboratory performance prior to contract award, or at least prior to initiation of project testing, when there is still an opportunity to correct problems.

3.8.2 Project-specific assessments

Project-specific audits and inspections should be performed at the onset of field activities with periodic follow-up inspections to correct any deficiencies previously observed and to verify that QA procedures are maintained throughout the process. The focus of these audits and inspections is to evaluate

the degree of conformance of activities with the project QAPP and contract SOWs. Any problems encountered should be discussed with the project manager and conveyed to contracting personnel.

Performance audits: Audit samples (also known as blind samples) should be representative of samples to be analyzed under the contract, and should be of a known or calculable value with a 95% confidence interval (preferably a 95% tolerance interval) established using a technically valid analytical procedure(s). The USACE has established an interlaboratory testing program, involving analysis of identical samples by multiple laboratories in order to assess the continuing capability, performance, and progress of each participating laboratory (USACE 1989). If a laboratory has never participated in the program, the results from participation in other audit sample programs may be evaluated as an indicator of performance or other accreditations considered.

Audit samples may be included for analysis with project samples. The QA Coordinator compares the results with the known values and possibly with values from other laboratories. If performance is unsatisfactory, the data from that laboratory should not be accepted until adequate performance has been demonstrated.

Historically, performance evaluation samples for chemical laboratories have been prepared by fully homogenizing and repeatedly testing either contaminated environmental samples or clean samples spiked with certified reference materials or primary standards. Split samples for physical, chemical, and biological laboratories have been prepared from fully homogenized environmental samples. Audit samples for sediments and water can be obtained from commercial suppliers. Audit samples for sediments and water can also be obtained from the U.S. Army Engineer Waterways Experiment Station through the appropriate USACE district.

Split samples: Split samples are project samples which have been split for concurrent analysis by two or more laboratories (see discussion in Attachment E-1). Because of the lack of sample homogeneity, field-split samples are not generally recommended for sediments. Sediment samples which have been homogenized in the laboratory are more suitable for split sample analysis.

The contractor is typically responsible for splitting and sending samples to the USACE or referee laboratory. The contractor and referee laboratories transmit results to the QA Coordinator of the contracting organization, who analyzes these results and verifies that they are within the predetermined

acceptable range using paired T-tests or scatter plots of the two laboratories results.

If performance is unacceptable, the laboratory should repeat the split sample analysis as part of the next sampling event. If performance is unacceptable on the second split field sample analysis, the laboratory should evaluate instrument and QC procedures, make necessary changes, and repeat the split field sample analysis as part of the next sampling event. If performance is unacceptable on the third split field sample analysis, this non-performance may be considered as a contributory cause for termination for default of the contract. The laboratory typically bears the cost of non-acceptable performance.

Laboratory inspections: Laboratory inspections may be necessary after contract award and during project implementation to assure compliance with requirements specified in the SOW and verify implementation and effectiveness of the corrective actions suggested in previous audits. For indefinite delivery (open-end) contracts, laboratory inspections should be performed at least every two years after award to monitor continued adherence to requirements of the contract. Unresolved inspection deficiencies may be considered as a contributory cause for termination by default of the contract.

Field inspections: A representative of the contracting organization should be present during all field sampling activities to assure compliance with the SOW and QAPP.

Assessment reports: Audit and inspection reports should include the date of the evaluation, information reviewed, person performing the evaluation, findings and problems, and corrective actions recommended to resolve problems. Specific examples of non-compliance or nonconformity should be documented in the report as well as possible reasons for such deficiencies. These reports should be submitted to the project manager immediately following any internal or external on-site inspection or upon receipt of the results of any performance evaluation audits.

3.8.3 Data validation

Validation is an audit of data quality (ADQ) that determines if the data is of known quality, defensible, free of transcription errors, and complete. Validation applies to all types of environmental data, and the procedures and persons responsible for validation should be specified in the QAPP according to the organization's QAMP and GLTEM recommendations.

For Great Lakes dredged material evaluations, a minimum of

10% of environmental data or one sample per batch, whichever is greater, should be validated. General guidance on data validation procedures is provided in Attachment E-4. When problems are found during validation of a data set, the frequency should be increased. The recommended frequency for new measurements and methods, critical parameters, and difficult analyses is 25%.

Validation should be performed by an independent reviewer (i.e., external to the organization that collected or analyzed the samples) using approved, method-specific SOPs. USACE district QA Coordinators will validate data collected by their contractors and data provided by permit applicants using the guidance provided in Attachment E-4 and SOPs developed in district QAMPs.

3.8.4 Corrective action

Corrective action may be required for two classes of problems; procedural and non-compliance. Procedural problems include equipment failures, breaks in custody, and documentation errors. Nonconformance with the established QA procedures in the QAPP or DCP should be identified and corrected in accordance with procedures in the QAPP and associated SOPs. Noncompliance problems include unapproved changes in sample design, data anomalies, and audit failures. A formal corrective action program should be determined and implemented when a noncompliance problem is identified.

The need for corrective action is identified by technical personnel who perform the daily activities. If the problem persists or cannot be resolved, the matter is referred to management and QA personnel for further investigation. Technical staff should not initiate corrective action without prior approval through the proper channels. Management should approve the change in writing or verbally prior to implementation, if feasible, through the same channels. Management is responsible for ensuring that corrective action are initiated by:

- evaluating all reported nonconformances,
- controlling additional work on nonconforming items,
- determining disposition or action to be taken,
- maintaining a log of nonconformances,
- reviewing nonconformance reports and corrective actions, and
- ensuring nonconformance reports and corrective action memos are included in the project file.

If corrective actions do not correct the problem, the manager should stop work until successful corrective action can be taken.

Corrective action for field sampling may include:

- recollecting the sample,
- sampling at a different location, or
- using a different sampling device/procedure,

Corrective action for field measurements may include:

- repeat the measurement to verify the error,
- check for proper adjustment for ambient conditions,
- check batteries,
- check calibration, and
- replace the instrument or measurement device.

Laboratory corrective action is dependent on the type of analysis and the event. Laboratory personnel are alerted that corrective actions may be necessary if:

- samples are received in improper/leaking containers without proper preservation or documentation,
- quality control data are outside the warning or acceptable windows for precision and accuracy,
- blanks contain target analytes or negative controls have responses above acceptable levels,
- undesirable trends are detected in spike recoveries or positive controls, or precision between replicates,
- there are unusual changes in method detection limits or organism sensitivity,
- performance and/or system deficiencies are detected by the QA personnel during internal or external audits, or
- inquiries concerning data quality are received.

Corrective actions for data management may be necessary during data review and data validation, such as:

- obtaining missing information or recovering lost data,
- recalculate data, or
- correcting transcription errors on forms, reports, and databases.

After assessing the data, the project manager may decide to repeat sample collection and/or analyses based on the extent of the deficiencies and their importance in the overall context of the project. Issues which may trigger additional work are:

- insufficient or nonrepresentative samples,
- samples lost due to breakage, loss of integrity (e.g., lack of preservation, exceed holding time) or insufficient volume for testing, or
- method not "in control", producing invalid results.

Nonconformances and corrective actions should be documented in field and laboratory log books. Changes may be requested verbally or by change request forms that are signed by the initiators and management. Nonconformance reports and corrective action memos should be prepared by field or laboratory management, and describe the nonconformance or noncompliance and its significance, recommended solution(s), results of corrective actions, and alternative corrective action (if necessary). Reports and memos should be submitted directly to the project manager. Nonconformance and corrective actions records should be sent with project results to the data validator.

4. REFERENCES

American Public Health Association (APHA), American Water Works Association (AWWA), Water Pollution Control Federation (WPCA). 1993. Standard methods for the analysis of water and wastewater, 18th edition, APHA/AWWA/WPCF, Washington, DC.

American Society of Testing Materials (ASTM). 1993. Annual Book of ASTM Standards, Volume 11.04, ASTM, Philadelphia, PA.

International Standards Organization (ISO)/ (IEC). 1990. "General Requirements for the Competence of Calibration and Testing Laboratories", ISO/IEC Guide 25, Geneva, Switzerland.

ISO/IEC Guide 38 "General Requirements for the Acceptance of Testing Laboratories".

Karn, R.A. and A. B. Strong. 1989. "Quality assurance guidelines for organic analysis," Technical Report EL-89-18, Water Quality Research Program, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Moore, D.W., Dillion T.M., Word, J.Q., and Ward, J.A. 1994. "Quality assurance/quality control (QA/QC) guidance for laboratory dredged material bioassays; Results of QA/QC workshop held May 26-27, 1993, in Seattle, Washington," Miscellaneous Paper D-94- , U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

National Institute of Standards and Technology (NIST). 1992. "Use of NIST SRMs for decisions on performance of analytical chemical methods and laboratories," Special Publication 829, NIST, Gaithersburg, MD.

NIST. 1993. "Handbook for SRM Users," Special Publication 260-100, NIST, Gaithersburg, MD.

North Central Division (NCD). 1994. "Quality assurance management program (QAMP) for environmental data collection," NCD Regulation NCDR 1110-2-27, U.S. Army Engineer Division, North Central, Chicago, IL.

Plumb, R.H., Jr. (editor). 1981. "Procedures for the handling and chemical analysis of sediments and water samples," Technical Report EPA/ACE-81-1, prepared by State University College at Buffalo, Great Lakes Laboratory, Buffalo, NY for U.S. Environmental Protection Agency and U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Sturgis, T.C. 1990. "Guidance for contracting biological and chemical evaluations of dredged material," DOTS Technical Report D-90-10, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

USACE. 1970. "Laboratory soils testing," Engineer Manual EM 1110-2-1906, Department of the Army, Headquarters, Office of the Chief of Engineers, Washington, DC.

USACE. 1974. "Laboratory investigations and material testing," Engineer Regulation ER 1110-1-8100, Department of the Army, Headquarters, Office of the Chief of Engineers, Washington, DC.

USACE. 1976. "Water quality data collection, interpretation and application activities," Engineer Regulation ER 1130-2-415, Department of the Army, Headquarters, Office of the Chief of Engineers, Washington, DC.

USACE. 1979. "USACE work for others; Laboratory investigations and material testing," Engineer Regulation ER 1140-1-210, Department of the Army, Headquarters, Office of the Chief of Engineers, Washington, DC.

USACE. 1980. "Quality control of water quality field sampling activities," Engineer Technical Letter ETL 1110-2-252, Department of the Army, Headquarters, Office of the Chief of Engineers, Washington, DC.

USACE. 1986a. "Contracting guidelines for water quality and other chemical analyses," Engineer Technical Letter ETL 1110-2-298, Department of the Army, Headquarters, Office of the Chief of Engineers, Washington, DC.

USACE. 1986b. "Quality assurance of laboratory testing procedures," Engineer Regulation ER 1110-1-261, Department of the Army, Headquarters, Office of the Chief of Engineers, Washington, DC.

USACE. 1987. "Safety and Health Requirements Manual," Engineer Manual EM 385-1-1, Department of the Army, Headquarters, Office of the Chief of Engineers, Washington, DC.

USACE. 1988. "Water and wastewater laboratory inspections," Engineer Technical Letter ETL 1110-2-309, Department of the Army, Headquarters, Office of the Chief of Engineers, Washington, DC.

USACE. 1989. "Interlaboratory testing program for chemical analyses," Engineer Regulation ER 1110-1-1401, Department of the Army, Headquarters, Office of the Chief of Engineers, Washington, DC.

USEPA. 1979. "Methods manual for bottom sediment sample collection," EPA 905/4-85-004, Central Regional Laboratory, Chicago, IL.

USEPA. 1982. "Handbook for sampling and sample preservation of water and wastewater," EPA-600/4-82-029, Environmental Systems and Monitoring Laboratory, Cincinnati, OH.

USEPA. 1983. "Methods for the chemical analysis of water and wastes," EPA-600/4-79-020, Environmental Systems Monitoring Laboratory, Cincinnati, OH.

USEPA. 1987. "Quality assurance and quality control (QA/QC) for 301(h) monitoring programs; Guidance on field and laboratory methods," EPA 430/9-86/004, Marine Operations Division, Washington, DC.

USEPA. 1991a. "Manual for the evaluation of laboratories performing aquatic toxicity tests," EPA/600/4-90/031, Environmental Monitoring Systems Laboratory, Cincinnati, OH.

USEPA. 1991b. "Methods for the determination of metals in environmental samples," EPA/600-4-91/010, Office of Research and Development, Washington, DC.

USEPA. 1991c. "Preparation aids for the development of category II quality assurance project plans," EPA/600/8-91/004, Office of Research and Development, Risk Reduction and Engineering Laboratory, Cincinnati, OH.

USEPA. 1993a. "Content requirements for quality assurance project plans, Revision 4," R5-QAS-93-001, Region 5, Quality Assurance Section, Chicago, IL.

USEPA. 1993b. "EPA requirements for quality management plans," EPA QA/R-2, Quality Assurance Management Staff, Washington, DC.

USEPA. 1993c. "EPA requirements for quality assurance project plans for environment data operations," EPA QA/R-5, Draft, Quality Assurance Management Staff, Washington, DC.

USEPA. 1993d. "Guidance for planning for data collection in support of environmental decision-making using the data quality objectives process," EPA QA/G-4, Quality Assurance Management Staff, Washington, DC.

USEPA. 1993e. "Methods for the determination of inorganic substances in environmental samples," EPA/600-R-93/100, Office of Research and Development, Washington, DC.

USEPA. 1993f. "Quality assurance manual for environmental data collection, Region 2, Environmental Services Division, Edison, NJ.

USEPA. 1993g. "Test methods for evaluating solid waste: Physical/chemical methods," SW-846, Third Ed., Including Update 1, Office of Solid Waste and Emergency Response, Washington, DC.

USEPA. 1993h. "Methods for the determination of nonconventional pesticides in municipal and industrial wastewater," Revision I, Volumes I and II, EPA-821-R-93-010-A/B, August 1993.

USEPA. 1993i. "Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms," Fourth Edition, C.I. Weber (editor), EPA-600/4-90-027F, Environmental Monitoring and Systems Laboratory, Cincinnati, OH.

USEPA. 1994a. "Guidance for preparing, conducting, and reporting the results of management system reviews," QA/G-3, Quality Assurance Management Staff, Washington, DC.

USEPA. 1994b. "Model quality assurance project plan for water programs (check title), R5-QAS/WD-94-003, Region 5, Environmental Sciences Division and Water Division, Chicago, IL.

USEPA. in prep. "Methods for measuring the toxicity and bioaccumulation of sediment- associated contaminants with freshwater invertebrates," EPA 600/R-94/924, Environmental Research Laboratory, Duluth, MN.

USEPA/USACE. 1998. "Evaluation of dredged material proposed for discharge in waters of the U.S.-Testing manual (Inland Testing Manual)," EPA-823-B-98-004, Office of Water, Washington DC.

ATTACHMENT E-1
Data Quality Indicators

1. Sensitivity and Method Detection Limit (MDL)

Definitions of sensitivity and method detection limit (MDL) are different for analytical procedures which measure concentrations/levels, biological tests which measure effects, and models which simulate processes.

1.1 Field measurements

For reasonably stable field measurement equipment, the MDL may be synonymous with the sensitivity of the equipment. This is typically an inherent quality in equipment design and can be obtained from manufacturer's specifications. Be aware, however, that manufacturer's specifications are set under strictly controlled conditions and may not be achievable under field conditions.

1.2 Physical and chemical analyses

Physical characterizations vary in complexity from simple procedures whose sensitivity is limited by the inherent quality of the equipment (similar to simple field equipment) to methods using instruments for which MDLs are statistically calculated. Each laboratory should determine the MDL at least annually for each sample matrix in each method and for each instrument which performs the analysis at the laboratory. The MDL should be re-determined after major changes in the method or instrument. Most method protocols contain procedures to verify the MDL periodically (e.g. daily, weekly). The actual MDL for a given sample is never determined and may be higher than the laboratory MDL due to interferences in the sample or as a result of diluting heavily contaminated samples so the instrument response is within the linear, calibrated range.

For chemical analytical procedures, sensitivity is the smallest incremental change which can be detected. Method detection limit is the smallest concentration which can be determined with a known degree of confidence. The MDL, a procedure adopted by USEPA, is similar to the Limit of Detection (LOD) used by the American Chemical Society (ACS) but is calculated differently. The MDL should not be confused with an instrument detection limit (IDL) which does not reflect the entire method/protocol.

A second limit commonly associated with the MDL (LOD) is the Minimum Level (ML). The ML, a procedure adopted by USEPA, is

similar to the Limit of Quantification (LOQ) used by the ACS. Historically, USACE conservatively defines the LOQ as 10 times the standard deviation observed for the low-level standard or blank sample which is equivalent to 3.18x the MDL. In practice, the ML (LOQ) equals the lowest calibration point.

Both the MDLs and LOQs are specific to a laboratory. For any given protocol, the MDL and associated LOQs varies with equipment, sample volume processed, and sample matrix and complexity. For 404(b)(1) projects, MDLs should be one-fifth to one-tenth, but no greater than one-third, the appropriate value critical to the decision making process (i.e. the "action level"). The MDL should be the reporting limit (RL). Sample values above the MDL but below the ML/LOQ are considered to be estimated data and should be used as a qualitative indicator of "presence" rather than a "quantitative value".

Because precision and accuracy vary with concentration, some laboratories may prefer to evaluate and set parameter MDLs (LODs) and associated MLs (LOQs) to achieve a uniform level of precision and accuracy for all parameters.

1.3 Toxicity and bioaccumulation tests

The sensitivity of biological evaluations cannot be evaluated in the traditional sense since the test measures a 'net effect' rather than response to any one sample characteristic or set of known components. However, the sensitivity (i.e. dose response) of a species to individual reference toxicants or reference material can be quantified.

Contrary to analytical methods, reference toxicant tests are performed on a routine basis (at least monthly) to monitor the sensitivity of the in-house culture or verify the sensitivity of shipped organisms. The laboratory should calculate acceptable limits and control charts for each reference toxicant and test organism. Controls charts are used to evaluate the cumulative trend of results from a series of samples. Endpoints from five tests are adequate for establishing the control charts. In this technique, a running plot is maintained for the values from successive tests. Control limits ($\pm$2 SD) are recalculated with each successive test result. Outliers, which are values falling outside the upper and lower control limits, and trends of increasing or decreasing sensitivity, are readily identified using control charts. Tests conducted during the time the of the outlier reference toxicant test should be considered as provisional and subject to careful review.

1.4 Model calculations

The sensitivity of model calculations cannot be evaluated in the traditional sense since the sensitivity of the output to changes in the input(s) will vary with the function of the input variable(s) in the algorithm. The sensitivity of a particular output will depend on the dominant input variable(s) for a project, and has to be evaluated on a parameter-specific basis.

2. Accuracy

Accuracy is the degree of agreement of a measurement (or an average of replicate measurements), X, with an accepted reference or true value, T. Accuracy is expressed as the difference between the two values, X-T, or the difference as a percentage of the reference or true value, 100 (X-T)/T, and sometimes expressed as a ratio, X/T. For an unknown sample, it is impossible to determine the true accuracy of the measurement. Therefore, accuracy is assessed through the analysis of negative controls/ blanks and positive controls/knowns, with the assumption that the method was calibrated and "in control" during the measurement.

2.1 Field measurements

The accuracy of simple measurements varies with the type of measurement and equipment. Most instrument manuals will provide an estimate of instrument accuracy, which does not include sampling variability. Accuracy of some field measurements may be impossible to measure because there are no standards to serve as references.

2.2 Sediment sample collection

Sources of sampling bias and imprecision cannot be measured because no standards exist to serve as references. Inappropriate equipment and cross contamination are the two most common sources of error. Potential sampling error can be minimized by controlling sample design and collection protocols. Blank and duplicate samples, which actually are a measure of sampling precision, are used to assess sampling accuracy for some environmental media.

Trip blanks are used to assess the potential for contamination of samples due to contaminant migration during sample shipment and storage. A clean sample is taken from the laboratory to the sampling site and returned to the laboratory unopened. Typically, this type of blank applies only to liquid samples collected for volatile analysis. Trip blanks are collected at a frequency of one per cooler or a minimum of one per 20 samples, whichever is greater.

Field blanks (equipment rinsates) are analyzed to check for procedural contamination at the facility which may cause sample contamination. Field blanks consist of a pouring analyte-free water over decontaminated sampling equipment as a check that the decontamination procedures has been adequately carried out and that there is no cross-contamination of samples occurring due to the equipment itself.

Analysis of field blanks is performed for all analytes of interest, but is typically required only when aqueous samples are being collected. Field blanks are not required for solid samples because it is difficult to interpret results and the associated QC costs for analysis of a different sampling matrix (water) can be prohibitive. The need for field blanks may be avoided by using the sample container as the sampling device, and pre-rinsing the container with the sample prior to sample collection.

One field blank should be collected for each type of equipment used each day field decontamination is performed, but are required only for liquid matrices. The rinse must be performed sequentially on all pieces of equipment used in the sampling protocol. The field blank should be collected at the beginning of the day prior to the sampling event and that blank must accompany those samples which were taken that day, at a minimum frequency of one for every ten or fewer investigative samples. This is a necessary procedure so that the blank will be associated with the proper samples during data validation.

For trip or field blanks to be acceptable for use with the accompanying samples, the concentration in the blank of any analyte of concern must, typically, be no higher than the highest of either:

- the method detection limit,
- 5% of the action level for that analyte, or
- 5% of the measured concentration in the sample.

Blank values are never subtracted from sample results, but are reported separately.

2.3 Physical and chemical analyses

Accuracy for laboratory measurements is typically assessed by analyzing laboratory blanks and known or blind reference materials and, for organic analyses, performing matrix spikes on selected samples and adding surrogates for each sample. However before accuracy for any sample set can be assessed using blank, spike, surrogate and reference results, equipment calibrations must be performed and accomplished within the established limits to define the accuracy of the equipment. In addition, test-

specific performance checks monitor test conditions during analysis of the samples.

Calibration: Calibration may be defined as a comparison of a measurement standard or instrument with known accuracy with other standard or instrument to eliminate deviations by adjustment. Calibration accuracy is critically dependent on the purity and reliability of the standard; standards should be traceable to a national standard. Standards may be prepared in the laboratory from neat materials or purchased as a pre-mixed concentrate.

Calibration must be performed under the same instrumental and chemical conditions as those that will exist during the measurement process. Initially, a minimum of three different concentrations of calibration standards should be measured, preferably at least five. The concentrations of the calibration standards must bracket the expected concentration of the analyte in the samples. Where possible, the calibration curve should be generated by suitable regression analysis of the net signal for the concentration. No data should be reported beyond the range of calibration.

For organic analysis, calibration standards may be external or internal. External standards are typically the target analyte being detected and are analyzed separate from environmental samples. Internal standards are compounds which simulates the analyte of interest (e.g. deuterated isotope) that are added to each QC and environmental sample analyzed. The ratio of internal standard response to the analyte response at the same concentration is called the response factor. The response factor must be relatively constant over the calibration range if it is to be used to calculate analyte concentrations.

Another technique, typically used for metal analysis, is the method of standard addition where successive, increasing known amounts of analytes are added to the sample or aliquots of it. It is essential to shown either the spiked chemicals equilibrate with the corresponding endogenous ones, or that the recovery of the spiked chemicals is the same as the recovery of the contaminant from samples (within experimental error) over the full range of concentration levels to be analyzed.

The frequency of calibration and calibration checks depends on the type of calibration (e.g. internal or external), accuracy requirements, stability of the instrument, sample load for the laboratory. External calibrations may be performed daily, weekly, or even monthly. If external calibration is not performed daily, a minimum of two calibration checks (at the beginning and end of the day) should be made. Unstable systems

may require additional checks after every 10th sample.

Test-specific performance checks: Additional instrument and method performance checks are specific to the equipment and method. Instrument and method performance check procedures, frequency, acceptance criteria and corrective actions may be found in instrument manuals and the method protocol.

Method blanks: The method blank is used to document contamination resulting from the analytical protocol. A method blank is a matrix to which all reagents and preservatives are added in the same volumes or proportions used in sample processing. The method blank must be carried through the complete preparation and analytical protocol.

The minimum frequency of method blanks is one per batch of samples processed within a work shift. If more than 20 samples are included in a batch, analyze one for every 20 samples. This frequency should be increased to a minimum of 10% for new parameters and methods. The method blank is typically acceptable if the concentration of any analyte of concern in the matrix is no higher than the highest of either:

- the method detection limit,
- 5% of the action level for that analyte, or
- 5% of the measured concentration in the sample.

Blank values are never subtracted from sample results, but are reported separately.

Matrix spike: A matrix spike is an aliquot of sample (blanks do not require separate matrix spike or duplicate analyses) spiked with a known concentration of target analytes. The spiking occurs prior to sample preparation and analysis. The added concentration should not be less than the background concentration of the sample selected. Ideally, the fortified analyte concentrations should be 10 times the MDL or the action level, whichever is less. A matrix spike is used to document the bias of a method in a given sample matrix.

Matrix spikes should be analyzed at a minimum frequency of one per 20 samples or one per sample batch, whichever frequency is greater. This frequency should be increased to a minimum of 10% for new parameters and methods.

Warning and control limits should be established using the mean value from a minimum of 20 to 30 analyses. The warning limit should be $\pm$ 2 standard deviations of the mean and the control limit should be $\pm$ 3 standard deviations of the mean. After each five to ten new measurements (i.e. daily), new limits

should be calculated using only the most recent 20 to 30 data points. These limits should never exceed those determined during the initial performance study. When measurements fall outside established control limits, that method is judged out-of-control and the source of the problem should be identified and resolved before continuing.

Surrogates: For organic chemical analyses, surrogates provide information about the effectiveness of the method to recover and detect the analyte. A surrogate must be similar to the target analyte(s) in chemical composition and method behavior, but should not be found in environmental samples. The surrogates are added to every sample aliquot, calibration standard, and blank in known amounts before extraction and are measured with the same procedures used to measure other sample components.

The purpose of the surrogate analyte is to monitor method performance with each sample. The recovery of the surrogates in each sample and blank should be evaluated with respect to laboratory control limits (established using a procedure similar to that used for matrix spikes) and continuously tracked. Minimum percent recoveries for each analyte is typically 70-130%.

Reference samples: The Internal Standards Organization (ISO) defines two types of reference samples: reference materials and certified reference materials.

A reference material (RM), not to be confused with the disposal site material used in dredged material evaluations, is a material or substance with one or more properties which are sufficiently well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials (ISO 1989). It is important to note that a given RM cannot be used for more than one purpose. Separate RMs must be obtained from different sources (i.e., vendors or lots of material) for instrument calibration and internal QC. For monitoring instrument accuracy, reference materials should be analyzed at least quarterly as well as with each large batch of samples.

A certified reference material (CRM) is a reference material with one or more property values certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body (ISO 1989). CRMs provide a QC test on the entire analytical process from sample preparation to the final reporting of results. For this reason, CRMs are typically used to document the bias of the analytical process during method validation and to compare performance among laboratories.

CRM values should be obtained through multi-lab (typically $\geq$20 laboratories) analysis using the method(s) specified on the Certificate of Analysis accompanying each sample. CRM values should be calculated using the $\pm$95% tolerance interval (TI) rather than a $\pm$95% confidence interval (CI). The TI estimates the uncertainty for the individual user unlike the CI which is a measure of certification of the participating labs and not the CRM. The TI is typically broader (2-6x) than the corresponding CI. CRM values based on 2 times the standard deviation of the mean are not statistical and should not be used.

Sources of CRMs are listed in USEPA (1994b). When CRMs are not available, materials that have been fully homogenized and repeatedly tested can be used. These materials may be contaminated environmental samples or clean samples spiked with a certified reference materials (or primary standards). Currently, CRMs are not available for physical sediment characterizations, all chemical pollutants in sediment, or for biological effects tests. CRMs issued by the National Institute of Standards and Technology (NIST) are called standard reference materials (SRMs).

Documentation accompanying reference samples should: describe applicable matrices and analytes; state if concentration levels are based on analyses of an entire subsample or analyses of an extraction fraction, and method of testing; describe homogeneity assessment of the final unit; describe minimum sample size for testing; describe how bulk material was processed; give handling and storage instructions, preparation and expiration dates (if applicable), and; list the name, address and phone number of the producer. Additional information on the preparation and application of CRMs (SRMs) can be found in NIST 1992; 1993).

2.4 Toxicity and bioaccumulation tests

Quantitative determination of precision and accuracy is difficult or may be impossible in some cases due, in part, to the many unknown variables which affect organism response. Determining the accuracy using field samples is not possible since the true values are not known. Since there is no acceptable reference material suitable for the determining the accuracy of these tests, their accuracy has not been determined.

Accuracy for biological evaluations can be assessed through the use of negative controls and long-term monitoring of the coefficients of variance among reference toxicants. These results, however, are valid only if organisms are appropriate (e.g. taxonomy verified, proper sex and age) and exhibit good health and normal behavior, and test conditions (e.g., temperature, dissolved oxygen) were maintained within pre-set

acceptance limits throughout the study. Test end point outliers are generally more important than test condition outliers.

Organism verification: Since taxonomic verification requires qualified experts (whose opinions may differ), reference toxicant response should be considered as the primary means of assessing test organism appropriateness. The source of test organisms should be documented as well as the response to reference toxicants. If possible, a subsample of test organisms should be preserved.

For each test, the age of the organisms should be documented. If age cannot be determined, the mean size or biomass at testing time should be recorded. Verification of loading rates via double counting is necessary as an internal QC check. At the end of a test, 10% of all endpoints should be verified by another observer.

Culture and test conditions: Environmental conditions for culturing/acclimation of organisms and during exposures should be monitored and maintained as specified in the test protocol. Parameters measured typically include water quality parameters and environmental conditions which affect organism health, care and handling.

Blanks: Negative controls consist of the water in which the organisms have been raised (elutriate test) or a control sediment (whole sediment test). The organism in the control samples should be required to equal or exceed specific criteria (e.g., 90% survival) indicating normal health and behavior stipulated in the testing protocol.

Laboratory water should be checked annually (more often, if necessary) for trace contaminants. In addition, when appropriate, test-organism food and tissues of test organisms held in culture should also be analyzed periodically for the presence of trace contaminants.

Reference toxicants and materials: The response of a given culture of organisms to a known quantity of reference toxicant or a reference material can be evaluated prior to a study, during a study, and over time. The reference toxicant chosen should have an established interlaboratory and intralaboratory database. Reference toxicants often used for freshwater systems are potassium chloride, copper, and zinc. Currently, there are no commercial reference materials available in the quantity required for biological evaluations.

Control charts are constructed by plotting successive toxicity values for each reference toxicant. The mean and

standard deviation are recalculated with each successive plot until the statistics stabilize. Control charts are used to assess whether test organism sensitivity to a given reference toxicant is within interlaboratory and intralaboratory control limits (±2 standard deviations) established for that reference toxicant. A significant change in response or a stable trend (n=7) requires investigation and possible replacement of the culture.

2.5 Model calculations

Accuracy for model calculations cannot be evaluated in the traditional sense. The verification of a model is a significant undertaking, requiring a substantial database and is not a reasonable burden for individual projects. Project data should be evaluated to confirm the chosen input values and assumptions were appropriate. The accuracy of input values should reflect the sensitivity of the model to specific parameters.

3. Precision

Precision is defined as the degree of mutual agreement among independent, similar, or repeated measurements. Various measures of precision exist depending upon the "prescribed similar conditions". Typically, precision is assessed through the use of replicate samples or measurements, and determining the statistical relationship among the results compared to the mean. For triplicate samples or measurements, the percent relative standard deviation (%RSD) is calculated.

3.1 Field measurements

Precision of field measurements is assessed by collecting replicate readings on a sample or standard at the frequency stated in the method. At a minimum, precision should be checked at the beginning and end of the day. Instrument calibration must be valid. Precision should be within the variance indicated in the instrument manual.

3.2 Sediment sample collection

Field duplicate: Field duplicates are collected to demonstrate the reproducibility of sampling technique in homogeneous material, or the degree of environmental heterogeneity. Independent samples are collected as close as possible to the same point in space and time using identical procedures. The two separate samples should be stored in separate containers and analyzed independently. These field QC samples must be treated as regular investigative samples concerning sample volume, containers and preservation.

Split samples: Split samples are aliquots of sample taken from the same container and analyzed independently. These are usually taken after homogenization and are used to document intralaboratory precision (in this case, also known as laboratory duplicates) or interlaboratory accuracy. Samples collected for analysis of volatiles cannot be splits, but must be taken as co-located grab samples. Split sample sets should include field duplicate samples as well as appropriate field blanks.

Because of the heterogeneity of sediments (*in situ*) and the inability to adequately homogenize samples in the field, field duplicates and split samples are not considered reliable indicators of precision in sediment sample collection. Sediment samples homogenized in the laboratory may be suitable for preparing split samples to assess interlaboratory accuracy. However, these would not provide information about sampling precision.

3.3 Physical and chemical analyses

Precision for laboratory measurements is usually assessed by analysis of laboratory duplicates or MS/MSD.

Laboratory duplicates: A laboratory duplicate is an intralaboratory split sample used to document the precision of a method in a given sample matrix. Laboratory duplicates are typically performed for analytes which are naturally occurring and/or frequently found in samples. Results document the precision of a method for a given sample matrix. Duplicates should agree within established laboratory control limits for similar matrices (typically $\leq$10-20%).

Matrix spike duplicates: A matrix spike duplicate is an intralaboratory split sample which is used to document the precision of a method for a given sample matrix. Matrix spike duplicates are typically performed for analytes which are not naturally occurring and/or not frequently found in sample. The intralaboratory split samples are spiked with identical concentrations of target analytes. The spiking occurs prior to sample preparation and analysis. Results document the precision of a method for a given sample matrix. Duplicates should agree within established laboratory control limits for similar matrices (typically $\leq$20-30%).

While both inorganic and organic analyses use matrix spikes, only the organic analyses requires additional sample volume. For this reason, sample and analysis tables list matrix spikes as investigative samples for organic analyses.

3.4 Toxicity and bioaccumulation tests

Biological evaluations are always performed in replicate, typically 3 to 5 with a minimum of 10 organisms per replicate. Precision is not only calculated, but is fundamental to interpretation of results. A measure of precision can be calculated using the mean and relative standard deviation (percent coefficient of variation = standard deviation/mean x 100) of the calculated endpoints from the replicated endpoints of a test. However, precision reported as the CV should not be the only approach used for evaluating precision of tests. Additional estimates of precision may include range of responses, minimum detectable differences compared to control survival or growth.

3.5 Model calculations

Precision of model outputs should be calculated by using each replicate data point rather than the average of the replicates. A minimum acceptable level of precision for the two models do not exist. However, if a sufficient number of replicates were tested, minimum acceptable levels of precision can be determined using a statistical test for outliers.

4. Completeness

Completeness is a measure of the amount of valid (i.e., meet or exceed the requirements of the project) samples collected or data obtained compared to the total amount necessary to make project decision(s) with confidence. Data completeness should be calculated as follows:

$$\% \text{ Completeness} = \frac{\text{Number of Valid Data or Samples}}{\text{Number of Data or Samples Planned}} \times 100$$

If completeness is less than stated, the sample or measurement may have to be repeated or best professional judgement used to assess the usefulness of the data for decision making purposes.

5. Representativeness

Representativeness expresses the degree to which data accurately and precisely represents a characteristic of a population, parameter variations at a sampling point, process condition, or an environmental condition. Representativeness is a qualitative parameter which is dependent upon proper choice of sampling design, and collection and testing protocols. Representativeness is maximized by performing all sampling and testing in a standardized manner, strictly adhering to procedures specified in the QAPP.

6. Comparability

Comparability expresses the confidence with which one data set can be compared with another. One way to ensure consistency is to require the use of similar procedures, SOPs, and standardized data forms. Data calculations and units should be consistent with the procedures and other organizations reporting similar data to allow for comparability. For laboratories, confidence in comparability can be enhanced by interlaboratory testing.

ATTACHMENT E-2
Guidance for Preparing Standard Operating Procedures

Standard operating procedures (SOPs) are written procedures that define how to carry out protocol-specified activities. Content may include, depending on the complexity and type of procedure:

General Information

- title and SOP number,
- version number and effective date,
- approval signature(s),
- serial page numbers and total number of pages, and
- person responsible for work (job title rather than name).

Procedural Information

- scope, application, and limitations of procedures,
- precautions, common problems, and interferences,
- facilities, equipment, organisms, and materials required (type, quality, and quantity),
- chronological description of required action steps and options for entire procedure from preparation through implementation and assessment to reporting,
- set-up, calibration, operation, and maintenance of ancillary equipment not part of the procedure's action steps,
- performance checks (type, frequency, acceptance criteria, corrective action),
- quality control checks (type, frequency, acceptance criteria, corrective action),
- recommended corrective and alternative actions (e.g. for equipment failure, procedural problems, documentation deficiencies, data anomalies, audit/inspection failures), and
- documentation requirements for each of the above.

The level of detail included depends mainly on the education, training, and experience of personnel. If written too restrictively, SOPs will need frequent revising. On the other hand, if the details are insufficient, instructions fail to provide adequate direction to study personnel. A compromise is to segregate all information that changes frequently as an appendix to the SOP, which may be easily updated.

SOPs for general activities (e.g. sample custody and sample collection) are typically less complex than SOPs for measurements.

ATTACHMENT E-3
Guidance for Preparing QAPPs

ELEMENT 1 "Title and Signature Page"

Page lists the project title, location of the site, project identification number, name of the QAPP preparer, for whom it was prepared, date prepared, and revision number. Signatures may include the project manager and QA personnel, field and lab managers and QA personnel, and Agency coordination personnel.

ELEMENT 2 "Table of Contents"

Table includes a serial listing of the 16 essential QAPP elements, tables, figures, attachments, references, and document distribution.

ELEMENT 3 "Project Description"

This project-specific information is likely to be provided in the Tier 1 evaluation and the DCP, and may be referenced. This element describes project scope and objectives, investigative approach, intended data use and associated data quality objectives, monitoring and sample network design and rationale, and project implementation issues and constraints.

Background information on the proposed dredging and disposal locations include:

- site specific features including location, size, borders, important physical features, topographic, geotechnical, geochemical and hydrodynamic data,
- historical contaminant data on sediments at the project,
- dredging and disposal history of the site,
- potential sources of contamination, and
- the list of contaminants of concern.

This information should be detailed in the Tier 1 evaluation report, and can be included in the QAPP by reference.

The scope of the proposed dredging project should be described, the decision to be made, and the data needed for a decision. The general design for data collection should be described, including:

- maps and tables documenting project monitoring and sample locations,
- management units delineation of the dredging site,
- methods and procedures for sample collection,
- methods and procedures for field measurements,
- the number and type of samples for each matrix,
- the number of samples for each parameter-matrix combination for all locations should include field blanks, spikes, and duplicates,
- testing scheduled for each sample, and the
- mechanism for making changes to the plan.

Individuals or organizations responsible for the implementation of sample collection and analysis should be identified, and limits on time and resources defined. These items, if fully described in the DCP or SOWs, can be included the QAPP by reference.

Data applications and modeling to be used in the evaluation should be identified along with data sources for input parameters.

The intended use of each type of data collected should be described and decision criteria identified. Project decisions and decision criteria for Great Lakes dredged material evaluations were detailed in the GLTEM and summarized in Section 3.3 of this appendix, and can be included in the QAPP by reference. Other decision criteria which need to be included in the QAPP are the appropriate State water quality standards and any project-specific criteria for Tier 4 testing.

ELEMENT 4 "Project Organization and Responsibility"

Project-specific information that must be provided in the QAPP include the following:

- key personnel/affiliation with planning, review, approval, implementation, and assessment authority,
- any special training or certification requirements for personnel in order to successfully complete the project task,
- lines of communication and authority between organizations and personnel, and
- a tentative schedule for preparation, review and approval of planning documents, data collection implementation, assessments and reporting,

Programmatic responsibilities of an organization that are provided in the organization's QAMP and project-specific

information contained in the DCP or other project planning documents may be included in the QAPP by reference.

ELEMENT 5 "Sampling and Measurement Quality Objectives"

The QAPP should include a description of project QA objectives, DQIs for field measurement data, sampling collection, laboratory measurements, model calculations or other types of data assessment as well as the means to achieve these objectives. This description should include:

- applicable technical, regulatory or project-required DQIs for each field and laboratory measurement for each sample matrix,
- how data quality will be measured and assessed to justify data usability,
- the type and frequency of internal QC samples and procedures, and
- how sample collection/handling, analysis, and reporting/ assessment ensure the representativeness and comparability of project samples and measurements.

The DQOs elaborated in the GLTEM and appendices can be included in the QAPP by reference. Project-specific DQOs, including DQIs and SOPs for new or modified procedures need to be described.

ELEMENT 6 "Sample Collection and Handling Procedures"

Information about sampling that must be provided in the QAPP include descriptions of the following:

- sampling equipment, any performance requirements and procedures for decontamination,
- sampling procedures, including field QC samples,
- criteria for retaining/discarding samples,
- sample containers and provisions to assure they are non-contaminated,
- sample packaging and shipment procedures, and
- procedures for sample homogenization and division.

This information, if detailed in the DCP or SOW can be included in the QAPP by reference.

ELEMENT 7 "Sample Documentation, Custody and Tracking"

Project-specific information that must be provided in the QAPP include descriptions of the following:

- project file, its location, custodian, storage and access procedures,
- sample numbering system and labeling method,
- how sampling activities will be documented,
- chain-of-custody procedures,
- sample receipt precautions and instructions,
- sample numbering system and labeling method for aliquoting bulk sample into individual sample containers (which may or may not be shipped to another lab),
- procedure(s) to ensure and document custody of the samples throughout the laboratory,
- laboratory sample storage conditions, and verification procedures,
- when and how to dispose of unused samples, and
- required subsequent corrective actions.

For most dredged material evaluations, these activities will be detailed in the organizational QAMP, DCP or contract SOW, and may be included in the QAPP by reference.

ELEMENT 8 "Calibration Procedures and Frequency"

For projects using field and laboratory methods in the GLTEM and appendices, these processes should be routine and may be included in the QAPP by reference. For modified or new methods, all tools, gauges, instruments, and other sampling, measuring, and test equipment that must be controlled and, at specified period, calibrated to maintain accuracy within specified limits should be identified. For each tool, gauge, instrument, or other equipment, the QAPP should:

- describe how to prepare standards and reagents,
- list the information concerning specific grades of material, appropriate glassware and containers for preparation and storage, and labeling and recordkeeping for stocks and dilutions should be included,
- describe the procedures for demonstrating proficiency for each method, including demonstrations of sensitivity, precision and accuracy of the method,
- define all terminology, procedures and frequency of determinations associated with the establishment of the sensitivity/MDL and the reporting limit,
- describe the initial and continuing calibration procedures (type of calibration, and concentration range and number of concentrations), calibration results and algorithm used to generate the calibration curve or response factor, initial and continuing calibration frequency, and initial and continuing calibration acceptance criteria, and
- indicate how calibration frequency, conditions, and

standards are documented and are traceable to the instrument.

ELEMENT 9 "Field and Laboratory Measurement Procedures"

The specific methods for field and laboratory measurements should be identified in the QAPP. For measurements made using the methods in the GLTEM and appendices, the methods can be included by reference. For measurements using new or modified methods, selected as discussed in Section 3.5, the following information should be included in the QAPP:

- an amendment to a standard method or a detailed SOP,
- cite by reference appropriate method validation data, or describe plans for conducting preliminary method validation studies as project subtasks if pertinent validation data are not available, and
- independent, validated, confirmatory methods for each critical measurement for which a multi-method confirmatory approach is applicable.

ELEMENT 10 "Internal Quality Control Checks"

Many of the field and laboratory methods detailed in the GLTEM and appendices include minimum QC procedures. These methods can be included in the QAPP by reference. For measurements using new or modified methods, or where QC procedures are not detailed, the following information should be included in the QAPP:

- identify all stages in sampling and measurement processes where internal QC checks are used to calculate the DQIs for sample collection, field measurements, laboratory analyses, and modeling efforts,
- describe or reference all specific QC samples and checks for each stage of field and laboratory activities, stating the frequency and required control limits for each QC sample or check,
- justify that QC procedures are compatible with the data specifications, and
- reference the required subsequent corrective action that should be described in detail in Element 15.

ELEMENT 11 "Data Reduction/Verification/Deliverables and Data Validation and Reporting"

Data reduction/verification, validation and reporting

procedures for approved laboratory methods are detailed in the GLTEM and appendices. These methods can be included in the QAPP by reference. For measurements using new or modified methods, or where data reduction/verification/validation procedures are not detailed in the GLTEM, the following information should be included in the QAPP:

- describe the reduction of field and laboratory raw data to final units, summarize reduction procedures, and any statistical approach used,
- describe the verification of field, laboratory and modeling results and summarize verification procedures,
- specify the reporting requirements for field, laboratory and modeling data, describe reporting format (including units), and content of data deliverable,
- describe the validation procedures for field, laboratory and modeling data, the criteria/guidelines/procedures to be used for data validation, and the procedures to determine outliers and define qualifying 'flags' used, and
- specify the format and content of data validation reports, any non-project specific reporting requirements, and annual reports.

The individual(s) responsible for data reduction/verification, validation and reporting should be identified in the QAPP.

ELEMENT 12 "Performance Audits and System Inspections"

Information about laboratory inspections and performance audits specific to the project data collection which should be provided include:

- specify the pre-award criteria and procedures,
- identify who is responsible for internal and external audits and inspections,
- specify the frequency of internal and external performance audits and system inspections,
- describe the audit and inspection procedures and criteria used to ensure work is performed as specified in the QAPP and that quality meet project requirements,
- reference the required subsequent corrective action, described in detail in Element 15, and
- specify the format and content of audit and inspection reports.

Routine procedures for performance audits and inspections for indefinite delivery laboratory contracts which are included in an organization's QAMP can be included in the QAPP by reference.

ELEMENT 13 "Equipment/Instrument Maintenance and Consumables Inspection"

These processes should be routine and, if documented in the organization's QAMP, SOPs or the DCP, may be included in the QAPP by reference. The information in these documents should include:

- identify the equipment and/or instruments requiring periodic maintenance (e.g. field monitors, sample equipment, laboratory equipment, and computer hardware),
- verify the availability of critical spare parts, necessary according to operating guidance or design specifications,
- describe the periodic preventative maintenance protocols for all equipment/instruments should be performed to ensure availability and satisfactory performance of the systems,
- discuss how repair of equipment/instruments will be performed (e.g. in-house, service contract),
- discuss how and by whom supplies and consumables are inspected and accepted for use in the project.
- identify the acceptance criteria for supplies and consumables in order to satisfy the technical and quality objectives of the project or task,
- discuss how inspections and acceptance testing, including use of QC samples, of environmental sampling and measurement systems and their components must be performed and documented to assure their use as specified by the design.
- identify and discuss how final acceptance of consumables is performed by independent personnel, and
- discuss how deficiencies will be resolved when acceptance criteria are not met, and how/when re-inspection occurs.

ELEMENT 14 "Procedures to Assess Data Usability"

The GLTEM and appendices provide considerable guidance on how to assess usability of data from approved methods. Assessing the usability of historic data is likely to require best professional judgement. Organizational QAMPs and SOPs may provide more specific procedures for assessing the usability of new or historic data. All of these procedures can be included in the QAPP by reference.

For data collection activities involving new or modified methods, and especially for any Tier 4 evaluations, the procedures for assessing data usability should be detailed in the QAPP, including the following information:

- describe the procedures to assess the usability of the samples collected, field and laboratory data, and
- discuss how issues will be resolved, by whom, and how

limitations on the data will be reported and used in decisions.

ELEMENT 15 "Corrective Action"

For projects using approved field and laboratory methods in the GLTEM and appendices, corrective action should be routine and may be included in the QAPP by reference. For new or modified methods, corrective actions should be defined in the SOP, including the following:

- list all activities potentially requiring corrective action during the course of the project,
- describe the mechanism to initiate, develop and approve corrective actions and identify the parties responsible,
- specify the predetermined limits for data acceptability beyond which corrective action is required for each procedure and/or measurement,
- describe the procedure to implement, document, and test effectiveness corrective actions, and
- specify the format and content of nonconformance reports and corrective action memos.

ELEMENT 16 "Quality Assurance Reports"

Procedures for QA reporting provided in organizational QAMPs can be included in the QAPP by reference. This information should include:

- identify the name and address of individuals submitting and receiving reports, number of copies and delivery date for draft and final QA reports,
- describe the type (e.g. written or oral, interim or final) and frequency of the QA report, and
- specify the contents of the various QA reports.

Project-specific information must always be provided in the QAPP.

ATTACHMENT E-4
Data Validation Guidance

1. Validation Activities

1.1 Check completeness and accuracy of deliverable

Field and laboratory deliverables should be reviewed to determine whether all documentation requirements in the QAPP, DCP and SOWs have been fulfilled. Complete records should exist for each activity. Emphasis on documentation helps assure sample integrity and sufficient technical information to recreate each event. Data validators are responsible for interacting with the data generator to obtain missing information and resolve data anomalies.

The results of the completeness check should be documented, and the data affected by incomplete records should be identified. Data validation cannot begin until the deliverable is complete.

1.2 Verify proper procedures followed

The data validator evaluates raw data and associated records to confirm all procedures were conducted according to the QAPP, DCP, and SOW. All deviations must be noted. The deliverable should be reviewed to verify:

- integrity and stability of samples,
- equipment operation and calibration,
- QC procedures and frequency,
- corrective action taken when necessary and was effective,
- internal verification performed, and
- calculations correct and no transcription errors exist.

1.3 Compare performance to acceptance criteria

Sensitivity/method detection limit, precision, and accuracy: The data validator quantitatively compares project results to acceptance criteria stated in the QAPP (element 5) and associated contract-SOWs and SOPs. Data not within control limits require corrective action, and the reviewer should check that corrective action reports, and the results of corrective action are available.

The data validator should determine whether samples associated with out-of-control quality control data are identified in a internal data verification report, and whether an assessment of the utility of such results is recorded.

The results, consequences, and documentation of performance and systems audits should also be considered in determining the validity of results.

Representativeness and comparability: The data validator qualitatively reviews field and laboratory records to detect problems affecting the representativeness and comparability of the data. Problems that may affect data representativeness are:

- choice of sample locations and subsamples,
- biases induced during field and laboratory preparation,
- exceedances of sample holding times,
- potential for contamination and degradation of sample during sample processing or analysis, and
- matrix interferences and effects.

The primary factor affecting data comparability is changes or modifications to sampling and analytical procedures specified in the QAPP and associated SOPs and contract-SOWs. The data validator assesses the consequences of these changes on the data. Conclusions should not be based on assumptions which cannot be tested and verified by data derived from the study.

2. Data Validation Report

Data validation reports identify samples and environmental data associated with poor or incorrect work. Data is either accepted or flagged with a qualifier. Qualifiers are letters which are placed next to the reported sample value to indicate there was, or could have been, a problem. Later, during data quality assessment, the reason for qualification should be considered when assessing the usability of qualified data.

Validation reports should include:

- case narrative describing any problems encountered and limitations on the use of the data, with a signature that authorizes the validation and release of the report,
- data assessment performed, including the number and type of samples evaluated, deviations from specified validation procedures, interpretation of test results and conclusions regarding the acceptability of data in terms of project objectives and method QA/QC,
- a summary of rejected samples or data,
- all qualifying flags used to mark the data in the validation report should be defined (a list typical data qualifiers is provided below), and
- a telephone record log and record of each communication.

Upon completion, data validation reports are forwarded to the PM for inclusion in the final report.

Data Qualifier Definitions

U	Nondetected. For chemical analysis, value reported is MDL.
J	Estimated results. Estimated data should be used with caution. For chemical analyses, concentrations between the MDL and LOQ are flagged with a "J".
R	Rejected due to deficiencies in the method or QC criteria.

GREAT LAKES
DREDGED MATERIAL TESTING AND EVALUATION MANUAL

APPENDIX F
METHODS FOR CHEMICAL AND PHYSICAL ANALYSES

prepared by:

Brian Schumacher
USEPA, Environmental Monitoring Systems Laboratory - Las Vegas

Russell Plumb, Jr.
Lockheed Environmental Systems & Technologies Company

Richard Fox[1]
USEPA, Great Lakes National Program Office

[1] affiliation no longer current

TABLE OF CONTENTS

Chemical Analyses of Waters and Elutriates (continued)

BACKGROUND ON PARAMETER AND METHOD SELECTION

Purpose

This appendix contains detailed methods for the physical and chemical analysis of sediments, water and elutriates to be used as part of the testing and evaluation of dredged material. The USEPA/USACE Task Group which developed the Great Lakes Dredged Material Testing and Evaluation Manual determined to have such detailed methods provided in this appendix for several reasons, including:

- identify analytical methods capable of meeting data quality objectives for dredged material testing,
- provide more uniformity and comparability of results between projects and laboratories, and
- enable districts and permit applicants to use methods as part of requirements in laboratory contracts.

The methods provided in this appendix should be capable of meeting the data quality objectives of the majority of dredged material testing projects in the Great Lakes. Alternate analytical methods may be considered for use in dredged material evaluations following the procedures described in Appendix E, Quality Assurance Guidance.

Parameter Selection

To provide guidance on Tier 2 evaluations, the USEPA/USACE Task Group developed a generic list of contaminants of concern. This list (table 1 in main text) is not intended to be all inclusive, but was developed as a "starting place" for project-specific evaluations. These contaminants were selected based on ecological or toxicological significance and their recorded presence in many Great Lake harbors and tributaries. In addition to the chemical parameters on this list, methods were selected for other physical and chemical parameters for the following reasons:

- the parameter was a reliable indicator of the presence of other contaminants (i.e., volatile solids and TPH), and
- the parameter was needed for use in TBP analysis or the STFATE mixing model (i.e., TOC, density, and grain size).

Additionally, the Task Group had to determine for which matrices (e.g., sediments, water/elutriate, or both) the methods would be prepared. The final list of selected parameters contained 22 parameters to be analyzed in sediments, water/elutriate, or both matrices (Table F-1). A method for the preparation of elutriates from sediment samples has also been included.

Table F-1. Parameters for Methods Selection

Parameter	Matrix[a]
Total Solids	S
Total Volatile Solids	S,E
Total Dissolved Solids	E
Total Suspended Solids	E
Specific Gravity	S
Grain Size	S
Ammonia	S,E
Cyanide	S,E
As	S,E
Cd	S,E
Cr	S,E
Cu	S,E
Hg	S,E
Ni	S,E
Pb	S,E
Zn	S,E
Total Organic Carbon	S
Chloro-Pesticides	S,E
Total PCBs	S,E
Total Petroleum Hydrocarbons	S,E
Phenolics	S,E
Polynuclear Aromatic Hydrocarbons	S,E

[a] S = sediment; E = water and elutriate.

It should be noted that evaluators need to develop a site-specific contaminants of concern list for each dredging project which may contain all, some part, or other parameters not identified in the methods manual appendix. It is the intention of the USEPA and USACE to prepare descriptions of additional physical and chemical analytical methods for sediments, water, elutriate, and animal tissues in future amendments to the Great Lakes Dredged Material Testing and Evaluation Manual.

Base Method Selection

The selection of appropriate published methods to be used as base methods for each parameter was also performed by committee. A new committee was selected of scientists from the USACE North Central Division; USACE Districts in Chicago, Detroit, and St. Paul; USACE Waterways Experiment Station; USEPA Regions 2, 3, and 5; USEPA Great Lakes National Program Office; USEPA Environmental Monitoring and Systems Laboratory - Las Vegas; and Lockheed Environmental Systems & Technologies Company.

To each member of the methods committee, a questionnaire was sent requesting a list of the most commonly used methods for each parameter in their laboratory, district, or Region. This list of commonly used methodologies was compiled and conference calls were held every other week to discuss and come to agreement on a common base method. After selection of the base method for each parameter, a final conference call was held to select an appropriate format for the methods presentation. For each parameter presented in this appendix, the method has been prepared in the agreed upon format by the methods committee and the base method has been referenced in the Scope and Application section (section 1.).

TOTAL SOLIDS

1.0 Scope and Application

This method is applicable to the determination of total solids in sediment samples.

The results of this analysis are used in the analytical laboratory to convert all results in which field-moist samples are used to oven-dry weight basis. Additionally, the end product or residue created from this procedure can be used in the determination of total volatile solids (TVS).

Since this method is based on the difference between two weighings, the range and sensitivity of the method is dependent upon the balance used.

This method is based on EPA Method 160.3 (USEPA, 1983).

2.0 Summary of Method

A well mixed aliquot of sediment is quantitatively transferred to a pre-weighed evaporating dish and evaporated to dryness at 103-105° C. Total solids is determined by expressing the sample weight loss as a percentage of the original field moist sample weight.

3.0 Interferences

Non-representative particulates, such as leaves, sticks, fish, and lumps of fecal matter, should be excluded from the sample if it is determined that their inclusion is not desired in the final result.

Residues dried at 103-105° C may retain not only water of crystallization but also some mechanically occluded water. Because removal of occluded water is marginal at this temperature, attainment of constant weight may be very slow (APHA, 1989).

Loss of CO_2 will result in conversion of bicarbonate to carbonate. Loss of organic matter by volatilization will usually be slight (APHA, 1989).

Results for samples high in oil and grease may be questionable because of the difficulty of drying to a constant weight in a reasonable time (APHA, 1989).

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Oven.
4. Thermometer, 0 to 200° C range, graduated to 1° C.
5. Desiccator and desiccant. Desiccants generally used include: anhydrous calcium sulfate, silica gel, or phosphorus pentoxide. Indicating desiccants are preferable since they show when the desiccant needs to be changed or regenerated.

4.2 Materials

1. Evaporating dishes, porcelain, 90 mm, 100 ml capacity. (Aluminum, Vycor, or platinum weighing dishes may be substituted and smaller size dishes may be used, if required.)

5.0 Reagents and Standards

No reagents are required for this procedure.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection to minimize loss of sample moisture prior to analysis. A holding time of 7 days is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C) to minimize microbiological decomposition of solids between sample collection and sample analysis.

All sample containers must be prewashed with detergents. If samples are to be analyzed for other parameters, acids and Type II water washings may also be required. Either glass or plastic containers can be used for the storage of samples to be analyzed for total solids.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The oven should be monitored to ensure that temperature fluctuation does not exceed ± 2° C.

8.0 Procedure

1. Heat the clean evaporating dish to 103-105° C for one hour prior to the determination of total solids of the sample. If TVS is also to be determined on the sample, the evaporating dish should be heated at 550 ± 50° C for one hour in a muffle furnace prior to use. Cool in a desiccator, weigh, and store in desiccator until ready for use.
2. Allow oven to equilibrate at 103 to 105° C overnight.
3. Tare each weighing dish to the nearest 0.01 g and record the weight.
4. Transfer 25 g of the homogenized, field-moist sediment sample into the pre-weighed dish. Weigh the dish + moist sample to the nearest 0.01 g and record the weight.
5. Place the sample in the oven and evaporate to dryness.
6. Dry the evaporated sample for at least 1 hour at 103-105° C. It is recommended that during the initial drying step, the samples be left in the oven for 2 to 3 hours.
7. Remove weighing pan from oven. Allow sample to cool in a desiccator for at least 1 hour. Weigh each sample to 0.01 g.

8. Repeat the cycle of drying at 103-105° C, cooling in a desiccator, weighing, and recording the weight until a constant weight is obtained or until observed weight loss on drying is less than 5% of the previous weight, or 0.5 mg, whichever is less.

   NOTE: Alternately, the sample may be place in the oven and dried at 103-105° C overnight and cooled in a desiccator. No differences in sample color, indicating moisture, should be noticeable upon visual examination of the sample. If differences are identified, continue drying the sample following the procedure in step 8.

9. Weigh the sample dish to the nearest 0.01 g and record final weight of the sample + dish.

9.0 Quality Control

9.1 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 10 percent or less.

9.2 Blanks

A minimum of one blank per sample batch should be analyzed. A blank for total solids consists of an empty dish. The weight change of the blank should not be greater than ± 0.5 mg.

10.0 Method Performance

In a study involving four sets of 10 determinations by two analysts, the method produced results with a calculated standard deviation of 5.2 mg/L at 15 mg/L, 24 mg/L at 242 mg/L, and 13 mg/L at 1707 mg/L. These results were derived with waste water samples rather than sediment samples (APHA, 1989) but indicate the precision that can be attained with this method.

11.0 Calculations and Reporting

Use the results of the individual weighings to calculate total solids as follows:

$$\text{Total Solids, \%} = \frac{(A - B) \times 100}{(A - D)}$$

where:

A = weight of moist sample + dish, g
B = weight of dried sample + dish, g
D = weight of evaporating dish, g

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition, APHA, New York, New York. p. 2-71 - 2-79.

U.S. Environmental Protection Agency. 1983. Methods for the Chemical Analysis of Water and Wastes. EPA-600/4-79-020. U.S. Environmental Protection Agency, Environmental Monitoring and Systems Laboratory, Cincinnati, Ohio.

TOTAL VOLATILE SOLIDS IN SEDIMENTS

1.0 Scope and Application

This method determines the weight of material associated with a sediment sample that is volatile or combustible at 550° C. The test is useful in obtaining a rough approximation of the amount of organic matter present in the solid fraction of bottom sediments.

The sediment used for the determination of total volatile solids may be obtained from the original sample or from the residue obtained in the determination of total solids.

> **NOTE**: If the aliquot is obtained from the original sample, then the procedure for total solids must be performed on the aliquot prior to ashing in the muffle furnace.

Since this method is based on the difference between two weighings, the range and sensitivity of the method is dependent upon the balance used.

This method is based on EPA Method 160.4 (USEPA, 1983).

2.0 Summary of Method

The residue obtained after the determination of total solids is ignited at 550° C in a muffle furnace. The loss of weight on ignition is reported as mg/kg or weight percent (%) of total volatile solids.

3.0 Interferences

Since the procedure is operationally defined and based on the difference between two weighings, the test is subject to errors due to loss of water of crystallization, loss of volatile organic matter prior to combustion, incomplete oxidation of certain complex organics, and decomposition of mineral salts during combustion.

The principal source of error in the determination is failure to obtain a representative sample.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.001 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Muffle furnace.
4. Thermocouple, 0 to 600° C range, graduated to 1° C.
5. Desiccator and desiccant. Desiccants generally used include: anhydrous calcium sulfate, silica gel, or phosphorus pentoxide. Indicating desiccants are preferable since they show when the desiccant needs to be changed or regenerated.

4.2 Materials

1. Evaporating dishes or crucibles, porcelain, 90 mm, 100 mL capacity. (Aluminum, Vycor, or platinum weighing dishes or crucibles may be substituted and smaller size dishes may be used, if required.)

5.0 Reagents and Standards

No reagents are required for this procedure.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection to minimize loss of sample moisture prior to analysis. A holding time of 7 days is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C) to minimize microbiological decomposition of solids between sample collection and sample analysis.

All sample containers must be prewashed with detergents. If samples are to be analyzed for other parameters, acids and Type II water washings may also be required. Either glass or plastic containers can be used for the storage of samples to be analyzed for total volatile solids.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The oven should be monitored to ensure that temperature fluctuation does not exceed ± 2° C.

8.0 Procedure

1. Heat muffle furnace to 550 ± 10° C.
2. Ignite residue produced from the determination of total solids at 550 ± 10° C to ash the sample overnight in a muffle furnace.
3. Remove the sample dish from the furnace and allow to partially cool until most of the heat has been dissipated (about 15 minutes).
4. Transfer the sample to a desiccator for final cooling.
5. Weigh sample dish to the nearest 0.01 g and record final weight of the sample + dish.

9.0 Quality Control

9.1 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 10 percent or less.

9.2 Blanks

A minimum of one blank per sample batch should be analyzed. A blank for total volatile solids consists of an empty dish. The weight change of the blank should not be greater than ± 0.5 mg.

10.0 Method Performance

A collaborative study involving three laboratories examining four samples by means of ten replicates produced a standard deviation of ± 11 mg/L at a volatile residue concentration of 170 mg/L (APHA, 1989; USEPA, 1983).

11.0 Calculations and Reporting

Use the results from the individual weighings to calculate the total volatile solid content of the sample as follows:

$$\text{Total Residue, \%} = \frac{(A - B) \times 100}{(A - C)}$$

where:

A = weight of dried residue plus dish before ignition, g.
B = weight of the ashed sample plus dish, g.
C = original weight of dish, g.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition, APHA, New York, New York. p. 2-71 - 2-79.

U.S. Environmental Protection Agency. 1983. "Methods for the Chemical Analysis of Water and Wastes". EPA-600/4-79-020. U.S. Environmental Protection Agency Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. March.

SPECIFIC GRAVITY

1.0 Scope and Application

This method is applicable to the determination of specific gravity of sediment samples. Unless otherwise required, specific gravity values will be based on water at 20° C.

The specific gravity of a sample for use in sediments engineering calculations is usually expressed in three different forms:

1. specific gravity of solids, G_s,
2. apparent specific gravity, G_a, and
3. bulk specific gravity, G_m.

The specific gravity of solids is applied to samples with a particle size that will pass through a No. 4 sieve (particles with mean diameters less than 4.76 mm or 0.187 in). The specific gravity of solids is not applied to coarse particles because they normally contain voids from which air cannot be displaced unless the samples are pretreated by grinding to reduce particle size and eliminate the voids. In contrast, the apparent and bulk specific gravities are applied to samples with particle sizes that will not pass through a No. 4 sieve. Thus, when dealing with coarser particles, it is more convenient to work with the apparent specific gravity of the particle mass.

The values of G_s or G_a are used in all calculations involving fundamental properties of a sediment mass. The bulk specific gravity is used in special calculations, such as corrections of density and water content, for sediments containing gravels.

This method is based on a procedure in Appendix IV of the Corps of Engineers engineering manual (1970).

2.0 Method Summary

An aliquot of sediment is weighed to determine its mass. The sample is then transferred to a volumetric flask to determine the volume of distilled water that would be displaced by the tared aliquot. The ratio of weight of sediment sample in air to the weight of displaced water is the specific gravity of the sample.

The types of specific gravity determinations that can be completed with this method include the following:

1. Specific Gravity of Solids. The specific gravity of a sediment sample, G_s, is the ratio of the weight in air of a given volume of sediment particles at a stated temperature to the weight in air of an equal volume of distilled water at the same stated temperature.

2. Apparent Specific Gravity. The apparent specific gravity of a sediment sample, G_a, is the ratio of the weight in air of a given volume of the impermeable portion of a permeable material (that is, the solid matter including its impermeable pores or voids) at a stated temperature to the weight in air of an equal volume of distilled water at the same stated temperature.

3. Bulk Specific Gravity. The bulk specific gravity of a sediment sample, G_m, is the ratio of the weight in air of a given volume of a permeable material (including both permeable and impermeable voids normal to the material) at a stated temperature to the weight in air of an equal volume of distilled water at the same stated temperature.

3.0 Interferences

Potential errors associated with specific gravity measurements include the following:

1. Imprecise weighing of flask and contents. Since the computation of the specific gravity of solids is based on a difference in weights which is small in comparison with the weights themselves, the same balance should be used for calibrating the volumetric flask and for determining the specific gravity whenever the calibration curve is used.
2. Temperature of flask and contents not uniform. Both in calibrating the flask and determining the specific gravity, utmost care should be taken to insure that measured temperatures are representative of the flask and contents during the times when the weighings are made.

3. Flask not clean. The calibration curve will not remain valid if accumulation of residue changes the tared weight of the flask. Also, if the inside of the neck is not clean, an irregular meniscus may form.
4. Moisture on outside of flask or inside of neck. When calibrating the flask for a temperature lower than room temperature, there is a tendency for condensation to form on the flask despite careful drying and rapid weighing. Whenever possible, weighing should be done at approximately the same temperature as that of the flask.
5. Meniscus not coincident with mark on neck of flask. One drop of water too much makes an error of approximately 0.05 g. This error can be minimized by taking the average of several readings at the same temperature. When the suspension is opaque, a strong light behind the neck is helpful in seeing the bottom of the meniscus.
6. Use of water containing dissolved solids. It is essential that ASTM Type I water be used exclusively to insure the continued validity of the flask calibration curve.
7. Incomplete removal of entrapped air from sample suspension. This is the most serious source of error in the specific gravity determination and will tend to lower the computed specific gravity. The suspension must be thoroughly evacuated or boiled and the absence of entrapped air verified as described in the note of step 5 in section 8.1.1. (It should be noted that air dissolved in the water will not affect the results, so it is not necessary to a apply vacuum to the flask when calibrating or after filling the flask to the calibration mark.)
8. Gain in moisture of oven-dried specimen before weighing. If the specimen is oven-dried before the specific gravity determination, it must be protected against a gain in moisture until it can be weighed and placed in the flask.
9. Loss of material from oven-dried specimen. If the specimen is oven-dried and weighed before being placed in the flask, any loss of material will lower the computed specific gravity.

Potential errors associated with apparent and bulk specific gravity measurements include the following:

1. Loss of moisture from saturated surface-dry particles before weighing. Unless the saturated surface-dry material is weighed

promptly, evaporation may cause an increase in the computed bulk specific gravity.

2. Failure to correct for the change in density of water with temperature. This correction is often overlooked when computing either the apparent or bulk specific gravity.

4.0 Apparatus and Materials

4.1 Specific Gravity Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Volumetric flask, 500 mL capacity.
4. Vacuum pump with vacuum gauge, piping and tubing for connections to each flask. The connection to each flask should include a trap to catch any water drawn from the flask.
5. Oven.
6. Thermometer, range 0 to 50° C, graduated in 0.1° degree
7. Evaporating dish, glass or porcelain, capable of holding 600 mL.
8. Water bath.
9. Sieve, U. S. Standard No. 4 conforming to ASTM Designation: E11, Standard Specifications for Sieves for Testing.

4.2 Apparent and Bulk Specific Gravity Apparatus

1. Balance, having capacity of 5 kg or more and sensitive to 1.0 g.
2. Wire basket of No. 6 mesh, approximately 20 cm in diameter and 20 cm high.
3. Suitable container for immersing the wire basket in water, and suitable apparatus for suspending the wire basket from the center of the balance scale pan.
4. Thermometer, range 0 to 50° C, graduated in 0.1° degree.

5.0 Reagents and Standards

1. ASTM Type I water (ASTM, 1984).

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Some samples, particularly those with a high organic content, are sometimes difficult to rewet after having been oven-dried. Therefore, it is recommended that samples be stored in their field-moist state until used in the procedure.

Samples should be stored at 4° C between sample collection and analysis.

All sample containers must be prewashed with detergents. If samples are to be analyzed for other parameters, acids and Type II water washings may also be required. Either glass or plastic containers can be used for the storage of samples to be analyzed for specific gravity.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The oven and water bath temperatures should be monitored to ensure that temperature fluctuation does not exceed ± 2° C.

The volumetric flask shall be calibrated for the weight of the flask and water at various temperatures. The flask and water are calibrated by direct weighing at the range of temperatures likely to be encountered in the laboratory. The calibration procedure is as follows:

1. Fill the flask with deaired water to slightly below the calibration mark and place in a water bath at a temperature between 30 and 35° C. Allow the flask to remain in the bath until the water in the flask reaches the temperature of the water bath. This may take several hours.
2. Remove the flask from the water bath and adjust the water level in the flask so that the bottom of the meniscus is even with the calibration mark on the neck of the flask.
3. Thoroughly dry the outside of the flask and remove any water adhering to the inside of the neck above the graduation
4. Weigh the flask and water to the nearest 0.01 g. Immediately after weighing, shake the flask gently and determine the temperature of the water to the nearest 0.1° C by immersing a thermometer to the mid-depth of the flask.
5. Repeat the procedure outlined in step 1 at approximately the same temperature. Make two additional determinations, one at room temperature and the other at approximately 5 degrees cooler than room temperature.
6. Draw a calibration curve showing the relation between temperature and corresponding weight of the flask + water.
7. Prepare a calibration curve for each flask used for specific gravity determined and maintain the curves as a permanent record.

8.0 Procedure

8.1 Specific Gravity Procedure

Prior to the determination of specific gravity, the sample to be tested should be sieved on a No. 4 sieve. That portion of the sample that passes the No. 4 sieve is used for the specific gravity determination and that portion of the sample retained by the No. 4 sieve is used for the apparent and bulk specific g gravity determination.

If the samples are sieved in a field-moist state, proceed as directed in section 8.1.1. If the samples are air-dried prior to being sieved, proceed as directed in paragraph 8.1.2.

8.1.1 Procedure for Field-Moist Samples.

1. Select a representative sample aliquot of the sieved sample passing a No. 4 sieve, ranging between 50 g (for cohesive sediments) and 150 g (for cohesionless sediments) and weigh the sample to the nearest 0.01 g in a dish or beaker.
2. Add 50 to 100 mL water to the sample and mix with a spatula to form a slurry.
3. Transfer the sample slurry to a calibrated volumetric flask and fill the flask approximately half full with water.
4. Connect the flask to the vacuum line and apply a vacuum of approximately 73.5 cm (29 in) mercury. Agitate the flask gently at intervals during the evacuation process.

   NOTE: The length of time that vacuum should be applied will depend on the properties of the sample being tested. Samples with a high plasticity or high organic content may require 6 to 8 hr and other samples may require considerably less time for removal of entrapped air.

   NOTE: To ensure continuous boiling, the temperature of the flask and contents may be elevated somewhat above room temperature by immersing in a water bath at approximately 35° C. Alternatively, entrapped air may be removed by boiling (see following note). Allow flask and contents to cool, preferably overnight, before filling and checking in step 5.

   NOTE: Air removal from organic sediments usually cannot be accomplished by the application of vacuum. In this case it will be necessary to boil the suspension contained in the flask for about 30 min, adding distilled or demineralized water carefully from time to time to prevent boiling the sample dry. The flask should at all times be approximately half full.

5. Fill the flask with deaired water to about 2 cm below the 500 mL graduation and apply a vacuum slightly less than that which will cause vigorous boiling in order to prevent loss of sample.

   NOTE: To determine if the suspension is deaired, slowly release the vacuum and observe the lowering of the water surface in the neck of the flask. If the water surface is lowered

less than 0.3 cm, the suspension can be considered sufficiently deaired. If the water surface is lowered more than 0.3 cm, Step 4 should be repeated.

6. Fill the flask until the bottom of the meniscus is coincident with the calibration line on the neck of the flask.
7. Thoroughly dry the outside of the flask and remove the moisture on the inside of the neck by wiping with a paper towel. Weigh the flask and contents to the nearest 0.01 g. Immediately after weighing, stir the suspension to assure uniform temperature, and determine the temperature of the suspension to the nearest 0.1° C by immersing a thermometer to the mid-depth of the flask.
8. Record the weight of the flask containing the sample suspension and the temperature of the sample.
9. Carefully transfer the contents of the flask to an evaporating dish.
10. Rinse the flask with distilled water to ensure removal of all of the sample from the flask.
11. Oven dry the sample to a constant weight at a temperature of 103-105° C. Allow the residue to cool to room temperature in a desiccator and determine the weight of the sample to the nearest 0.01 g.
12. Record all weights.

8.1.2 Procedure for Air-Dried Samples

1. Oven dry that portion of the sieved sample passing a No. 4 sieve at 103-105° C and cool to room temperature in a desiccator.
2. Select a sample aliquot ranging between 50 g (for cohesive sediments) and 150 g (for cohesionless sediments) and weigh the sample to the nearest 0.01 g. Quantitatively transfer the sediment to a volumetric flask, taking care not to lose any material during this operation.

   NOTE: To avoid possible loss of preweighed sediment, the sample may be weighed after transfer to the flask.

3. Fill the flask approximately half full with deaired distilled water and allow the suspension to stand overnight.

4. Connect the flask to the vacuum line and apply a vacuum of approximately 73.5 cm (29 in) mercury for approximately 2 to 4 hr.

   NOTE: Entrapped air may also be removed by boiling as previously discussed. However, the process should be observed closely to avoid loss of material during boiling. Allow flask and contents to cool, preferably overnight, before filling and checking.

5. Fill the flask with deaired distilled water to about 2 cm below the 500 mL graduation and apply a vacuum slightly less than that which will cause vigorous boiling in order to prevent loss of sample.

   NOTE: To determine if the suspension is deaired, slowly release the vacuum and observe the lowering of the water surface in the neck of the flask. If the water surface is lowered less than 0.3 cm, the suspension can be considered sufficiently deaired. If the water surface is lowered more than 0.3 cm, Step 4 should be repeated.
6. Fill the flask until the bottom of the meniscus is coincident with the calibration line on the neck of the flask.
7. Thoroughly dry the outside of the flask and remove the moisture on the inside of the neck by wiping with a paper towel. Weigh the flask and contents to the nearest 0.01 g. Immediately after weighing, stir the suspension to assure uniform temperature, and determine the temperature of the suspension to the nearest 0.1° C by immersing a thermometer to the mid-depth of the flask.
8. Record the weight of the flask containing the sample suspension and the temperature of the sample.
9. Record all weights.

8.2 Apparent Specific Gravity and Bulk Specific Gravity

1. Wash the sample material retained on a No. 4 sieve thoroughly with water to remove dust or other coatings from the surface of the sample.
2. Immerse the sample material in water at 15 to 25° C for a period of 24 hr.

3. Remove the sample material from the water and roll it in a large absorbent cloth or tissue until all visible films of water are removed, although the surfaces of the particles may still appear to be damp. Wipe large particles individually.

   NOTE: Take care to avoid excess evaporation during the operation of surface drying.

4. Transfer the sample to a tared beaker or weighing dish to obtain the weight of the saturated, surface-dry material. These results, and those of all subsequent weighings, should be reported to the nearest 1.0 g.
5. Immediately after weighing, place the sample in the wire basket and determine the weight of the sample in water. Measure and record the temperature of the water in which the specimen is immersed to the nearest 0.1° C.
6. Remove the sample from the wire basket and transfer to a beaker. Oven-dry the sample to a constant weight at a temperature of 103-105° C. After cooling to room temperature, weigh the sample.
7. Record all weights.

9.0 Quality Control

9.1 Replicates

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 10 percent or less.

10.0 Method Performance

None identified.

11.0 Calculations

11.1 Specific Gravity

The specific gravity of the sample is calculated to two decimal places using the following formula:

$$G_s = \frac{W_s \times K}{W_s + W_{bw} - W_{bws}}$$

where:

G_s = the specific gravity of the sample, g/cm^3
W_s = the dry weight of the sample, g
K = correction factor based on the density of water at 20° C from Table 1. Unless otherwise required, specific gravity values should be based on water at 20° C.
W_{bw} = weight of flask plus water at test temperature, g (obtained from calibration curve)
W_{bws} = weight of flask plus water plus wet sample at test temperature, g.

11.2 Apparent Specific Gravity

The apparent specific gravity of the sample is calculated to two decimal places using the following formula:

$$G_a = \frac{A \times K}{A - C}$$

where:

G_a = the apparent specific gravity of the sample, g/cm^3
A = weight of dry sample, g
K = correction factor based on the density of water at 20° C from Table 1
C = weight of saturated sample suspended in water, g

11.3 Bulk Specific Gravity

The bulk specific gravity of the sample is calculated to two decimal places using the following formula:

$$G_m = \frac{A * K}{B - C}$$

where:

G_m = the bulk specific gravity of the sample, g/cm^3
A = weight of dry sample, g
K = correction factor based on the density of water at 20° C from Table 1
B = weight of saturated, surface dry sample, g.
C = weight of saturated sample suspended in water, g.

11.4 Combined Fraction Specific Gravity

When a sample is composed of particles both larger and smaller than the openings of a No. 4 sieve, the specific gravity of the sample for use in engineering calculations should be computed as follows:

$$G = 100 / \left[\frac{\text{(\% passing No. 4 sieve)}}{G_s} + \frac{\text{(\% retained on No. 4 sieve)}}{G_a} \right]$$

where all terms are as defined above.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. Army Corps of Engineers. 1970. Specific Gravity. *In* Engineering Manual, Laboratory Soils Testing. EM 1110-2-1906. Compiled by Headquarters, Department of the Army, Office of the Chief Engineer. Updated by Change 1, May 1, 1980 and Change 2, August 29, 1986.

Table 1. Relative Density of Water and Correction Factor (K) for Various Temperatures[1].

Temp °C	Relative Density	Correction Factor, K[2]	Temp °C	Relative Density	Correction Factor, K	Temp °C	Relative Density	Correction Factor, K
18.0	0.99862	1.0004	23.0	0.99756	0.9993	28.0	0.99626	0.9980
.1	60	4	.1	54	3	.1	23	0
.2	58	4	.2	51	3	.2	20	0
.3	56	3	.3	49	3	.3	17	0
.4	54	3	.4	46	2	.4	14	79
.5	52	3	.5	44	2	.5	11	9
.6	50	3	.6	42	2	.6	08	9
.7	49	3	.7	39	2	.7	06	8
.8	47	2	.8	37	1	.8	03	8
.9	45	2	.9	34	1	.9	00	8
19.0	0.99843	1.0002	24.0	0.99732	0.9991	29.0	0.99597	0.9977
.1	41	2	.1	29	1	.1	94	7
.2	39	2	.2	27	0	.2	91	7
.3	37	1	.3	24	0	.3	88	6
.4	35	1	.4	22	0	.4	85	6
.5	33	1	.5	20	0	.5	82	6
.6	31	1	.6	17	89	.6	79	6
.7	29	1	.7	14	9	.7	76	5
.8	27	0	.8	12	9	.8	73	5
.9	25	0	.9	09	9	.9	70	5
20.0	0.99823	1.0000	25.0	0.99707	0.9988	30.0	0.99567	0.9974
.1	21	0	.1	04	8	.1	64	4
.2	19	0	.2	02	8	.2	61	4
.3	17	0.9999	.3	699	8	.3	58	3
.4	15	9	.4	97	7	.4	55	3
.5	13	9	.5	94	7	.5	52	3
.6	10	9	.6	91	7	.6	49	3
.7	08	8	.7	89	7	.7	46	2
.8	06	8	.8	87	6	.8	43	2
.9	04	8	.9	84	6	.9	40	2
21.0	0.99802	0.9998	26.0	0.99681	0.9986	31.0	0.99537	0.9971
.1	00	8	.1	78	6	.1	33	1
.2	798	8	.2	76	5	.2	30	1
.3	96	7	.3	73	5	.3	27	0
.4	93	7	.4	70	5	.4	24	0
.5	91	7	.5	68	4	.5	21	0
.6	89	7	.6	65	4	.6	18	69
.7	87	6	.7	63	4	.7	15	9
.8	85	6	.8	60	4	.8	12	9
.9	83	6	.9	57	3	.9	09	9
22.0	0.99780	0.9996	27.0	0.99654	0.9983	32.0	0.99505	0.9968
.1	78	6	.1	51	3	.1	02	8
.2	75	5	.2	48	2	.2	499	8
.3	73	5	.3	46	2	.3	96	7
.4	70	5	.4	43	2	.4	93	7
.5	68	5	.5	40	2	.5	90	7
.6	65	4	.6	37	1	.6	86	6
.7	63	4	.7	34	1	.7	83	6
.8	61	4	.8	32	1	.8	80	6
.9	58	4	.9	29	1	.9	77	5

[1] - Relative density of water based on density of water at 4° C equal to unity. The values given are numerically equal to the absolute density in grams/milliliter (for sediment testing purposes, g/mL = g/cm^3). Data obtained from Smithsonian Tables, compiled by various authors.

[2] - Correction factor, K, is found by dividing the relative density of water at the test temperature by the relative density of water at 20° C.

PARTICLE-SIZE ANALYSIS

1.0 Scope and Application

This method is applicable to the determination of particle-size distribution in sediment samples. Particle-size distributions are determined by a combination of sieving for particles retained on a No. 200 mesh sieve (particles with mean diameters greater than 0.074 mm or 0.0029 in) and hydrometer analysis for particles that pass through the No. 200 mesh sieve.

The method is based on a procedure in Appendix V of the U.S. Army Corps of Engineers engineering manual (1970).

2.0 Summary of Method

Particle-size analysis, or grain-size analysis, is a process in which a frequency distribution or a cumulative frequency distribution of discrete sized particles in a sediment sample is determined. This process is conducted by passing a known sample through a series of sieves with progressively smaller openings. The percentage of particles larger than a specific size is calculated as the weight of material retained on a sieve of that size divided by the weight of sample originally placed on the nest of sieves.

The particle-size distribution for the fraction of the original sample that passes the smallest sieve (No. 200) is determined using a hydrometer. The hydrometer method of analysis is based on Stokes' law, which relates the terminal velocity of a sphere falling freely through a fluid to the diameter. The relation is expressed according to the equation:

$$v = \frac{(\gamma_s - \gamma_f) \times D^2 \times g}{18\,\eta}$$

where:

v = terminal velocity of sphere, cm/sec
γ_s = density of particle, g/cm^3
γ_f = density of fluid, g/cm^3
D = diameter of sphere, cm
g = gravitational acceleration, cm/sec^2
η = viscosity of fluid, g/(sec × cm)

It is assumed that Stokes' law can be applied to a mass of dispersed sediment particles of various shapes and sizes. The hydrometer is used to determine the percentage of dispersed sediment particles remaining in suspension at a given time. The maximum grain size equivalent to a spherical particle is computed for each hydrometer reading using Stokes' law.

3.0 Interferences

Potential errors associated with the sieve analyses include the following:

1. aggregations of particles not thoroughly broken. If the material contains plastic fines, the sample should be slaked before sieving.
2. overloading sieves. This is the most common and most serious error associated with the sieve analysis and will tend to indicate that a material is coarser than it actually is. Large samples may have to be sieved in several portions and the portions retained on each sieve recombined afterwards for weighing.
3. sieves shaken for too short a period or with inadequate horizontal or jarring motions. The sieves must be shaken so that each particle is exposed to the sieve openings with various orientations and has every opportunity to fall through.
4. broken or deformed sieve screens. Sieves must be frequently inspected to ensure they contain no openings larger than the specified size for the sieve. Rips and tears commonly occur on the finer screen meshes particularly around the edge where the screen is welded to the brass frame.
5. loss of material when removing sediment from each sieve.

Potential errors associated with the hydrometer analyses include the following:

1. sediment oven-dried before test. With the exception of inorganic sediments of low dry strength, oven-drying may cause permanent changes in the particle sizes.
2. unsatisfactory type or quantity of dispersing agent. Whenever new or unusual sediments are tested, trials may be necessary to determine the type and quantity of chemical which gives the most effective dispersion and deflocculation.
3. incomplete dispersion of sediment into suspension.

4. insufficient shaking or agitating of suspension in cylinder at start of test.
5. too much sediment in suspension. The results of the hydrometer analysis will be affected in the size of the sample exceeds the approximately 150 g.
6. disturbance of suspension while inserting or removing hydrometer. Such disturbance is most likely to result when the hydrometer is withdrawn too rapidly after a reading.
7. stem of hydrometer not clean. Dirt or grease on the stem may prevent full development of the meniscus.
8. nonsymmetrical heating or cooling of suspension.
9. excessive variation in temperature of suspension during test.

Potential errors associated with the overall analyses include the following:

1. insufficient washing of material over the No. 200 sieve. The dispersing agent should be added to the water in which the sample is soaked and the sediment-water mixture should be frequently manipulated to aid the separation of particles. Coarser particles may be removed from the mixture and washed free of fines by hand to reduce the quantity of material to be washed on the sieve. While the additional water used for washing should be held to a minimum, enough must be added to insure adequate removal of the fines.
2. loss of suspension material passing the No. 200 sieve.

4.0 Apparatus and Materials

4.1 Apparatus Specific to Sieve Analyses

1. A series of U.S. standard sieves with openings ranging from 76 mm to 0.074 mm (No. 200), including a cover plate and bottom pan. Sieves with an 20 cm (8 in) diameter are generally sufficient for all particle-size analyses. The following sieves are generally used for the determination of particle-size distribution:

Sieve Mesh	Opening Size (------mm------)	Opening Size (--------in-------)
-	76	3
-	6.35	0.75
4	4.76	0.187
10	2.00	0.0787
35	0.50	0.0197
200	0.074	0.0029

Sieve sizes were selected according to the standard particle-size limits following the ASTM classification scheme (ASTM, 1985). Sieves should conform to ASTM Designation: E11, Standard Specifications for Sieves for Testing. Additional sieves may be used for testing a given sample depending upon the intended use of the particle-size distribution curve.

2. Sieve shaker, 1.25 cm vertical and lateral movement, and 500 oscillations per minute, or equivalent. Unit must accommodate a nest of sieves.
3. Paintbrush, 2.54 cm (1 in), or soft wire brush, for cleaning sieves. Brush hairs should be softer than the screen material.

4.2 Apparatus Specific to Hydrometer Analyses.

1. Standard hydrometer, ASTM no. 152H or equivalent, calibrated at 20° C (68° F), graduated in grams per liter with a range of 0 to 50, respectively. The accuracy of the hydrometer should be ± 1 unit.
2. Electric stirrer. A mechanically operated stirring device (milk shake mixer) in which a suitably mounted electric motor turns a vertical shaft at a speed of not less than 10,000 rpm without load. The shaft shall be equipped with a replaceable stirring paddle of metal, plastic, or hard rubber. An acceptable stirrer is available from Soil Test, Inc., Evanston, IL, or other sources[1].
3. Metal dispersion cup with internal baffles.
4. Sedimentation cylinders with a 1 L mark at 36 ± 2 cm from the bottom of the inside.

[1]Trade names are used solely for the purpose of providing specific information. Mention of trade names or commercial products does not constitute endorsement of recommendation for use.

5. Centigrade thermometer, range 0 to 50° C, accurate to ± 0.5° C.
6. Timing device, a watch or clock with a second hand.
7. Constant temperature bath, optional if room temperature is controlled. Either the room of bath should be controlled to ± 1° C.

4.3 Apparatus for Overall Particle-Size Analyses.

1. Analytical balance, capable of weighing to 0.001 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Drying oven.
4. Thermometer, 0 to 200° C range, graduated to 1° C.
5. 600 mL glass beakers.

5.0 Reagents

1. ASTM Type I water (ASTM, 1984).
2. Dispersing Agent.

   Suspensions of fine grain particles will tend to flocculate (i.e., to adhere with sufficient force that they settle together as a particle of apparently larger size). Consequently, a dispersing agent is added to the sediment suspensions to prevent flocculation of the individual particles during the hydrometer analysis. The following dispersing agents, listed in approximate order of effectiveness, have been found to be satisfactory for this purpose:

   a. Sodium tripolyphosphate, 0.4 N. Dissolve 29 g solids in 1 L water.
   b. Sodium polyphosphate, 0.4 N. Dissolve 36 g solids in 1 L water.
   c. Sodium tetraphosphate, 0.4 N. Dissolve 31 g solids in 1 L water.
   d. Sodium hexametaphosphate (sodium metaphosphate), 0.4 N. Place 41 g of sodium hexametaphosphate and 2.1 g of $NaCO_3$ in a 1 L container with approximately 900 mL water. Dilute the solution to 1 L with Type I water.

NOTE: Sodium hexametaphosphate is the most commonly used dispersant.

NOTE: Phosphate solutions are somewhat unstable and therefore should not be stored for extended periods of time. A fresh solution should be prepared at least once a month.

NOTE: The chemical product Calgon, available in retail outlets, should not be used as a dispersing agent as it no longer contains sodium hexametaphosphate. Also, sodium silicate should not be used as a dispersing agent since it gives unsatisfactory dispersion while at the same time permitting flocculation to a point where it is not apparent to visual examination.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Samples should be stored at 4° C between sample collection and analysis. It is recommended that particle-size samples not be frozen or oven-dried prior to analysis. Freezing-thawing cycles or sample drying may cause an irreversible change in the particle-size distribution in the sample.

All sample containers must be prewashed with detergents. If samples are to be analyzed for other parameters, acids and Type II water washings may also be required. Either glass or plastic containers can be used for the storage of samples to be analyzed for particle-size distribution.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The oven should be monitored to ensure that temperature fluctuation does not exceed ± 2° C.

7.1 Hydrometer Calibration

The hydrometer must be calibrated[2] to establish the relationship between the true depth and the hydrometer reading using the following procedure:

1. Determine the volume of the hydrometer bulb, V_R. This may be determined in either of two ways:

    a. Measure the volume of water displaced. Fill a 1000 mL graduated cylinder with water to approximately 700 mL. The water should be at a temperature of about 20° C. Observe and record the reading of the water level. Insert the hydrometer and again observe and record the reading. The difference in these two readings equals the volume of the bulb plus the part of the stem that is submerged. The error due to inclusion of this latter quantity is so small that is may be neglected for practical purposes.

    b. By determining the volume from the weight of the hydrometer. Weigh the hydrometer to 0.01 g on the laboratory balance. Since the specific gravity of a hydrometer is about unity, the weight in grams may be recorded as the volume in mL. This volume includes the volume of the bulb plus the volume of the stem. The error due to inclusion of the stem volume is negligible.

2. Determine the area, in cm^2, of the graduated cylinder in which the hydrometer is to be used by measuring the distance between two graduations (measured distance should be between approximately 0.32 and 0.38 cm). The area, A, is equal to the volume included between the graduations divided by the measured distance.
3. Measure and record the distances from the lowest calibration mark on the stem of the hydrometer to each of the other major calibration marks, R, in cm.

---

[2] ASTM hydrometers 151 H or 152 H (ASTM Designation: E 100) have a uniform size; therefore, only a single calibration is required, which can be applied to all ASTM hydrometers of this type.

4. Measure and record the distance from the neck of the bulb to the lowest calibration mark in cm. The distance, H_1, corresponding to a reading, R, equals the sum of the two distances measured in steps (3) and (4).
5. Measure the distance from the neck to the tip of the bulb. Record this as h, the height of the bulb in cm. The distance, h/2, locates the center of volume of a symmetrical bulb. If a nonsymmetrical bulb is used, the center of volume can be determined with sufficient accuracy by projecting the shape of the bulb on a sheet of paper and locating the center of gravity of this projected area.
6. Compute the true distances, H_R, corresponding to each of the major calibration marks, R, from the formula:

   $$H_R = H_1 + 0.5 [h - (V_R/A)]$$

   where:

   H_R = true distance of suspension above center of hydrometer bulb, cm
   H_1 = distance between reading mark on hydrometer and neck of the hydrometer bulb (equals the sum of the distances measured in steps 3 and 4 above)
   h = distance between tip and neck of hydrometer bulb, cm
   V_R = volume of hydrometer, cm^3 (mL)
   A = inside area of graduated cylinder, cm^2

7. Plot the calibration curve expressing the relation between H_R and R (the hydrometer reading), with H_R on the y-axis of the graph. The relation is essentially a straight line for hydrometers having a streamlined shape.

Once calibrated, the hydrometer can generally be considered to be calibrated for the its life time of use. If the hydrometer readings in the blank start to vary by more than 2 g/L (one line of the hydrometer neck markings) and the variance can not be explained by temperature differences or differences in the composition of the dispersing agent, then a recalibration or disposal (if a crack in the glass is noticed) of the hydrometer is necessary.

7.2 Meniscus Correction

As part of the calibration process, a meniscus correction must be determined for each hydrometer. This is necessary because hydrometers are calibrated for the surface of the liquid but sediment suspensions must be read at the upper rim of the meniscus rather than at the surface. The meniscus correction, C_m, which is a constant for a given hydrometer, is determined by:

1. immersing the hydrometer in water.
2. observing the height to which the meniscus rises on the stem above the water surface.

For most hydrometers, it will be found that C_m is equal to approximately 0.5. This value can be assumed for routine testing.

8.0 Procedure

1. Transfer 50 g of the homogenized, field-moist sediment sample into a 600 mL beaker. If the sample is dominated by particle sizes finer that the No. 4 sieve, a 50 g sample is generally sufficient for these analyses. If the sample is dominated by sands, a sample size of 75 to 100 g may be needed to obtain reproducible results for the fine fraction.

   However, the size of the sample to be used will depend on the maximum particle size in the sample and the requirement that the sample be representative of the material to be tested. The sample should be limited in weight so that no sieve in the series will be overloaded. Overloading of a sieve should be avoided because it can result in incomplete separation of the sample particles and subsequent errors in the particle size distribution of the sample. Maximum sieve loads on 20 cm (8 in) sieves are presented in Table 1.

   The following tabulation will be used as a guide in obtaining a minimum-weight sample:

Maximum Particle Size	Minimum Weight of Sample, g
3 in.	64,000 g
2 in.	19,000 g
1.5 in.	8,000 g
1 in.	2,400 g
0.75 in.	1,000 g
0.5 in.	300 g
0.375 in.	150 g
0.187 (No. 4)	50 g

Additionally, the size of the beaker may need to be increased as the weight of the sample increases due to the presence of larger particle sizes.

NOTE: Highly organic sediments require special treatment and it may be necessary to oxidize the organic matter in order to perform a hydrometer analysis on these sediments. Oxidation is accomplished by mixing the sample with a solution of 30 percent hydrogen peroxide until effervescence is no longer observed. H_2O_2 should be added in increments of 5 mL. When frothing has ceased, heat to 90° C to remove excess peroxide and water (do not take sample to dryness). If only small amounts of organic matter are present, treatment with hydrogen peroxide may be omitted.

2. Add 100 mL[3] of the dispersing agent and approximately 250 mL of water to the sediment. Stir and allow to stand overnight.

NOTE: If the sample contains more than about 10%, by weight, of sizes larger than the No. 4 sieve and more than 150 g of sample is required to obtain a representative sample (i.e., maximum particle size is greater than 0.375 in.), it is generally advisable to separate the material (after dispersing the sample - step 2) on the No. 4 sieve,

[3]The original method by the U.S. Army Corps of Engineers (USACE, 1970) indicates that the addition of 15 mL of dispersing agent should be adequate for most instances. Upon addition of the dispersing agent, the suspension must be observed for reflocculation of the sample (after 2 to 3 hours). If flocculation is observed, then additional 15 mL increments must be added. To eliminate the additional time required to determine if the suspension is adequately dispersed, Gee and Bauder (1986) use a one time addition of 100 mL of dispersing agent. It is for the sake of saving time and effort that dispersion following the method of Gee and Bauder will be used here.

retaining both fractions, and recombine for the sieve analysis starting in step 13. Continue with step 3 after sieving.

3. Transfer the treated sediment to the metal dispersing cup, washing any residue from the beaker with water. Add water, if necessary, until the water surface is 5 to 7.6 cm (2 to 3 inches) below the top of the cup.
4. Mechanically mix for 5 min on electric stirrer at 10,000 rpm. Additional time may be required for highly plastic sediments (an additional 5 min is recommended for these sediments).

   NOTE: If more than 10% of the sample is expected to be retained on the No. 4 sieve and is expected to contain less than 50 g of fines, it is generally advisable to separate the sample on a No. 4 sieve, retaining both fractions for the sieve and hydrometer analysis. To perform this separation, place the No. 4 sieve in a large funnel over the sedimentation cylinder. Quantitatively transfer the suspension from the dispersing cup to the sedimentation cylinder. Gently wash the portion of the sample retained on the No. 4 sieve using a wash bottle or gentle stream of water. Bring the suspension volume in the cylinder to approximately 800 mL. Remove the funnel and sieve. Add water until the volume of the suspension equals 1000 mL. Save the fraction retained on the No. 4 sieve and recombine with the sample in step 13. Proceed to step 6 after the suspension has had sufficient time to thermally equilibrate to the temperature expected to prevail during the test.

5. Quantitatively transfer the suspension into a 1000-mL sedimentation cylinder and add water until the volume of the suspension equals 1000 mL. The suspension should be brought to the temperature expected to prevail during the test.
6. One minute before starting the test, take the sedimentation cylinder in one hand and using a suitable rubber stopper, shake the suspension vigorously for a few seconds in order to transfer the sediment on the bottom of the cylinder into a uniform suspension. Continue the agitation for the remainder of the minute by turning the cylinder upside down and back. It may be necessary to loosen the sediment at the bottom of the cylinder by means of a glass rod before shaking to assist in the resuspension of the settled sediments.

Alternatively, the suspension may be agitated by means of a hand agitator/plunger for one minute prior to testing. A uniform distribution of the sediment particles in the suspension is accomplished by moving the hand agitator up and down through the suspension for one minute. This process also prevents the accumulation of sediment on the base and sides of the sedimentation cylinder.

NOTE: Add a drop of amyl alcohol if the surface of the suspension is covered with foam (Gee and Bauder, 1986).

7. Slowly immerse the hydrometer in the suspension 20 to 25 sec before each reading.

   NOTE: Care should be taken when inserting and removing the hydrometer to prevent disturbance of the suspension.

8. Observe and record the hydrometer readings after 1 and 2 min have elapsed from the time the mixing (either by cylinder inversion or plunging) has been completed. After the 2 min reading, carefully remove the hydrometer from the suspension and place it in a graduated cylinder of clean water). (If a hydrometer is left in a sediment suspension for any length of time, material will settle on or adhere to the hydrometer bulb and this will cause a significant error in the reading).

   NOTE: Make all hydrometer readings at the top of the meniscus.

9. At the end of 2 min and after each subsequent hydrometer reading, place a thermometer in the suspension and record the temperature. The temperature should be recorded to ± 0.5° C. Temperature changes of the sediment suspension during the test will affect the test results. Variations in temperature should be minimized by keeping the suspension away from heat sources such as radiators, sunlight, or open windows. A constant-temperature bath or constant temperature room provide convenient means for controlling temperature variation effects. Temperatures should be controlled within ± 1° C.

10. Insert the hydrometer in the suspension and record readings after elapsed times of 4, 15, 30, 60, 120[4], 240, and 1440 min, removing the hydrometer from the suspension after each reading and placing it in a graduated cylinder of clean water.
11. Quantitatively transfer the sediment and suspension from the 1000 mL sedimentation cylinder to a 200-mesh (0.074 mm) sieve using a wash bottle or gentle stream of water.
12. Gently wash the sands and other coarser particles until all the fines have passed through the 200-mesh sieve.

    NOTE: The disposal of the fine fraction should be performed according to State regulations.

13. Transfer the sample to a tared beaker.
14. Oven-dry the sample at 103 to 105° C, allow to cool, and weigh. If the sample weighs less than 500 g, weigh it to the nearest 0.1 g. If the sample weighs over 500 g, weigh to the nearest 1 g. Record the dry weight of the sample.
15. Arrange the nest of sieves according to size with decreasing opening diameters from top to bottom. Attach the bottom pan to the bottom of the smallest sieve opening diameter used.
16. Place the sample on the top sieve of the nest and put the cover plate over the top sieve.
17. Place the nest of sieves in the shaking machine and shake for 10 min, or until additional shaking does not produce appreciable changes in the amounts of material on each sieve.

    If a shaking machine is not available, the nest of sieves may be shaken by hand. In the hand operation, shake the nest of sieves with a lateral and vertical motion, accompanied by jarring, to keep the material moving continuously over the surfaces of the sieves. Jarring is accomplished by occasionally dropping the nest lightly on several thicknesses of magazines/papers. The nest should not be broken to rearrange particles or to manipulate them through a sieve by hand. Hand-shaking should be continued for at least 15 min.

---

[4] A final reading after 120 min is sufficient for most soils when hydrometer analysis is used for classification purposes. Additional readings at 240 and 1440 min may be made to further separate the fines into individual silt and clay fractions.

18. Remove the nest of sieves from the mechanical shaker, if used. Beginning with the top sieve, transfer the contents of the sieve to a piece of heavy paper approximately 30 cm^2 (1 ft^2). Carefully invert the sieve on the paper and gently brush the bottom of the sieve to remove all the sample.
19. Transfer the sample from the paper to the balance and weigh in accordance with requirements in step 14. Record the weight of material retained on each sieve to the nearest 0.001 g.

    NOTE: Care should be exercised that no loss of material occurs during the transfer.

20. Repeat steps 18 and 19 for each sieve.

9.0 Quality Control

9.1 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 10 percent or less.

NOTE: Precision for the coarser fractions naturally tends to be more variable than for the finer fractions due to fewer particles accounting for the weight of these fractions. Therefore, precision for the largest particle sizes may not meet the acceptance criterion. However, the whole analysis (i.e., all fractions) should be examined to determine if acceptable precision has been obtained with a majority of the fractions should meet the acceptance criterion.

9.2 Blanks

The addition of a dispersing agent to the sediment suspension results in an increase in density of the liquid and necessitates a correction to the observed hydrometer reading. The correction factor, C_d, is determined by adding to a 1000 mL sedimentation cylinder partially filled with water, 100 mL of dispersing agent, filling the cylinder to the 1000 mL mark with water, inserting the hydrometer, and observing the reading. The correction factor, C_d, is equal to the difference between this reading and the hydrometer reading in pure water (i.e., no dispersing agent added).

One blank should be prepared and analyzed for each batch of dispersing agent to account for the slight differences in dispersing agent concentrations and resultant density changes.

9.3 Internal Consistency Checks

The sum of the weights retained on each sieve should equal the weight of the coarse fraction obtained in steps 13 and 14 within ± 1%. If the difference is greater than 1 percent, sample processing should be discontinued until the source of the error is identified and a decision made to repeat the analyses.

10.0 Method Performance

None identified.

11.0 Calculations

11.1 Percent Retained on No. 200 Sieve

Compute the percentage retained on the No. 200 sieve for the total sample used in the sieve and hydrometer combined analysis as follows:

$$\text{Percent retained on No. 200 sieve, \%} = \frac{W_d \times 100}{B \times [(1-C)/(100+C)]}$$

where:

W_d = dry weight of sample retained on No. 200 sieve from steps 13 and 14, g
B = weight of field-moist sample, g
C = moisture content as determined by previous method, %

11.2 Sieve Analysis Calculations

The percentage of material by weight retained on the various sieves is computed as follows:

$$\text{Percent retained, \%} = \frac{A \times 100}{B \times [(1-C)/(100+C)]}$$

where:

A = weight of fraction retained on the sieve, g
B = weight of field-moist sample, g
C = moisture content as determined by previous method, %

11.3 Hydrometer Analysis Calculations

Compute the corrected hydrometer readings, R, for use in computing particle diameter by adding the meniscus correction, C_m, to the actual hydrometer readings, R'. Record the corrected reading, R.

Calculate the particle diameter corresponding to a given hydrometer reading on the basis of Stokes' equation, using the nomograph shown in Fig. 1 (nomograph). The R-scale corresponding to the distances H_R is prepared using the hydrometer calibration curves as determined in section 7.0 - step 6. The R-scale should be designed for the particular hydrometer used in the test. A key showing the steps to follow in computing D for various values of R is shown on the chart. Record the particle diameters, D, for each time interval of sampling.

To calculate the percent finer by weight, use the following formula:

$$\text{Percent finer by weight} = \frac{(R - C_d + m) \times 100}{B \times [(1-C)/(100+C)]}$$

where:

R = corrected hydrometer reading from the hydrometer calibration chart
C_d = dispersing agent correction factor from section 9.2
m = temperature correction factor from Table 2.
B = weight of field-moist sample, g
C = moisture content as determined by previous method, %

NOTE: Calculations for routine work can be greatly facilitated by using charts, tables, and other simplifying aids based on a given oven-dry weight of the sample and average specific gravity values for the major sediment types.

The results of the particle-size analysis, in terms of particle diameter and total percent finer by weight, are generally presented either in the form of particle-size distribution curves on a semi-logarithmic chart or as tables. The particle-size distribution curves obtained from the sieve and hydrometer analyses are joined by constructing a smooth curve between all points.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

American Society for Testing and Materials. 1985. Standard Test Method for Classification of Soils for Engineering Purposes. D 2487-83. Annual Book of ASTM Standards 04.08:395-408. ASTM, Philadelphia, PA.

Gee, G.W., and J.W. Bauder. 1986. Particle-Size Analysis. *In* Klute, A. (ed.) Methods of Soil Analysis, Part 1. Agronomy 9. 2nd ed. American Society of Agronomy, Madison, WI.

Plumb, R.H., Jr. 1981. Procedure for Handling and Chemical Analysis of Sediment and Water Samples. Technical Report EPA/CE-81-1. U.S. Army Engineering Waterways Experiment Station, Vicksburg, MS.

U.S. Army Corps of Engineers. 1970. Grain-Size Analysis. *In* Engineering Manual, Laboratory Soils Testing. EM 1110-2-1906. Compiled by Headquarters, Department of the Army, Office of the Chief Engineer. Updated by Change 1, May 1, 1980 and Change 2, August 29, 1986.

Table 1. Maximum Sieve Loads on 20 cm Sieves (after Plumb, 1981).

Sieve Mesh	Opening Size (------mm------)	Maximum Load (----------g---------)
-	76	N/A[a]
-	6.35	200
4	4.76	175.
10	2.00	110.
35	0.50	60
200	0.074	25

a - N/A = not applicable due to large individual particle size.

Table 2. Temperature Correction Factor, m, for Use in Computing Percent Finer.

Degrees (C)	Degrees (F)	Correction	Degrees (C)	Degrees (F)	Correction
14.0	57.2	-0.9	24.5	76.1	+0.9
14.5	58.1	-0.8	25.0	77.0	+1.0
15.0	59.0	-0.8	25.5	77.9	+1.1
15.5	59.9	-0.7	26.0	78.8	+1.3
16.0	60.8	-0.6	26.5	79.7	+1.4
16.5	61.7	-0.6	27.0	80.6	+1.5
17.0	62.6	-0.5	27.5	81.5	+1.6
17.5	63.5	-0.4	28.0	82.4	+1.8
18.0	64.4	-0.4	28.5	83.3	+1.9
18.5	65.3	-0.3	29.0	84.2	+2.1
19.0	66.2	-0.2	29.5	85.1	+2.2
19.5	67.1	-0.1	30.0	86.0	+2.3
20.0	68.0	0.0	30.5	86.9	+2.5
20.5	68.9	+0.1	31.0	87.8	+2.6
21.0	69.8	+0.2	31.5	88.7	+2.8
21.5	70.7	+0.3	32.0	89.6	+2.9
22.0	71.6	+0.4	32.5	90.5	+3.0
22.5	72.5	+0.5	33.0	91.4	+3.2
23.0	73.4	+0.6	33.5	92.3	+3.3
23.5	74.3	+0.7	34.0	93.2	+3.5
24.0	75.2	+0.8			

AMMONIA NITROGEN IN SEDIMENTS (COLORIMETRIC, AUTOMATED)

1.0 Scope and Application

This method covers the determination of ammonia in sediments. Ammonia is distilled from a sediment slurry and determined with an automated colorimetric method. This range is for photometric measurements made at 630-660 ηm in a 15 mm or 50 mm tubular flow cell. Higher concentrations can be determined by sample dilution. Approximately 20 to 60 samples per hour can be analyzed.

This procedure is based on Method 350.1 (APHA, 1989).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Ammonia is distilled from a sediment-distilled water slurry and trapped in a boric acid solution. The distillate is analyzed with an automated method in which alkaline phenol and hypochlorite are reacted with ammonia to form indophenol blue. The blue color formed is intensified with sodium nitroprusside and is proportional to the ammonia concentration. The intensity of the color is automatically determined by measuring sample absorbance at 630 ηm.

3.0 Interferences

Sulfide that may be present in sediments can interfere with ammonia analysis. This interference can be reduced or eliminated by boiling the acidified sediment slurry prior to distilling off the ammonia under neutral conditions (Section 8.5). The sulfide interference can also be removed by precipitation with lead carbonate.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.

2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Technicon AutoAnalyzer Unit (AAI or AAII) consisting of:
   a. sampler,
   b. manifold (AAI) or analytical cartridge (AAII),
   c. proportioning pump,
   d. heating bath with double delay coil (AAI),
   e. colorimeter equipped with 15 mm tubular flow cell and 630-660 ηm filters,
   f. recorder, and
   g. digital printer for AAII (optional).
4. Kjeldahl digestion apparatus.

4.2 Materials

1. Boiling chips
2. Erlenmeyer flask, 1 L.
3. Erlenmeyer flask, 100 mL.
4. Kjeldahl flask, 800 mL.
5. Volumetric flask, class A, 100 mL.
6. Volumetric flask, class A, 1 L.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.

   NOTE: Type II water: Special precaution must be taken to insure that this Type II water is free of ammonia. Such water is prepared by passage of Type II water 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 instruction of the manufacturer. All solutions must be made using ammonia-free Type II water.

2. Boric acid solution. Dissolve 20 g anhydrous boric acid (H_3BO_3) in ammonia-free Type II water. Dilute to 1 liter with Type II water.

3. Disodium ethylenediamine-tetraacetate (EDTA), 5%. Dissolve 50 g of EDTA (disodium salt) and approximately six pellets of NaOH in 1 liter of Type II water.
4. Phosphate buffer solution, pH 7.4. Dissolve 14.3 g anhydrous potassium dihydrogen phosphate (KH_2PO_4), and 68.8 g anhydrous dipotassium hydrogen phosphate (K_2HPO_4). Dilute to 1 liter with ammonia-free Type II water.
5. Sodium hypochlorite (NaOCl) solution. Dilute 250 mL of a bleach solution containing 5.25% NaOCl (such as "Clorox") to 500 mL with Type II water. Available chlorine level should approximately 2 to 3%.

   NOTE: Since "Clorox" is a proprietary product, its formulation is subject to change. The analyst must remain alert to detecting any variation in this product significant to its use in this procedure. Due to the instability of this product, storage over an extended period should be avoided.
6. Sodium hydroxide (NaOH), 1 N: Dissolve 40 g NaOH in ammonia-free Type II water. Dilute to 1 liter with Type II water.
7. Sodium nitroprusside ($Na_2Fe(CN)_5NO \cdot H_2O$), 0.05%. Dissolve 0.5 g of sodium nitroprusside in 1 liter of Type II water.
8. Sodium phenolate. Using a 1 liter Erlenmeyer flask, dissolve 83 g phenol (C_6H_5OH) in 500 mL of Type II water. In small increments, cautiously add with agitation, 32 g of NaOH. Periodically cool flask under water faucet. When cool, dilute to 1 liter with Type II water.
9. Concentrated sulfuric acid (H_2SO_4), reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
10. Sulfuric acid (H_2SO_4), 5 N, for use as the air scrubber solution. Carefully add 139 mL of concentrated sulfuric acid to approximately 500 mL of ammonia-free Type II water. Cool to room temperature and dilute to 1 liter with ammonia-free Type II water.
11. Ammonia stock solution. Dissolve 3.819 g of anhydrous ammonium chloride (NH_4Cl), dried at 105° C, in Type II water. Dilute to 1 liter with Type II water. (1.0 mg/mL NH_3-N).
12. Standard solution A. Dilute 10.0 mL of ammonia stock solution to 1 liter with Type II water. (0.01 mg/mL NH_3-N).
13. Standard solution B. Dilute 10.0 mL of standard solution A to 1 liter with Type II water. (0.001 mg/mL NH_3-N).

6.0 Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Samples should be stored in a field moist condition which should help minimize the possible loss of ammonia by volatilization. In addition, samples should be processed within a week to minimize possible losses of ammonia due to volatilization or transformation.

A holding time of 28 days after sample collection is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of sediment samples to be analyzed for ammonia.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Using standard solutions A and B, prepare the following standards in 100 mL volumetric flasks (prepare fresh daily):

NH_3-N, mg/L	mL Standard Solution/100 mL
	Solution B
0.01	1.0
0.02	2.0
0.05	5.0
0.10	10.0
	Solution A
0.20	2.0
0.50	5.0
0.80	8.0
1.00	10.0
1.50	15.0
2.00	20.0

8.0 Procedure

8.1 Preparation of Equipment

1. Add 500 mL of Type II water to an 800 mL Kjeldahl flask.

   NOTE: The addition of boiling chips which have been previously treated with dilute NaOH will prevent bumping.

2. Steam out the distillation apparatus until the distillate shows no trace of ammonia with Nessler reagent.

8.2 Sample Distillation

1. Weigh a 0.5 to 1.0 g sample of homogenized, field-moist sediment and quantitatively transfer the sample to a 100 mL Erlenmeyer flask
2. Add approximately 50 ml ammonia-free Type II water and 3 to 4 drops concentrated sulfuric acid.

NOTE: Addition of a few drops of concentrated sulfuric acid will stabilize the ammonia. The procedure can be interrupted at this point, if necessary.

3. Transfer the acidified sediment slurry to a 800 mL Kjeldahl flask.
4. Add 500 mL ammonia-free Type II water and a few boiling stones to an 800 mL Kjeldahl flask.
5. Boil the sample for a few minutes to remove any sulfides that may be present.

   NOTE: This step will also remove any volatile organics such as formaldehyde that may interfere with the Nesslerization determination of ammonia.

   NOTE: Sulfide interferences may also be removed by precipitating the sulfide with lead carbonate.

6. Neutralize the pH of the sediment slurry with 1 N NaOH to a pH of about 6.6.
7. Add 10 mL phosphate buffer.
8. Distill over 300 mL of sample, at a rate of 6 to 10 mL/minute, and collect in 50 mL boric acid solution.
9. Dilute the final distillate to 500 mL with ammonia free water. The samples are now ready for analysis.

8.3 Automated Colorimetric Analysis

NOTE: The intensity of the color developed between ammonia and the colorimetric reagents is pH dependent. In order to compensate for this effect, the pH of the samples, the standard ammonia solutions, and the wash water should be similar. This can be accomplished by either (1) adding 2 mL concentrated H_2SO_4 per liter to the standards and wash water or (2) neutralizing the pH of the samples with NaOH or KOH.

1. Select the appropriate manifold for the automated analyses to be run. For a working range of 0.01 to 2.00 mg NH_3-N/L use the AAI set up. For a working range of.01 to 1.0 mg NH_3-N/L, use the AAII set up. Higher concentrations may be accommodated by sample dilution.

   NOTE: Manifold flow rates for the AAI set up are as follows:

Wash Water	2.0 mL/min.
Sample	0.42 mL/min.
EDTA	0.8 mL/min.
Air*	0.23 mL/min.
Na-phenolate	0.42 mL/min.
Na-hypochlorite	0.32 mL/min.
Na-nitroprusside	0.42 mL/min.

NOTE: Manifold flow rates for the AAII set up are as follows:

Wash Water	2.9 mL/min.
Sample	2.0 mL/min.
EDTA	0.8 mL/min.
Air*	2.0 mL/min.
Na-phenolate	0.6 mL/min.
Na-hypochlorite	0.6 mL/min.
Na-nitroprusside	0.6 mL/min.

* = air should be scrubbed through 5 N H_2SO_4

2. Allow both colorimeter and recorder to warm up for 30 minutes.
3. Obtain a stable baseline with all reagents, feeding Type II water through sample line.
4. For the AAI system, sample at a rate of 20/hr. 1:1. For the AAII, use a 60/hr 6:1 cam with a common wash.
5. Arrange ammonia standards in the sampler in order of decreasing ammonia concentration.
6. Complete loading of the sampler tray with routine and quality assurance/quality control samples.
7. Switch sample line from distilled water to sampler and begin analysis.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for ammonia in sediments is 100 µg/kg.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured ammonia concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

10.0 Method Performance

In a single laboratory (EMSL-CIN), using surface water samples at concentrations of 1.41, 0.77, 0.59 and 0.43 mg NH_3-N/L, the standard deviation was ± 0.005. In a second single laboratory study (Alberta Pollution Control Laboratory), the calculated coefficient of variation for surface water samples with ammonia concentrations of 0.029, 0.060, and 0.093 mg/L NH_3-N were 4.7, 2.0, and 1.1%, respectively.

In a single laboratory (EMSL-CIN), using surface water samples at concentrations of 0.16 and 1.44 mg NH_3-N/L, recoveries were 107 and 99%, respectively. In a second single laboratory study (Alberta Pollution Control Laboratory), using surface water samples with NH_3-N concentrations of 0.008, 0.015, and 0.039 mg/L, the recoveries were 104, 97, and 105%, respectively.

11.0 Calculations and Reporting

The resultant ammonia concentrations can obtained by comparison of distillate peak heights to the standard curve of peak heights of the standards plotted against their corresponding known concentration values. The ammonia concentration of the original field moist sample is then calculated as:

$$\text{Ammonia nitrogen, mg/kg (wet weight)} = \frac{X \times Y \times 1000}{g}$$

where:
X = ammonia concentration in distillate, mg/L
Y = final volume of sediment distillate, L
g = wet weight of sediment sample, g

12.0 References

Alberta Environmental Centre. 1981. Methods Manual for Chemical Analysis of Water and Wastes. Environment Canada, Vegreville, Alberta, Canada.

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. Method 4500-NH_3 H. 17th Edition, APHA, New York, New York. p. 4-126.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Booth, R. L., and L.B. Lobring. 1973. Evaluation of the AutoAnalyzer II: A Progress Report". In Advances in Automated Analysis 8:7-10. 1972 Technicon International Congress. Mediad Incorporated, Tarrytown, N.Y.

Fiore, J., and J.E. O'Brien. 1962. Ammonia Determination by Automatic Analysis. Wastes Engineering 33:352.

Hiller, A., and D. Van Slyke. 1933. Determination of Ammonia in Blood. J. Biol. Chem. 102:499.

O'Connor, B., R. Dobbs, B. Villiers, and R. Dean. 1967. Laboratory Distillation of Municipal Waste Effluents. JWPCF 39:25.

AMMONIA NITROGEN IN SEDIMENTS (COLORIMETRIC, MANUAL)

1.0 Scope and Application

This method covers the determination of ammonia in sediments. Ammonia is distilled from a sediment slurry and determined colorimetrically. Spectrophotometric measurements are made at a wavelength of 425 ηm. The ammonia concentrations determined by this method should be $\leq$1.0 mg NH_3-N/L. Higher concentrations can be determined by sample dilution.

This procedure is based on Method 350.2 (APHA, 1989).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Ammonia is distilled from a sediment-distilled water slurry and trapped in a boric acid solution. The distillate is analyzed after nesslerization using a spectrophotometer. The intensity of the color is automatically determined by measuring sample absorbance at 425 ηm.

3.0 Interferences

Sulfide that may be present in sediments can interfere with ammonia analysis. This interference can be reduced or eliminated by boiling the acidified sediment slurry for a few minutes prior to distilling off the ammonia under neutral conditions (Section 8.5). The sulfide interference can also be removed by precipitation with lead carbonate.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Kjeldahl distillation apparatus.

4. Spectrophotometer or filter photometer for use at 425 ηm and providing a light path of 1 cm or more.

4.2 Materials

1. Boiling chips
2. Erlenmeyer flask, 100 mL.
3. Kjeldahl flask, 800 mL.
4. Nessler tubes. Matched Nessler tubes (APHA Standard) about 300 mm long, 17 mm inside diameter, and marked at 225 mm ± 1.5 mm inside measurement from bottom.
5. Volumetric flask, class A, 100 mL.
6. Volumetric flask, class A, 1 L.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.

   NOTE: Type II water: Special precaution must be taken to insure that this Type II water is free of ammonia. Such water is prepared by passage of Type II water 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 instruction of the manufacturer. All solutions must be made using ammonia-free Type II water.

2. Boric acid solution, 2%. Dissolve 20 g anhydrous boric acid (H_3BO_3) in ammonia-free Type II water. Dilute to 1 liter with Type II water.
3. Nessler reagent. Dissolve 100 g of mercuric iodide (HgI) and 70 g of potassium iodide (KI) in a small amount of water. Add this mixture slowly, with stirring, to a cooled solution of 160 g of NaOH in 500 mL of Type II water. Dilute to 1 liter with Type II water.

   NOTE: If this reagent is stored in a Pyrex bottle out of direct sunlight, it will remain stable for a period of up to 1 year.

NOTE: This reagent should give the characteristic color with ammonia within 10 minutes after addition and should not produce a precipitate with small amounts of ammonia ($\leq$0.04 mg/50 mL).

4. Phosphate buffer solution, pH 7.4. Dissolve 14.3 g anhydrous potassium dihydrogen phosphate (KH_2PO_4), and 68.8 g anhydrous dipotassium hydrogen phosphate (K_2HPO_4). Dilute to 1 liter with ammonia-free Type II water.
5. Sodium hydroxide (NaOH), 1 N: Dissolve 40 g NaOH in ammonia-free Type II water. Dilute to 1 liter with Type II water.
6. Concentrated sulfuric acid (H_2SO_4), reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
7. Ammonia stock solution. Dissolve 3.819 g of anhydrous ammonium chloride (NH_4Cl), dried at 105° C, in Type II water. Dilute to 1 liter with Type II water. (1.0 mg/mL NH_3-N).
8. Ammonia standard solution. Dilute 10.0 mL of ammonia stock solution to 1 liter with Type II water. (0.01 mg/mL NH_3-N).

6.0 Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Samples should be stored in a field moist condition which should help minimize the possible loss of ammonia by volatilization. In addition, samples should be processed within a week to minimize possible losses of ammonia due to volatilization or transformation.

A holding time of 28 days after sample collection is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of sediment samples to be analyzed for ammonia.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Prepare a series of Nessler tube standards as follows:

mL of Standard (0.01 mg/mL NH_3-N)	mg NH_3-N/50.0 mL
0.0	0.0
0.5	0.005
1.0	0.01
2.0	0.02
3.0	0.03
4.0	0.04
5.0	0.05
8.0	0.08
10.0	0.10

Dilute each tube to 50 mL with Type II water. Add 2.0 mL of Nessler reagent. Mix. After 20 minutes read the absorbance at 425 ηm against the blank. From the values obtained, plot absorbance vs. mg NH_3-N for the standard curve.

The sulfuric acid standard solution must be standardized following one of the two following methods:

a. Standardize the approximately 0.02 N acid against 0.0200 N Na_2CO_3 solution. This sodium carbonate solution is prepared by dissolving 1.060 g anhydrous Na_2CO_3, oven-dried at 140° C, and diluting to 1 liter with CO_2-free Type II water.
b. Standardize the approximately 0.1 N H_2SO_4, solution against a 0.100 N Na_2CO_3 solution. By proper dilution, the 0.02 N acid can then be prepared.

The later method (b.) is preferable.

8.0 Procedure

8.1 Preparation of Equipment

1. Add 500 mL of Type II water to an 800 mL Kjeldahl flask.

   NOTE: The addition of boiling chips which have been previously treated with dilute NaOH will prevent bumping.

2. Steam out the distillation apparatus until the distillate shows no trace of ammonia with Nessler reagent.

8.2 Sample Distillation

1. Weigh a 0.5 to 1.0 g sample of homogenized, field-moist sediment and quantitatively transfer the sample to a 100 mL Erlenmeyer flask
2. Add approximately 50 ml ammonia-free Type II water and 3 to 4 drops concentrated sulfuric acid.

   NOTE: Addition of a few drops of concentrated sulfuric acid will stabilize the ammonia. The procedure can be interrupted at this point, if necessary.

3. Transfer the acidified sediment slurry to a 800 mL Kjeldahl flask.
4. Add 500 mL ammonia-free Type II water and a few boiling stones to an 800 mL Kjeldahl flask.
5. Boil the sample for a few minutes to remove any sulfides that may be present.

   NOTE: This step will also remove any volatile organics such as formaldehyde that may interfere with the Nesslerization determination of ammonia.

   NOTE: Sulfide interferences may also be removed by precipitating the sulfide with lead carbonate.

6. Neutralize the pH of the sediment slurry with 1 N NaOH to a pH of about 6.6.
7. Add 10 mL phosphate buffer.
8. Distill over 300 mL of sample, at a rate of 6 to 10 mL/minute, and collect in 50 mL boric acid solution.
9. Dilute the final distillate to 500 mL with ammonia free water. The samples are now ready for analysis.

8.3 Colorimetric Analysis

1. Allow the spectrophotometer to warm up for 30 minutes.
2. Add 2.0 mL of Nessler reagent to 50 mL of the distillate in a Nessler tube. Mix.
3. After 20 minutes, read the absorbance at 425 ηm.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for ammonia in sediments is 100 µg/kg.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured ammonia concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

10.0 Method Performance

In a single laboratory (EMSL-CIN), using surface water samples at concentrations of 1.41, 0.77, 0.59 and 0.43 mg NH_3-N/L, the standard deviation was ± 0.005. In a second single laboratory study (Alberta Pollution Control Laboratory), the calculated coefficient of variation for surface water samples with ammonia concentrations of 0.029, 0.060, and 0.093 mg/L NH_3-N were 4.7, 2.0, and 1.1%, respectively.

In a single laboratory (EMSL-CIN), using surface water samples at concentrations of 0.16 and 1.44 mg NH_3-N/L, recoveries were 107 and 99%, respectively. In a second single laboratory study (Alberta Pollution Control Laboratory), using surface water samples with NH_3-N concentrations of 0.008, 0.015, and 0.039 mg/L, the recoveries were 104, 97, and 105%, respectively.

11.0 Calculations and Reporting

The resultant ammonia concentrations can obtained by comparison of distillate peak heights to the standard curve of peak heights of the standards plotted against their corresponding known concentration values. The ammonia concentration of the original field moist sample is then calculated as:

$$\text{Ammonia nitrogen, mg/kg (wet weight)} = \frac{X \times Y \times 1000}{g}$$

where:
X = ammonia concentration in distillate, mg/L
Y = final volume of sediment distillate, L
g = wet weight of sediment sample, g

12.0 References

Alberta Environmental Centre. 1981. Methods Manual for Chemical Analysis of Water and Wastes. Environment Canada, Vegreville, Alberta, Canada.

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. Method 4500-NH_3 H. 17th Edition, APHA, New York, New York. p. 4-126.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

CYANIDE IN SEDIMENTS
(COLORIMETRIC, AUTOMATED UV)

1.0 Scope and Application

This method is used to determine the concentration of inorganic cyanide in sediments. The method detects inorganic cyanides that may be present as either simple soluble salts or complex radicals.

The colorimetric method is sensitive to approximately 0.02 mg/L of cyanide and is recommended for concentrations below 1 mg/L. The range of the procedure can be adjusted by modifying the sample preparation technique or the cell path length. However, the amount of sodium hydroxide in the standards and the sample to be analyzed must be the same.

This procedure is based on SW-846 Method 9012 (EPA, 1986).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

An aliquot of the sediment sample to be analyzed is placed in 500 mL of acidified distilled water. The resulting suspension is heated to distill hydrocyanic acid (HCN) from the acidic suspension and into a sodium hydroxide trap. The cyanide concentration of the final distillate is determined with an automated UV colorimetric procedure.

Cyanide in the distillate is reacted with chloramine-T at a pH less than 8 to produce cyanogen chloride (CNCl). After this reaction is complete, the addition of pyridine-barbituric acid reagent produces a red-blue color that is proportional to the cyanide concentration. The intensity of the color is automatically determined by measuring sample absorbance at 570 ηm. The concentration of NaOH must be the same in the standards, the sample distillate, and any dilutions of the original sample distillate to obtain colors of comparable intensity.

3.0 Interferences

Sulfides adversely affect the development of color in the analytical procedure. This interference can be reduced or eliminated by adding bismuth nitrate to the samples to precipitate the sulfide prior to distillation (Section 8.1.4). Samples that contain hydrogen sulfide, metal sulfides, or other compounds that may produce sulfide during the distillation procedure should also be treated with bismuth nitrate prior to distillation.

Nitrate and/or nitrite in samples can act as a positive interference when present at concentrations above 10 mg/L and in the presence of certain organic compounds. These nitrogen compounds can form nitrous acid during the distillation process which will react with some organic compounds to form oxides. These oxides will decompose under conditions developed in the colorimetric procedure to generate HCN. This interference is eliminated by treating the samples with sulfamic acid prior to distillation (Section 8.1.5).

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Reflux distillation apparatus. The boiling flask should be of 1-liter size with inlet tube and provision for condenser. The gas absorber is a Fisher-Milligan scrubber (Fisher Catalog #07-513), or equivalent.
4. Automated continuous-flow analytical instrument with:
   a. sampler,
   b. manifold with UV digestor,
   c. proportioning pump,
   d. heating bath with distillation coil,
   e. distillation head,
   f. colorimeter equipped with a 15 mm flow cell and 570 ηm filter, and
   g. recorder.
5. Extractor. Any suitable device that sufficiently agitates a sealed container with a capacity of one liter or more. For the purpose of this procedure, agitation must be sufficient to: (1) maintain

continuous contact between all sample particles and the extraction fluid, and (2) prevent stratification of the sample and the extraction fluid.

6. Buchner funnel, 500 mL capacity.
7. Vacuum filtration flask, 1 L.
8. Glass fiber filter pads.
9. Vacuum source, preferably a water driven aspirator. A value or stopcock is needed to release the vacuum.
10. Top loading balance, capable of weighing 0.1 g.

4.2 Materials

1. Potassium iodide-starch test paper.
2. Volumetric flasks, class A, 250 mL.
3. Volumetric flasks, class A, 100 mL.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Ascorbic acid ($C_6H_8O_6$), analytical reagent grade crystals.
3. Bismuth nitrate solution ($Bi(NO_3)_3$). Dissolve 30.0 grams of $Bi(NO_3)_3$ in 100 mL of Type II water. While stirring, add 250 mL of glacial acetic acid. Stir until dissolved. Dilute to 1 liter with Type II water.
4. Chloramine-T solution. Dissolve 1.0 g of white, water-soluble, chloramine-T in 100 mL of Type II water. Refrigerate until ready to use.
5. Concentrated acetic acid ($C_4H_6O_3$), glacial, reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
6. Concentrated sulfuric acid (H_2SO_4), reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
7. Sulfuric acid (1:1). Slowly add 500 mL of concentrated H_2SO_4 to 500 mL of Type II water.

   CAUTION: This is an exothermic reaction.

8. Magnesium chloride solution ($MgCl_2 \cdot 6H_2O$). Dissolve 510 g of $MgCl_2 \cdot 6H_2O$ into a 1 liter flask. Dilute to 1 liter with Type II water.
9. Pyridine-barbituric acid reagent. Place 15 g of barbituric acid ($C_4H_4O_3N_2$) in a 250 mL volumetric flask. Add just enough Type II water to wash the sides of the flask and wet the barbituric acid. Add 75 mL of pyridine (C_5H_5N) and mix. Add 15 mL of concentrated HCl. Allow to cool to room temperature. Dilute to 250 mL with Type II water. This reagent is stable for approximately six months, if stored in a cool, dark place.
10. Sodium dihydrogenphosphate ($NaH_2PO_4.H_2O$), 1 M. Dissolve 138 g of $NaH_2PO_4.H_2O$ in 1 liter of Type II water.
11. Sodium hydroxide solution (NaOH), 1.25 N. Dissolve 50 g of NaOH in Type II water. Dilute to 1 liter with Type II water.
12. Sodium hydroxide solution (NaOH), 1 N. Dissolve 40 g of NaOH in Type II water. Dilute to 1 liter with Type II water.
13. Sodium hydroxide dilution water and receptacle wash water (NaOH), 0.25 N. Dissolve 10.0 g NaOH in 500 mL of Type II water. Dilute to 1 liter with Type II water.
14. Sulfamic acid solution (NH_2SO_3H). Dissolve 40 g of sulfamic acid in Type II water. Dilute to 1 liter with Type II water.
15. Cyanide stock solution. Dissolve 2.51 g of KCN and 2 g KOH in 900 mL of Type II water. Standardize with 0.0192 N $AgNO_3$. Dilute to appropriate concentration of 1 mg/mL.
16. Intermediate standard cyanide solution. Dilute 100.0 mL of stock cyanide solution to 1 liter with Type II water (100 µg/mL CN).
17. Working standard cyanide solution. Prepare fresh daily by diluting 100.0 mL of intermediate cyanide solution to 1 liter with Type II water (10.0 µg/mL CN). Store in a glass-stoppered bottle.

    NOTE: All working standards should contain 2 mL of 1 N NaOH per 100 mL.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection. A holding time of 14 days is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of aqueous samples to be analyzed for cyanide.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Two methods are given for preparing a standard cyanide curve. Section 7.1 should be followed if the samples do not contain sulfide and Section 7.2 should be followed if the samples to be analyzed contain sulfide. The difference between these two methods is that all the cyanide standards must be carried through the sample distillation process when sulfide is present.

7.1 Standard Curve for Samples without Sulfide.

1. Prepare a series of standards by pipetting suitable volumes of the working standard cyanide solution into 250 mL volumetric flasks. To each standard add 50 mL of 1.25 N sodium hydroxide and dilute to 250 mL with Type II water. Prepare as follows:

mL of Working Standard Solution (1 mL = 10 µg CN)	Concentration (µg CN/250 mL)
0.0	BLANK
1.0	10
2.0	20
5.0	50
10.0	100
15.0	150
20.0	200

It is not imperative that all standards be distilled in the same manner as the samples. It is recommended that at least two standards (a high and a low) be distilled and compared with similar values on the curve to ensure that the distillation technique is reliable. If distilled standards do not agree within ± 10% of the undistilled standards, the analyst should find the cause of the apparent error before proceeding.

2. Prepare a standard curve by plotting absorbances of standards vs. cyanide concentrations.
3. To check the efficiency of the sample distillation, add an increment of cyanide from either the intermediate standard cyanide solution or the working standard cyanide solution to 500 mL of sample to ensure a level of 20 µg/L. Proceed with the analysis as in Section 8.1 - Sample Distillation.

7.2 Standard Curve for Samples with Sulfide

1. All standards must be distilled in the same manner as the samples. A minimum of three standards shall be distilled.
2. Prepare a standard curve by plotting absorbance of standards vs. cyanide concentration.

8.0 Procedure

8.1 Sample Distillation

1. Weigh a 1 to 5 g aliquot of the homogenized field-moist sample and quantitatively transfer the sample to a 1 liter boiling flask containing 500 mL ASTM Type II water.
2. Pipet 50 mL of 1.25 N sodium hydroxide solution into the absorbing tube of the reflux distillation apparatus. Connect the boiling flask, condenser, absorber, and trap in the train.
3. Adjust the vacuum source to allow a slow stream of air to enter the boiling flask. Approximately two bubbles of air per second should enter the boiling flask.
4. Use lead acetate paper to check the sample for the presence of sulfide. A positive test is indicated by a black color on the paper.

NOTE: If test is positive, treat the sample by adding 50 mL of bismuth nitrate solution through the air inlet tube after the air rate is set. Mix for 3 minutes prior to addition of H_2SO_4 in step 6.

5. If samples are suspected to contain NO_3 and/or NO_2, add 50 mL of sulfamic acid solution after the air rate is set through the air inlet tube. Mix for 3 min prior to addition of H_2SO_4.
6. Slowly add 50 mL 1:1 H_2SO_4 through the air inlet tube.
7. Rinse the tube with Type II water and allow the airflow to mix the flask contents for 3 min.
8. Pour 20 mL of magnesium chloride into the air inlet and wash down with stream of water.
9. Heat the solution to boiling. Reflux for 1 hr. Turn off heat and continue the airflow for at least 15 min. After cooling the boiling flask, disconnect absorber and close off the vacuum source.
10. Drain the solution from the absorber into a 250 mL volumetric flask. Wash the absorber with Type II water and add the washings to the flask. Dilute to the 250 mL with Type II water. The sample is now ready for analysis.

8.2 Aqueous Extraction

1. Weigh out a 25 g aliquot of the well-mixed sediment sample and quantitatively transfer the sediment to a wide-mouthed bottle containing 500 mL of ASTM Type II water.
2. Add 5 mL 50% sodium hydroxide solution and cap the bottle.

NOTE: The pH of the extract must be maintained above 10 throughout the extraction step and subsequent filtration. Since some sediment samples may be acidic, the pH must be monitored as follows: shake the extraction bottle and check the pH after one minute. If the pH is below 12, add 50% sodium hydroxide solution in 5 mL increments until it is at least 12. Recap the bottle and repeat this process until the pH does not drop.

3. Place the samples in a tumbler with enough foam insulation to cushion each bottle. Turn the tumbler on and allow the extraction to continue for approximately 16 hours.
4. Prepare the Buchner funnel apparatus with a glass fiber filter pad. Decant the extract to the Buchner funnel. Measure the volume of collected filtrate.

5. Place 500 mL of filtrate, or an aliquot diluted to 500 mL, in a 1 liter boiling flask.
6. Pipet 50 mL of 1.25 N sodium hydroxide solution into the absorbing tube of the reflux distillation apparatus. Connect the boiling flask, condenser, absorber, and trap in the train.
7. Adjust the vacuum source to allow a slow stream of air to enter the boiling flask. Approximately two bubbles of air per second should enter the boiling flask.
8. Use lead acetate paper to check the sample for the presence of sulfide. A positive test is indicated by a black color on the paper.

   NOTE: If test is positive, treat the sample by adding 50 mL of bismuth nitrate solution through the air inlet tube after the air rate is set. Mix for 3 minutes prior to addition of H_2SO_4 in step 6.

9. If samples are suspected to contain NO_3 and/or NO_2, add 50 mL of sulfamic acid solution after the air rate is set through the air inlet tube. Mix for 3 min prior to addition of H_2SO_4.
10. Slowly add 50 mL 1:1 H_2SO_4 through the air inlet tube.
11. Rinse the tube with Type II water and allow the airflow to mix the flask contents for 3 min.
12. Pour 20 mL of magnesium chloride into the air inlet and wash down with stream of water.
13. Heat the solution to boiling. Reflux for 1 hr. Turn off heat and continue the airflow for at least 15 min. After cooling the boiling flask, disconnect absorber and close off the vacuum source.
14. Drain the solution from the absorber into a 250 mL volumetric flask. Wash the absorber with Type II water and add the washings to the flask. Dilute to the 250 mL with Type II water. The sample is now ready for analysis.

8.3 Automated Colorimetric Determination

1. Set up the manifold in a hood or a well-ventilated area.
2. Allow colorimeter and recorder to warm up for 30 min.
3. Run a baseline with all reagents feeding Type II water through the sample line.
4. Place appropriate standards in the sampler in order of decreasing concentration.
5. Complete loading of the sampler tray with unknown and quality assurance/quality control samples.

6. When the baseline becomes steady, begin the analysis.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for cyanide in sediments is 2000 µg/kg.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured cyanide concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the instrument detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared

from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - cyanide, to the 100 mL aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The matrix spike should be prepared by adding cyanide from the working standard or intermediate cyanide standard to ensure a final concentration of approximately 40 µg/L. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

10.0 Method Performance

In a single laboratory study, recoveries of cyanide of 60 to 90% were reported for solid samples. The reported coefficients of variation were less than 13%.

11.0 Calculations and Reporting

The method of standard additions shall be used for the analysis of all samples that suffer from matrix interferences, such as those that contain sulfide (see section 11.1). The results of all other colorimetric analyses can obtained by comparison of sample peak heights to the standard curve of peak heights of the standards plotted against their corresponding known concentration values.

11.1 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of

known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. Method 4500-CN C and 4500-CN E. 17th Edition, APHA, New York, New York. p. 4-29 - 4-31.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Goulden, P.D., B.K. Afghan, and P. Brooksbank. 1972. Determination of Nanogram Quantities of Simple and Complex Cyanides in Water. Anal. Chem. 44:1845-49.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

CYANIDE IN SEDIMENTS
(COLORIMETRIC, MANUAL)

1.0 Scope and Application

This method is used to determine the concentration of inorganic cyanide in sediments. The method detects inorganic cyanides that may be present as either simple soluble salts or complex radicals.

The colorimetric method is sensitive to approximately 0.02 mg/L of cyanide in the final sediment distillate. The range of the procedure can be adjusted by modifying the sample preparation technique or the cell path length. However, the amount of sodium hydroxide in the standards and the sample to be analyzed must be the same.

This procedure is based on SW-846 Method 9010A (EPA, 1986).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

An aliquot of the sediment sample to be analyzed is placed in 500 mL of acidified distilled water. The resulting suspension is heated to distill hydrocyanic acid (HCN) from the acidic suspension and into a sodium hydroxide trap. The cyanide concentration of the final distillate is determined with a colorimetric procedure.

Cyanide in the distillate is reacted with chloramine-T at a pH less than 8 to produce cyanogen chloride (CNCl). After this reaction is complete, the addition of pyridine-barbituric acid reagent produces a red-blue color that is proportional to the cyanide concentration. The intensity of the color is measured by measuring sample absorbance at 578 ηm. The concentration of NaOH must be the same in the standards, the sample distillate, and any dilutions of the original sample distillate to obtain colors of comparable intensity.

3.0 Interferences

Sulfides adversely affect the development of color in the analytical procedure. This interference can be reduced or eliminated by adding bismuth nitrate to the samples to precipitate the sulfide prior to distillation (Section 8.1.4). Samples that contain hydrogen sulfide, metal sulfides, or other compounds that may produce sulfide during the distillation procedure should also be treated with bismuth nitrate prior to distillation.

Nitrate and/or nitrite in samples can act as a positive interference when present at concentrations above 10 mg/L and in the presence of certain organic compounds. These nitrogen compounds can form nitrous acid during the distillation process which will react with some organic compounds to form oxides. These oxides will decompose under conditions developed in the colorimetric procedure to generate HCN. This interference is eliminated by treating the samples with sulfamic acid prior to distillation (Section 8.1.5).

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Reflux distillation apparatus. The boiling flask should be of 1-liter size with inlet tube and provision for condenser. The gas absorber is a Fisher-Milligan scrubber (Fisher Catalog #07-513), or equivalent.
4. Spectrophotometer. Suitable for measurements at 578 ηm with a 1.0 cm cell or larger.
5. Extractor. Any suitable device that sufficiently agitates a sealed container with a capacity of one liter or more. For the purpose of this procedure, agitation must be sufficient to: (1) maintain continuous contact between all sample particles and the extraction fluid, and (2) prevent stratification of the sample and the extraction fluid.
6. Buchner funnel, 500 mL capacity.
7. Vacuum filtration flask, 1 L.
8. Glass fiber filter pads.

9. Vacuum source, preferably a water driven aspirator. A value or stopcock is needed to release the vacuum.
10. Top loading balance, capable of weighing 0.1 g.

4.2 Materials

1. Potassium iodide-starch test paper.
2. Volumetric flasks, class A, 250 mL.
3. Volumetric flasks, class A, 100 mL.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Bismuth nitrate solution ($Bi(NO_3)_3$). Dissolve 30.0 grams of $Bi(NO_3)_3$ in 100 mL of Type II water. While stirring, add 250 mL of glacial acetic acid. Stir until dissolved. Dilute to 1 liter with Type II water.
3. Chloramine-T solution. Dissolve 1.0 g of white, water-soluble, chloramine-T in 100 mL of Type II water. Refrigerate until ready to use.
4. Concentrated acetic acid ($C_4H_6O_3$), glacial, reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
5. Concentrated sulfuric acid (H_2SO_4), reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
6. Sulfuric acid (1:1). Slowly add 500 mL of concentrated H_2SO_4 to 500 mL of Type II water.

   CAUTION: This is an exothermic reaction.

7. Magnesium chloride solution ($MgCl_2 \cdot 6H_2O$). Dissolve 510 g of $MgCl_2 \cdot 6H_2O$ into a 1 liter flask. Dilute to 1 liter with Type II water.
8. Pyridine-barbituric acid reagent. Place 15 g of barbituric acid ($C_4H_4O_3N_2$) in a 250 mL volumetric flask. Add just enough Type II water to wash the sides of the flask and wet the barbituric acid. Add 75 mL of pyridine (C_5H_5N) and mix. Add 15 mL of concentrated HCl. Allow to cool to room temperature. Dilute to 250 mL with Type II water. This

reagent is stable for approximately six months, if stored in a cool, dark place.

9. Sodium dihydrogenphosphate ($NaH_2PO_4.H_2O$), 1 M. Dissolve 138 g of $NaH_2PO_4.H_2O$ in 1 liter of Type II water.
10. Sodium hydroxide solution (NaOH), 1.25 N. Dissolve 50 g of NaOH in Type II water. Dilute to 1 liter with Type II water.
11. Sodium hydroxide solution (NaOH), 50% (w/v). Dissolve 50 g of NaOH in 50 mL Type II water..
12. Sulfamic acid solution (NH_2SO_3H). Dissolve 40 g of sulfamic acid in Type II water. Dilute to 1 liter with Type II water.
13. Cyanide stock solution. Dissolve 2.51 g of KCN and 2 g KOH in 900 mL of Type II water. Standardize with 0.0192 N $AgNO_3$. Dilute to appropriate concentration of 1 mg/mL.
14. Intermediate standard cyanide solution. Dilute 100.0 mL of stock cyanide solution to 1 liter with Type II water (100 µg/mL CN).
15. Working standard cyanide solution. Prepare fresh daily by diluting 100.0 mL of intermediate cyanide solution to 1 liter with Type II water (10.0 µg/mL CN). Store in a glass-stoppered bottle.

    NOTE: All working standards should contain 2 mL of 1 N NaOH per 100 mL.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection. A holding time of 14 days is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of sediment samples to be analyzed for cyanide.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Two methods are given for preparing a standard cyanide curve. Section 7.1 should be followed if the samples do not contain sulfide and Section 7.2 should be followed if the samples to be analyzed contain sulfide. The difference between these two methods is that all the cyanide standards must be carried through the sample distillation process when sulfide is present.

7.1 Standard Curve for Samples without Sulfide.

1. Prepare a series of standards by pipetting suitable volumes of the working standard cyanide solution into 250 mL volumetric flasks. To each standard add 50 mL of 1.25 N sodium hydroxide and dilute to 250 mL with Type II water. Prepare as follows:

mL of Working Standard Solution (1 mL = 10 µg CN)	Concentration (µg CN/250 mL)
0.0	BLANK
1.0	10
2.0	20
5.0	50
10.0	100
15.0	150
20.0	200

It is not imperative that all standards be distilled in the same manner as the samples. It is recommended that at least two standards (a high and a low) be distilled and compared with similar values on the curve to ensure that the distillation technique is reliable. If distilled standards do not agree within ± 10% of the undistilled standards, the analyst should find the cause of the apparent error before proceeding.

2. Prepare a standard curve by plotting absorbances of standards vs. cyanide concentrations.

3. To check the efficiency of the sample distillation, add an increment of cyanide from either the intermediate standard cyanide solution or the working standard cyanide solution to 500 mL of sample to ensure a level of 20 µg/L. Proceed with the analysis as in Section 8.1 - Sample Distillation.

7.2 Standard Curve for Samples with Sulfide

1. All standards must be distilled in the same manner as the samples. A minimum of three standards shall be distilled.
2. Prepare a standard curve by plotting absorbance of standards vs. cyanide concentration.

8.0 Procedure

Two procedures are presented to isolate cyanide from the sediment matrix prior to analysis. The first is a direct distillation of the cyanide from a sediment-distilled water slurry (section 8.1) and the second is an aqueous extraction of cyanide from the sediment sample (section 8.2).

8.1 Sample Distillation

1. Weigh a 1 to 5 g aliquot of the homogenized field-moist sample and quantitatively transfer the sample to a 1 liter boiling flask containing 500 mL ASTM Type II water.
2. Pipet 50 mL of 1.25 N sodium hydroxide solution into the absorbing tube of the reflux distillation apparatus. Connect the boiling flask, condenser, absorber, and trap in the train.
3. Adjust the vacuum source to allow a slow stream of air to enter the boiling flask. Approximately two bubbles of air per second should enter the boiling flask.
4. Use lead acetate paper to check the sample for the presence of sulfide. A positive test is indicated by a black color on the paper.

   NOTE: If test is positive, treat the sample by adding 50 mL of bismuth nitrate solution through the air inlet tube after the air rate is set. Mix for 3 minutes prior to addition of H_2SO_4 in step 6.

5. If samples are suspected to contain NO_3 and/or NO_2, add 50 mL of sulfamic acid solution after the air rate is set through the air inlet tube. Mix for 3 min prior to addition of H_2SO_4.
6. Slowly add 50 mL 1:1 H_2SO_4 through the air inlet tube.
7. Rinse the tube with Type II water and allow the airflow to mix the flask contents for 3 min.
8. Pour 20 mL of magnesium chloride into the air inlet and wash down with stream of water.
9. Heat the solution to boiling. Reflux for 1 hr. Turn off heat and continue the airflow for at least 15 min. After cooling the boiling flask, disconnect absorber and close off the vacuum source.
10. Drain the solution from the absorber into a 250 mL volumetric flask. Wash the absorber with Type II water and add the washings to the flask. Dilute to the 250 mL with Type II water. The sample is now ready for analysis.

8.2 Aqueous Extraction

1. Weigh out a 25 g aliquot of the well-mixed sediment sample and quantitatively transfer the sediment to a wide-mouthed bottle containing 500 mL of ASTM Type II water.
2. Add 5 mL 50% sodium hydroxide solution and cap the bottle.

   NOTE: The pH of the extract must be maintained above 10 throughout the extraction step and subsequent filtration. Since some sediment samples may be acidic, the pH must be monitored as follows: shake the extraction bottle and check the pH after one minute. If the pH is below 12, add 50% sodium hydroxide solution in 5 mL increments until it is at least 12. Recap the bottle and repeat this process until the pH does not drop.

3. Place the samples in a tumbler with enough foam insulation to cushion each bottle. Turn the tumbler on and allow the extraction to continue for approximately 16 hours.
4. Prepare the Buchner funnel apparatus with a glass fiber filter pad. Decant the extract to the Buchner funnel. Measure the volume of collected filtrate.
5. Place 500 mL of filtrate, or an aliquot diluted to 500 mL, in a 1 liter boiling flask.

6. Pipet 50 mL of 1.25 N sodium hydroxide solution into the absorbing tube of the reflux distillation apparatus. Connect the boiling flask, condenser, absorber, and trap in the train.
7. Adjust the vacuum source to allow a slow stream of air to enter the boiling flask. Approximately two bubbles of air per second should enter the boiling flask.
8. Use lead acetate paper to check the sample for the presence of sulfide. A positive test is indicated by a black color on the paper.

   NOTE: If test is positive, treat the sample by adding 50 mL of bismuth nitrate solution through the air inlet tube after the air rate is set. Mix for 3 minutes prior to addition of H_2SO_4 in step 6.

9. If samples are suspected to contain NO_3 and/or NO_2, add 50 mL of sulfamic acid solution after the air rate is set through the air inlet tube. Mix for 3 min prior to addition of H_2SO_4.
10. Slowly add 50 mL 1:1 H_2SO_4 through the air inlet tube.
11. Rinse the tube with Type II water and allow the airflow to mix the flask contents for 3 min.
12. Pour 20 mL of magnesium chloride into the air inlet and wash down with stream of water.
13. Heat the solution to boiling. Reflux for 1 hr. Turn off heat and continue the airflow for at least 15 min. After cooling the boiling flask, disconnect absorber and close off the vacuum source.
14. Drain the solution from the absorber into a 250 mL volumetric flask. Wash the absorber with Type II water and add the washings to the flask. Dilute to the 250 mL with Type II water. The sample is now ready for analysis.

8.3 Manual Spectrophotometric Analysis

1. Withdraw 50 mL, or a smaller aliquot diluted to 50 mL with 1.25 N sodium hydroxide solution, of the final sample distillate and transfer to a 100 mL volumetric flask.
2. Add 15.0 mL of sodium dihydrogenphosphate solution. Mix.
3. Add 2 mL of Chloramine-T. Mix.

   NOTE: Some distillates may contain compounds that have a chlorine demand. One minute after the addition of chloramine-T, test for residual chlorine with KI-starch paper. If the test is

negative, add an additional 0.5 mL chloramine-T. Recheck after 1 min.

NOTE: Temperature of reagents may affect the response factor of the colorimetric determination. The reagents stored under refrigerated conditions should be warmed to ambient temperature before use. Also, samples should not be left in a warm instrument to develop color but, instead, should be aliquoted to a cuvette immediately prior to reading the sample absorbance.

4. After 1 to 2 min, add 5 mL of pyridine-barbituric acid solution. Mix.
5. Dilute to 100 mL with Type II water. Mix.
6. Allow 8 min for color development and then read absorbance at 578 ηm in a 1-cm cell within 15 min.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for cyanide in sediments is 2000 µg/kg.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured cyanide concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the instrument detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - cyanide, to the 100 mL aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The matrix spike should be prepared by adding cyanide from the working standard or intermediate cyanide standard to ensure a final concentration of approximately 40 µg/L. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

10.0 Method Performance

In a single laboratory study, recoveries of cyanide of 60 to 90% were reported for solid samples. The reported coefficients of variation were less than 13%.

11.0 Calculations and Reporting

The method of standard additions shall be used for the analysis of all samples that suffer from matrix interferences, such as those that contain sulfide (see section 11.1). The results of all other colorimetric analyses can obtained by

comparison of sample peak heights to the standard curve of peak heights of the standards plotted against their corresponding known concentration values.

11.1 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The

abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. Method 4500-CN C and 4500-CN E. 17th Edition, APHA, New York, New York. p. 4-29 - 4-31.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Goulden, P.D., B.K. Afghan, and P. Brooksbank. 1972. Determination of Nanogram Quantities of Simple and Complex Cyanides in Water. Anal. Chem. 44:1845-49.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

ARSENIC IN SEDIMENTS

1.0 Scope and Application

This method is applicable to the determination of arsenic in sediment samples.

This procedure is based on EPA SW-846 Methods 3050 and 7060 (USEPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

An aliquot of the sediment sample is digested with nitric acid and hydrogen peroxide. Following dissolution, the digestate is spiked with a nickel nitrate solution and placed in a graphite furnace tube. The sample is then slowly evaporated to dryness, charred (ashed), and atomized. The absorption of hollow cathode or electrodeless discharge lamp (EDL) radiation during sample atomization is proportional to the arsenic concentration in the digestate.

3.0 Interferences

Elemental arsenic and many of its compounds are volatile; therefore, samples may be subject to potential loss of arsenic during the sample preparation procedure. Spiked samples and relevant standard reference materials should be processed to demonstrate the performance of the sample preparation technique.

Caution should also be employed when selecting the temperature and duration of the sample drying and charring (ashing) cycles. A nickel nitrate solution must be added to all digestates prior to analysis to minimize volatilization losses during drying and ashing.

In addition to the normal interferences experienced during graphite furnace analysis, arsenic analysis can suffer from severe nonspecific absorption and light scattering caused by matrix components during atomization. Arsenic analysis is particularly susceptible to these problems because of its low analytical wavelength (193.7 ηm). Simultaneous background correction must be employed to avoid

erroneously high results. Aluminum is a severe positive interferant in the analysis of arsenic, especially using D_2 arc background correction. Zeeman background correction is very useful in this situation.

If the analyte is not completely volatilized and removed from the furnace during atomization, memory effects will occur. If this situation is detected by means of blank burns, the tube should be cleaned by operating the furnace at full power at regular intervals in the analytical scheme.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, 3-5 weights covering expected weight range.
3. Drying oven, capable of maintaining 30° C.
4. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
4. Thermometer, 0 to 100° C range.
5. Centrifuge and centrifuge tubes.
6. Atomic absorption spectrophotometer, single or dual channel, single- or double-beam instrument having a grating monochromator, photo-multiplier detector, adjustable slits, a wavelength range of 190 to 800 ηm, and provisions for simultaneous background correction and interfacing with a strip-chart recorder.
7. Arsenic hollow cathode lamp, or electrodeless discharge lamp (EDL). EDLs are recommended since they provide better sensitivity for arsenic analyses.
8. Graphite furnace. Any graphite furnace device with the appropriate temperature and timing controls.
9. Strip-chart recorder. A recorder is strongly recommended for furnace work so that there will be a permanent record and so that any problems with the analysis such as drift, incomplete atomization, losses during charring, and changes in sensitivity can easily be recognized.

4.2 Materials

1. Conical Phillips beakers, 250 mL, or equivalent.
2. Watch glasses ribbed, or equivalent.
3. Volumetric flasks, class A, 10 mL.
4. Whatman no. 41 filter paper or equivalent.
5. Pipets. Microliter with disposable tips. Sizes can range from 5 to 1,000 µL, as required.

5.0 Reagents and Standards

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid is <MDL, then the acid can be used.
3. Hydrogen peroxide, 30% (H_2O_2). Oxidant should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the H_2O_2 is <MDL, then the acid can be used.
4. Arsenic standard stock solution (1,000 mg/L). Either procure a certified aqueous standard from a supplier and verify by comparison with a second standard, or dissolve 1.320 g of arsenic trioxide (As_2O_3, analytical reagent grade), or equivalent, in 100 mL of Type II water containing 4 g NaOH. Acidify the solution with 20 mL concentrated HNO_3. Dilute to 1 liter with Type II water (1 mL = 1 mg As).
5. Nickel nitrate solution, 5%. Dissolve 24.780 g of ACS reagent grade $Ni(N0_3)_2 \bullet 6H_2O$, or equivalent, in Type II water and dilute to 100 mL.
6. Nickel nitrate solution, 1%. Dilute 20 mL of the 5% nickel nitrate to 100 mL with Type II water.
7. Arsenic working standards. Prepare dilutions of the stock solution to be used as calibration standards at the time of the analysis. Withdraw appropriate aliquot of the stock solution, add 1 mL of concentrated HNO_3, 2 mL of 30% H_2O_2, and 2 mL of the 5% nickel nitrate solution. Dilute to 100 mL with Type II water.

6.0 Sample Handling and Preservation

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection. A holding time of 6 months is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of samples to be analyzed for arsenic in sediments.

Special containers (e.g., containers used for volatile organic analysis) may have to be used if the samples are to be analyzed for very volatile arsenic compounds.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The oven or hot plate should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

Prepare a method blank and at least three standards in the appropriate concentration range to correlate arsenic concentrations with the atomic absorption spectrophotometer's linear response range. Prepare standards for instrument calibration by appropriate dilution of the stock arsenic solution. These standards should be prepared fresh on the day of use. Match the sample matrix and that of the standards as closely as possible.

Inject a suitable portion of each standard into the graphite furnace in order of increasing concentration. It is recommended that each standard solution be analyzed in triplicate in order to assess method precision. Instrument calibration curves should be composed of a minimum of a blank and three standards. A calibration curve should be prepared every day of continuous sample analysis and prior to the initiation of the project's routine sample analysis.

Construct an analytical curve by plotting the average peak absorbance or peak area for the standard solutions as a function of sample concentration on a linear graph. Prepare this graph daily when new initial calibration information is obtained. Alternatively, electronic instrument calibration can be used if the instrument is appropriately equipped.

8.0 Procedure

1. Weigh a 1.00 to 2.00 g aliquot of the homogenized, field-moist sediment to the nearest 0.01 g and quantitatively transfer the sample to a 250 mL beaker.
2. Add 10 mL 1:1 HNO_3, mix the slurry, and cover the beaker opening with a watch glass.
3. Heat the sample to 95° C and reflux for 10 to 15 minutes without boiling.
4. Allow the sample to cool. Add 5 mL concentrated HNO_3, replace the watch glass, and reflux for an additional 30 minutes.
5. Allow the sample to cool. Add 5 mL concentrated HNO_3 and cover the flask with a ribbed watch glass. Allow the solution to evaporate to a final volume of 5 mL without boiling while maintaining a covering of solution over the bottom of the beaker.
6. After the sample has cooled, add 2 mL Type II water and 3 mL H_2O_2. Cover the beaker with a watch glass and return the covered beaker to a hot plate or oven for warming and to initiate the peroxide reaction. Heat until the effervescence subsides. Allow the beaker to cool.

   NOTE: Exercise care to ensure that sample is not lost due to excessively vigorous effervescence.

7. Continue to add 30% H_2O_2 in 1 mL aliquots until the effervescence is minimal or until the general sample appearance is unchanged.

   NOTE: The total volume of 30% H_2O_2 added should not exceed 10 mL.

8. Cover the sample flask with a ribbed watch glass and continue heating until the volume of the acid-peroxide digestate has been reduced to approximately 5 mL. After cooling, dilute the final digestate to 100 mL with Type II water.
9. Suspended particulates should be removed by filtration, centrifugation, or by allowing the sample to settle prior to analysis.

    a. Filtration. Filter digest through Whatman no. 41 filter paper (or equivalent) and dilute to 100 mL with Type II water.

    b. Centrifugation. Centrifugation at 2,000 to 3,000 rpm for 10 minutes is usually sufficient to produce a clear supernatant.

10. Pipet 5 mL of the final sample digest into a 10-mL volumetric flask, add 1 mL of 1% nickel nitrate solution, and dilute to 10 mL with Type II water. The sample is now ready for injection into the furnace.
11. The 193.7-ηm wavelength line and a background correction system are required. Follow the manufacturer's suggestions for all other spectrophotometer parameters.
12. Furnace parameters suggested by the manufacturer should be employed as guidelines. Because temperature-sensing mechanisms and temperature controllers can vary between instruments or with time, the validity of the furnace parameters must be periodically confirmed by systematically altering the furnace parameters while analyzing a standard. In this manner, losses of analyte due to overly high temperature settings or losses in sensitivity due to less than optimum settings can be minimized. Similar verification of furnace parameters may be required for complex sample matrices.
13. Inject a measured microliter (µL) aliquot of sample digest into the furnace and atomize. If the digest concentration is greater than the highest standard, or if the instrument response falls on the plateau of the calibration curve, the sample should be diluted in the same acid matrix and reanalyzed. The use of multiple injections can improve accuracy and help detect furnace pipetting errors.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for arsenic in sediments is 1000 µg/kg (dry weight).

The detection limit specified in this method is presented to account for typical sediment background contents of chromium in the Great Lakes.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured arsenic concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards 2704 - Buffalo River sediment and 1646 - Estuarine sediment, should be run to monitor the life and performance of the graphite tube and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a routine sediment sample collected and homogenized in bulk that has undergone multiple analyses by the analytical laboratory. Alternately, a LCS can be created by spiking a known quantity of the contaminant(s) of concern into a clean sediment, homogenizing the bulk sample, followed by multiple analyses at the analytical laboratory. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the instrument detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 15 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - arsenic, to a 1-2 g sample aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 3 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

10.0 Method Performance

The optimal concentration range for this method is 5-100 µg/L.

The data shown in Table 1 were obtained from records of state and contractor laboratories. The data provide an estimate of the precision that can be attained with the combined sample preparation and analysis method.

11.0 Calculations and Reporting

Calculate metal concentrations by (1) the method of standard additions, or (2) from a calibration curve, or (3) directly from the instrument's concentration readout. All dilution or concentration factors must be taken into account.

Prepare a standard curve based on the absorbance and concentration of the arsenic standards. Determine the arsenic concentration in each of the sediment digests by comparing the digest absorbance with the standard curve.

Calculate the arsenic concentration of the sediment sample as follows:

$$\text{As, } \mu g/kg \text{ (wet weight)} = \frac{X \times V \times 1000}{g}$$

$$\text{As, } \mu g/kg \text{ (dry weight)} = \frac{X \times V \times 1000}{g \times \%S}$$

where:

X = is the arsenic concentration in the final sediment digest, µg/L.
V = the final sediment digest volume, 0.1 L.
g = the weight of wet sediment digested, g.
%S = the percent solids concentration of the field moist sediment sample expressed as a decimal fraction.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Gaskill, A., 1986. Compilation and Evaluation of RCRA Method Performance Data, Work Assignment No. 2, EPA Contract No. 68-01-7075.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Table 1. Method Performance Data (after Gaskill, 1986).

Sample Matrix	Preparation Method	Laboratory Replicates
Contaminated soil	3050	2.0, 1.8 µg/g
Oily soil	3050	3.3, 3.8 µg/g
NBS estuarine sediment (SRM 1646)	3050	8.1, 8.33 µg/g[a]
Emission control dust	3050	430, 350 µg/g

a = Bias of -30 and -28% from expected, respectively.

CADMIUM IN SEDIMENTS
(ICP)

1.0 Scope and Application

Inductively coupled plasma atomic emission spectroscopy (ICP) determines cadmium and numerous other elements that are present in solution. In order to apply this technique to sediments, the samples must be digested with nitric acid and hydrogen peroxide prior to analysis.

Detection limits, sensitivity, and optimum concentration ranges for cadmium, and other metals stable in a mixed standard solution with cadmium, will vary with the sample aliquot size, the spectrophotometer being used, and possible matrix interferences. Based on the estimated detection limit of 4 µg/L for cadmium in aqueous samples (Table 1), a sample size of 2 g, and a final digestate volume of 100 mL, the estimated detection limit for sediment samples is 200 µg/kg.

The method of standard addition (MSA) shall be used for the analysis of all sample digests unless either serial dilution or matrix spike addition demonstrates that it is not required (see section 9).

This method should only be used by spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

This procedure is based on SW-846 Methods 3050 (for digestion) and 6010 for quantitation (EPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

A well-mixed, representative 1 to 2 g (wet weight) sample of sediment is digested in nitric acid and hydrogen peroxide. The digestate is then refluxed using hydrochloric acid and diluted to volume.

NOTE: A separate sample shall be dried for a total solids determination in order to express the results on a dry-weight basis.

This instrumental method measures light emitted by cadmium in the final sediment digest by optical spectrometry. The digested samples are nebulized and the resulting aerosol is transported to a plasma torch. Cadmium-specific atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

The possibility of additional interferences named in section 3.0 should also be recognized and appropriate corrections made when necessary. The procedures that can be used for this purpose are described in section 9.

3.0 Interferences

Sediments represent diverse matrix types and samples from each location may present its own analytical challenge. Spiked samples and any relevant standard reference material should be processed to aid in determining whether the digestion procedure is appropriate for a specific sediment sample or there are matrix or other effects interfering with the analysis of the resultant sediment digestate.

3.1 Spectral Interferences

Spectral interferences are caused by: (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of simultaneous multi-element instruments must verify the absence of spectral interference from an element in a sample for which there is no instrument detection channel. Potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferants can be assumed.

The magnitude of the interference effects summarized in Table 2 are expressed as analyte concentration equivalents (i.e., false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that Cd is to be determined (at 226.502 ηm) in a sample containing approximately 10 mg/L of Fe. According to Table 2, 100 mg/L of Fe would yield a false signal for Cd equivalent to approximately 0.03 mg/L. Therefore, the presence of 10 mg/L of Fe would result in a false signal for Cd equivalent to approximately 0.003 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, and argon flow rate.

The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.2 Physical Interferences

Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, or by using the standard additions method. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Further, it has been reported that better control of the argon flow rate improves instrument performance. Improved argon flow rate control can be accomplished with the use of mass flow controllers.

3.3 Chemical Interferences

Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, etc.), buffering of the sample, matrix matching, and standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Drying oven, capable of maintaining 30° C.
4. Thermometer, 0 to 200° C.
5. Centrifuge and centrifuge tubes.
6. Inductively coupled argon plasma emission spectrometer.
7. Computer-controlled emission spectrometer with background correction.
8. Radio frequency generator.
9. Argon gas supply, welding grade or better.

4.2 Materials

1. Conical Phillips beakers, 250 mL, or equivalent.
2. Watch glasses.
3. Whatman No. 41 filter paper, or equivalent.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.

3. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
4. Hydrogen peroxide, 30% (H_2O_2). Oxidant should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the H_2O_2 is <MDL, then the acid can be used.
5. Nitric acid (1:1). Add 500 mL concentrated HNO_3 to 400 mL Type II water. Dilute to 1 liter with Type II water.
6. Hydrochloric acid (1:1). Add 500 mL concentrated HCl to 400 mL Type II water. Dilute to 1 liter with Type II water.
7. Standard stock solutions. May be purchased or prepared from ultrahigh purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hr at 105° C, unless otherwise specified.

   CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

8. Beryllium standard stock solution (100 µg/mL). Dissolve 1.970 g $BeSO_4 \cdot 4H_2O$ (analytical reagent grade, undried), in 100 mL of Type II water, add 10.0 ml concentrated HNO_3. Dilute to 1 liter with Type II water.
9. Cadmium standard stock solution (100 µg/mL). Dissolve 0.1100 g CdO (analytical reagent grade) in a minimum amount of 1:1 HNO_3. Heat to increase rate of dissolution. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
10. Lead standard stock solution (100 µg/mL). Dissolve 0.1600 g $Pb(NO_3)_2$ (analytical reagent grade) in a minimum amount of 1:1 HNO_3. Add 10 mL 1:1 HNO_3. Add 10 mL 1:1 HNO_3. Dilute to 1 liter with Type II water.
11. Manganese standard stock solution (100 µg/mL). Dissolve 0.1000 g of manganese metal, in an acid mixture of 10 mL concentrated HCl and 1 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
12. Selenium standard stock solution (100 µg/mL). Dissolve 0.1700 g H_2SeO_3 (analytical reagent grade, undried) in 100 mL of Type II water. Dilute to 1 liter with Type II water.
13. Zinc standard stock solution (100 µg/mL). Dissolve 0.1200 g ZnO in a minimum amount of 1:1 HNO_3. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
14. Mixed calibration standard solutions. Care should be taken when preparing mixed standards for ICP analysis to ensure that the elements

in the final mixed standard are compatible and stable together. One set of mixed calibration standards that has been found to be useful is listed in Table 3. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interferences or the presence of impurities.

To prepare the mixed calibration standard for cadmium, combine appropriate volumes of the individual stock solutions indicated in Table 3 in volumetric flask. Add 2 mL 1:1 HNO_3 and 10 mL of 1:1 HCl and dilute to 100 mL with Type II water. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see section 9.9) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should be scanned using a sequential spectrometer to verify the absence of interelement spectral interference in the recommended mixed standard solutions.

TABLE 3. MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo, and Si
IV	Al, Ca, Cr, K, Na, and Ni

NOTE: Premixed standard solutions (traceable to NIST) containing the combined elements as listed in solutions I through IV are available from a number of commercial vendors.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection. A holding time of 6 months is generally cited for this parameter. Samples may be stored for a longer period of time by air-drying or freezing. However, these samples should not be used for the preparation of sediment elutriates because the process of freezing and thawing the samples may influence the migration potential of sediment-associated constituents.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of samples to be analyzed for cadmium in sediments.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The oven or hot plate should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

Prepare a calibration blank (see section 9.5.1) and at least three mixed calibration standards in the appropriate concentration range to correlate cadmium concentrations with the ICP's linear response range. Prepare standards for instrument calibration as instructed in section 5.14. Match the sample matrix and that of the standards as closely as possible.

Calibrate the instrument according to the instrument manufacturer's recommended procedures using typical mixed calibration standard solutions. Flush the system with the calibration blank between each standard. Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.

8.0 Procedure

8.1 Sample Digestion

1. Weigh a 1.00 to 2.00 g aliquot of the homogenized, field-moist sediment to the nearest 0.01 g and quantitatively transfer the sample to a 250 mL conical beaker.
2. Add 10 mL of 1:1 HNO_3, mix the slurry, and cover with a watch glass.
3. Heat the sample to 95° C and reflux for 10 to 15 min without boiling.
4. Allow the sample to cool. Add 5 mL of concentrated HNO_3, replace the watch glass, and reflux for 30 min. Repeat this last step to ensure complete oxidation of the sample. Using a ribbed watch glass, allow the solution to evaporate to 5 mL without boiling, while maintaining a covering of solution over the bottom of the beaker.
5. After the sample has cooled, add 2 mL of Type II water and 3 mL of 30% H_2O_2. Cover the beaker with a watch glass and return the covered beaker to the hot plate for warming and to start the peroxide reaction. Heat until effervescence subsides. Allow the beaker to cool.

   NOTE: Exercise care to ensure that sample is not lost due to excessively vigorous effervescence.

6. 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: The total volume of the 30% H_2O_2 should not exceed 10 mL.

7. Add 5 mL of concentrated HCl and 10 mL of Type II water, return the covered beaker to the hot plate, and reflux for an additional 15 min without boiling. After cooling, dilute to 100 mL with Type II water.
8. Suspended particulates should be removed by filtration, by centrifugation, or by allowing the sample to settle prior to analysis.

   a. Filtration: Filter through Whatman No. 41 filter paper (or equivalent) and dilute to 100 mL with Type II water.

   b. Centrifugation: Centrifugation at 2,000 to 3,000 rpm for 10 min is usually sufficient to clear the supernatant.

9. The diluted sample has an approximate acid concentration of 5.0% (v/v) HCl and 5.0% (v/v) HNO_3. The sample is now ready for analysis.

8.2 Sample Analysis

1. The analyst should follow the instructions provided by the instrument's manufacturer. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 min of operation prior to calibration). For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within instrument linear range where coordination factors are valid. The analyst must: (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.

2. Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5% (or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this conditions.

3. Flush the system with the calibration blank solution for at least 1 min before the analysis of each sample. Analyze samples.

   NOTE: Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for cadmium in sediments is 1000 µg/kg (dry weight).

The detection limit specified in this method is presented to account for typical sediment background contents of chromium in the Great Lakes.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured cadmium concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards 2704 - Buffalo River sediment and 1646 - Estuarine sediment, should be run to monitor performance of the ICP and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a routine sediment sample collected and homogenized in bulk that has undergone multiple analyses by the analytical laboratory. Alternately, a LCS can be created by spiking a known quantity of the contaminant(s) of concern into a clean sediment, homogenizing the bulk sample, followed by multiple analyses at the analytical laboratory. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

9.5.1 Calibration Blank

The calibration blank is prepared by diluting 2 mL of 1:1 HNO_3 and of 1:1 HCl to 100 mL with Type II water. Prepare a sufficient quantity to flush the system between standards and samples.

The calibration blank should be analyzed prior to routine sample analysis, after every 10 samples, and at the end of the analytical run. The measured concentration in the calibration blank should be less than or equal to the method detection limit.

9.5.2 Reagent Blank

The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - cadmium, to a 1-2 g sample aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

9.8 Interference Check Sample

The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the method detection limit for each element. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

The interference check sample should be analyzed at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Results should be within ± 20% of the known concentration.

9.9 Calibration Control Sample

A calibration control sample should be prepared in the same acid matrix as the calibration standards at 10 times the method detection limit. This sample should be prepared in accordance with the instructions provided by the supplier. The calibration control sample is used to verify the integrity of the calibration standards on a weekly basis.

9.10 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor

negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

9.10.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the instrumental detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.10.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.

4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 µg/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported in µg/kg with up to three significant figures.

The concentrations determined are to be reported on the basis of the actual weight of the sample. If a dry weight analysis is desired, then the percent solids of the sample must also be provided.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Winge, R.K., V.J. Peterson, and V.A. Fassel. 1979. Inductively coupled plasma-atomic emission spectroscopy: Prominent lines, final report, March 1977 - February 1978, Ames laboratory, Ames, IA. EPA-600/4-79-017. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Table 1. Recommended Wavelengths and Estimated Instrumental Detection Limits.

Element	Wavelength[a] (ηm)	Estimated Detection Limit[b] (µg/L)
Beryllium	313.042	0.3
Cadmium	226.502	4
Lead	220.353	42
Manganese	257.610	2
Selenium	196.026	75
Zinc	213.856	2

a - The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see section 3.1).

b - The estimated instrumental detection limits are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

Table 2. Analyte Concentration Equivalents arising from Interference at the 100 mg/L Level.

		Interferant[a,b]									
	Wavelength										
Analyte	(ηm)	Al	Ca	Cr	Cu	Fe	Mg	Mn	Mi	Tl	V
Beryllium	313.042	-	-	-	-	-	-	-	-	0.04	0.05
Cadmium	226.502	-	-	-	-	0.03	-	-	0.02	-	-
Lead	220.353	0.17	-	-	-	-	-	-	-	-	-
Manganese	257.610	0.005	-	0.01	-	0.002	0.002	-	-	-	-
Selenium	196.026	0.23	-	-	-	0.09	-	-	-	-	-
Zinc	213.856	-	-	-	0.14	-	-	-	0.29	-	-

a - Dashes indicate that no interference was observed even when interferants were introduced at the following levels:

Al - 1000 mg/L,	Mg - 1000 mg/L,
Ca - 1000 mg/L,	Mn - 200 mg/L,
Cr - 200 mg/L,	Tl - 200 mg/L,
Cu - 200 mg/L,	V - 200 mg/L, and
Fe - 1000 mg/L.	

b - The figures recorded as analyte concentrations are not the actual observed concentrations. To obtain those figures, add the listed concentration to the interferant figure.

Table 4. ICP Precision and Accuracy Data[a]

	Sample No. 1			Sample No. 2			Sample No. 3		
Element	True Value (µg/L)	Mean Reported Value (µg/L)	Mean SD[b] (%)	True Value (µg/L)	Mean Reported Value (µg/L)	Mean SD[b] (%)	True Value (µg/L)	Mean Reported Value (µg/L)	Mean SD[b] (%)
Be	750	733	6.2	20	20	9.8	180	176	5.2
Mn	350	345	2.7	15	15	6.7	100	99	3.3
Cd	50	48	12	2.5	2.9	16	14	13	16
Pb	250	236	16	24	30	32	80	80	14
Zn	200	201	5.6	16	19	45	80	82	9.4
Se[c]	40	32	21.9	6	8.5	42	1`0	8.5	8.3

a - Not all elements were analyzed by all laboratories.
b - SD = standard deviation.
c - Results for Se are from two laboratories.

CHROMIUM IN SEDIMENTS
(ICP)

1.0 Scope and Application

Inductively coupled plasma atomic emission spectroscopy (ICP) determines chromium and numerous other elements that are present in solution. In order to apply this technique to sediments, the samples must be digested with nitric acid and hydrogen peroxide prior to analysis.

Detection limits, sensitivity, and optimum concentration ranges for chromium, and other metals stable in a mixed standard solution with chromium, will vary with the sample aliquot size, the spectrophotometer being used, and possible matrix interferences. Based on the estimated detection limit of 7 µg/L for chromium in aqueous samples (Table 1), a sample size of 2 g, and a final digestate volume of 100 mL, the estimated detection limit for sediment samples is 350 µg/kg.

The method of standard addition (MSA) shall be used for the analysis of all sample digests unless either serial dilution or matrix spike addition demonstrates that it is not required (see section 9).

This method should only be used by spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

This procedure is based on SW-846 Methods 3050 (for digestion) and 6010 for quantitation (EPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

A well-mixed, representative 1 to 2 g (wet weight) sample of sediment is digested in nitric acid and hydrogen peroxide. The digestate is then refluxed using hydrochloric acid and diluted to volume.

NOTE: A separate sample shall be dried for a total solids determination in order to express the results on a dry-weight basis.

This instrumental method measures light emitted by chromium in the final sediment digest by optical spectrometry. The digested samples are nebulized and the resulting aerosol is transported to a plasma torch. Chromium-specific atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

The possibility of additional interferences named in section 3.0 should also be recognized and appropriate corrections made when necessary. The procedures that can be used for this purpose are described in section 9.

3.0 Interferences

Sediments represent diverse matrix types and samples from each location may present its own analytical challenge. Spiked samples and any relevant standard reference material should be processed to aid in determining whether the digestion procedure is appropriate for a specific sediment sample or there are matrix or other effects interfering with the analysis of the resultant sediment digestate.

3.1 Spectral Interferences

Spectral interferences are caused by: (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of simultaneous multi-element instruments must verify the absence of spectral interference from an element in a sample for which there is no instrument detection channel. Potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferants can be assumed.

The magnitude of the interference effects summarized in Table 2 are expressed as analyte concentration equivalents (i.e., false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that Cd is to be determined (at 267.716 ηm) in a sample containing approximately 10 mg/L of Mn. According to Table 2, 100 mg/L of Mn would yield a false signal for Cr equivalent to approximately 0.04 mg/L. Therefore, the presence of 10 mg/L of Mn would result in a false signal for Cr equivalent to approximately 0.004 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, and argon flow rate.

The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.2 Physical Interferences

Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, or by using the standard additions method. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Further, it has been reported that better control of the argon flow rate improves instrument performance. Improved argon flow rate control can be accomplished with the use of mass flow controllers.

3.3 Chemical Interferences

Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, etc.), buffering of the sample, matrix matching, and standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Drying oven, capable of maintaining 30° C.
4. Thermometer, 0 to 200° C.
5. Centrifuge and centrifuge tubes.
6. Inductively coupled argon plasma emission spectrometer.
7. Computer-controlled emission spectrometer with background correction.
8. Radio frequency generator.
9. Argon gas supply, welding grade or better.

4.2 Materials

1. Conical Phillips beakers, 250 mL, or equivalent.
2. Watch glasses.
3. Whatman No. 41 filter paper, or equivalent.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.

3. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
4. Hydrogen peroxide, 30% (H_2O_2). Oxidant should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the H_2O_2 is <MDL, then the acid can be used.
5. Nitric acid (1:1). Add 500 mL concentrated HNO_3 to 400 mL Type II water. Dilute to 1 liter with Type II water.
6. Hydrochloric acid (1:1). Add 500 mL concentrated HCl to 400 mL Type II water. Dilute to 1 liter with Type II water.
7. Standard stock solutions. May be purchased or prepared from ultrahigh purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hr at 105° C, unless otherwise specified.

   CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

8. Aluminum standard stock solution (100 µg/mL). Dissolve 0.1000 g of aluminum metal in an acid mixture of 4 mL of 1:1 HCl and 1 mL of concentrated HNO_3 in a beaker. Warm gently to effect solution. When solution is complete, transfer quantitatively to a liter flask, add an additional 10 mL of 1:1 HCl. Dilute to 1 liter with Type II water.
9. Calcium standard stock solution (100 µg/mL). Suspend 0.2500 g $CaCO_3$ dried at 180° C for 1 hr before weighing in Type II water and dissolve cautiously with a minimum amount of 1:1 HNO_3. Add 10.0 mL of concentrated HNO_3. Dilute to 1 liter with Type II water.
10. Chromium standard stock solution (100 µg/mL). Dissolve 0.1900 g CrO_3 in Type II water. When solution is complete, acidify with 10 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
11. Potassium standard stock solution (100 µg/mL). Dissolve 0.1900 g KCl dried at 110° C in Type II water. Dilute to 1 liter with Type II water.
12. Sodium standard stock solution (100 µg/mL). Dissolve 0.2500 g NaCl in Type II water. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
13. Nickel standard stock solution (100 µg/mL). Dissolve 0.1000 g of nickel metal in 10.0 mL hot concentrated HNO_3. Cool. Dilute to 1 liter with Type II water.
14. Mixed calibration standard solutions. Care should be taken when preparing mixed standards for ICP analysis to ensure that the elements

in the final mixed standard are compatible and stable together. One set of mixed calibration standards that has been found to be useful is listed in Table 3. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interferences or the presence of impurities.

To prepare the mixed calibration standard for chromium, combine appropriate volumes of the individual stock solutions indicated in Table 3 in volumetric flask. Add 2 mL 1:1 HNO_3 and 10 mL of 1:1 HCl and dilute to 100 mL with Type II water. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see section 9.9) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should be scanned using a sequential spectrometer to verify the absence of interelement spectral interference in the recommended mixed standard solutions.

TABLE 3. MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo, and Si
IV	Al, Ca, Cr, K, Na, and Ni

NOTE: Premixed standard solutions (traceable to NIST) containing the combined elements as listed in solutions I through IV are available from a number of commercial vendors.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection. A holding time of 6 months is generally cited for this parameter. Samples may be stored for a longer period of time by air-drying or freezing. However, these samples should not be used for the preparation of sediment elutriates because the process of freezing and thawing the samples may influence the migration potential of sediment-associated constituents.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of samples to be analyzed for chromium in sediments.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The oven or hot plate should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

Prepare a calibration blank (see section 9.5.1) and at least three mixed calibration standards in the appropriate concentration range to correlate chromium concentrations with the ICP's linear response range. Prepare standards for instrument calibration as instructed in section 5.14. Match the sample matrix and that of the standards as closely as possible.

Calibrate the instrument according to the instrument manufacturer's recommended procedures using typical mixed calibration standard solutions. Flush the system with the calibration blank between each standard. Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.

8.0 Procedure

8.1 Sample Digestion

1. Weigh a 1.00 to 2.00 g aliquot of the homogenized, field-moist sediment to the nearest 0.01 g and quantitatively transfer the sample to a 250 mL conical beaker.
2. Add 10 mL of 1:1 HNO_3, mix the slurry, and cover with a watch glass.
3. Heat the sample to 95° C and reflux for 10 to 15 min without boiling.
4. Allow the sample to cool. Add 5 mL of concentrated HNO_3, replace the watch glass, and reflux for 30 min. Repeat this last step to ensure complete oxidation of the sample. Using a ribbed watch glass, allow the solution to evaporate to 5 mL without boiling, while maintaining a covering of solution over the bottom of the beaker.
5. After the sample has cooled, add 2 mL of Type II water and 3 mL of 30% H_2O_2. Cover the beaker with a watch glass and return the covered beaker to the hot plate for warming and to start the peroxide reaction. Heat until effervescence subsides. Allow the beaker to cool.

   NOTE: Exercise care to ensure that sample is not lost due to excessively vigorous effervescence.

6. 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: The total volume of the 30% H_2O_2 should not exceed 10 mL.

7. Add 5 mL of concentrated HCl and 10 mL of Type II water, return the covered beaker to the hot plate, and reflux for an additional 15 min without boiling. After cooling, dilute to 100 mL with Type II water.
8. Suspended particulates should be removed by filtration, by centrifugation, or by allowing the sample to settle prior to analysis.

   a. Filtration: Filter through Whatman No. 41 filter paper (or equivalent) and dilute to 100 mL with Type II water.

   b. Centrifugation: Centrifugation at 2,000 to 3,000 rpm for 10 min is usually sufficient to clear the supernatant.

9. The diluted sample has an approximate acid concentration of 5.0% (v/v) HCl and 5.0% (v/v) HNO_3. The sample is now ready for analysis.

8.2 Sample Analysis

1. The analyst should follow the instructions provided by the instrument's manufacturer. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 min of operation prior to calibration). For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within instrument linear range where coordination factors are valid. The analyst must: (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.
2. Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5% (or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this conditions.
3. Flush the system with the calibration blank solution for at least 1 min before the analysis of each sample. Analyze samples.

   NOTE: Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for chromium in sediments is 20 mg/kg (dry weight).

The detection limit specified in this method is presented to account for typical sediment background contents of chromium in the Great Lakes.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured arsenic concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards 2704 - Buffalo River sediment and 1646 - Estuarine sediment, should be run to monitor the performance of the ICP and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a routine sediment sample collected and homogenized in bulk that has undergone multiple analyses by the analytical laboratory. Alternately, a LCS can be created by spiking a known quantity of the contaminant(s) of concern into a clean sediment, homogenizing the bulk sample, followed by multiple analyses at the analytical laboratory. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

9.5.1 Calibration Blank

The calibration blank is prepared by diluting 2 mL of 1:1 HNO_3 and of 1:1 HCl to 100 mL with Type II water. Prepare a sufficient quantity to flush the system between standards and samples.

The calibration blank should be analyzed prior to routine sample analysis, after every 10 samples, and at the end of the analytical run. The measured concentration in the calibration blank should be less than or equal to the method detection limit.

9.5.2 Reagent Blank

The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - chromium, to a 1-2 g sample aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and

analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of ≤ 20%.

9.8 Interference Check Sample

The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the method detection limit for each element. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

The interference check sample should be analyzed at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Results should be within ± 20% of the known concentration.

9.9 Calibration Control Sample

A calibration control sample should be prepared in the same acid matrix as the calibration standards at 10 times the method detection limit. This sample should be prepared in accordance with the instructions provided by the supplier. The calibration control sample is used to verify the integrity of the calibration standards on a weekly basis.

9.10 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

9.10.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the instrumental detection limit after dilution), an analysis of a 1:4 dilution

should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.10.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The

abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 µg/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported in µg/kg with up to three significant figures.

The concentrations determined are to be reported on the basis of the actual weight of the sample. If a dry weight analysis is desired, then the percent solids of the sample must also be provided.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Winge, R.K., V.J. Peterson, and V.A. Fassel. 1979. Inductively coupled plasma-atomic emission spectroscopy: Prominent lines, final report, March 1977 -

February 1978, Ames laboratory, Ames, IA. EPA-600/4-79-017. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Table 1. Recommended Wavelengths and Estimated Instrumental Detection Limits.

Element	Wavelength[a] (ηm)	Estimated Detection Limit[b] (µg/L)
Aluminum	308.215	45
Chromium	267.716	7
Copper	324.754	6
Nickel	231.604	15
Potassium	766.491	See footnote c
Sodium	588.995	29

a - The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see section 3.1).
b - The estimated instrumental detection limits are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.
c - Highly dependent on operating conditions and plasma position.

Table 2. Analyte Concentration Equivalents arising from Interference at the 100 mg/L Level.

Analyte	Wavelength (ηm)	Interferant[a,b] Al	Ca	Cr	Cu	Fe	Mg	Mn	Mo	Tl	V
Aluminum	308.215	-	-	-	-	-	-	0.21	-	-	1.4
Calcium	317.933	-	-	0.08	-	0.01	0.01	0.04	-	0.03	0.03
Chromium	267.716	-	-	-	-	0.003	-	0.04	-	-	0.04
Nickel	231.604	-	-	-	-	-	-	-	-	-	-
Sodium	588.995	0.30	-	-	-	-	-	-	-	-	-

a - Dashes indicate that no interference was observed even when interferants were introduced at the following levels:

Al - 1000 mg/L,	Mg - 1000 mg/L,
Ca - 1000 mg/L,	Mn - 200 mg/L,
Cr - 200 mg/L,	Tl - 200 mg/L,
Cu - 200 mg/L,	V - 200 mg/L, and
Fe - 1000 mg/L.	

b - The figures recorded as analyte concentrations are not the actual observed concentrations; to obtain those figures, add the listed concentration to the interferant figure.

Table 4. ICP Precision and Accuracy Data[a]

Element	Sample No. 1 True Value (µg/L)	Sample No. 1 Mean Reported Value (µg/L)	Sample No. 1 Mean SD[b] (%)	Sample No. 2 True Value (µg/L)	Sample No. 2 Mean Reported Value (µg/L)	Sample No. 2 Mean SD[b] (%)	Sample No. 3 True Value (µg/L)	Sample No. 3 Mean Reported Value (µg/L)	Sample No. 3 Mean SD[b] (%)
Cr	150	149	3.8	10	10	18	50	50	3.3
Al	700	695	5.6	60	62	33	160	161	13
Ni	250	245	5.8	30	28	11	60	55	14

a - Not all elements were analyzed by all laboratories.
b - SD = standard deviation.

COPPER IN SEDIMENTS
(ICP)

1.0 Scope and Application

Inductively coupled plasma atomic emission spectroscopy (ICP) determines copper and numerous other elements that are present in solution. In order to apply this technique to sediments, the samples must be digested with nitric acid and hydrogen peroxide prior to analysis.

Detection limits, sensitivity, and optimum concentration ranges for copper, and other metals stable in a mixed standard solution with copper, will vary with the sample aliquot size, the spectrophotometer being used, and possible matrix interferences. Based on the estimated detection limit of 6 µg/L for copper in aqueous samples (Table 1), a sample size of 2 g, and a final digestate volume of 100 mL, the estimated detection limit for sediment samples is 300 µg/kg.

The method of standard addition (MSA) shall be used for the analysis of all sample digests unless either serial dilution or matrix spike addition demonstrates that it is not required (see section 9).

This method should only be used by spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

This procedure is based on SW-846 Methods 3050 (for digestion) and 6010 for quantitation (EPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

A well-mixed, representative 1 to 2 g (wet weight) sample of sediment is digested in nitric acid and hydrogen peroxide. The digestate is then refluxed using hydrochloric acid and diluted to volume.

NOTE: A separate sample shall be dried for a total solids determination in order to express the results on a dry-weight basis.

This instrumental method measures light emitted by copper in the final sediment digest by optical spectrometry. The digested samples are nebulized and the resulting aerosol is transported to a plasma torch. Copper-specific atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

The possibility of additional interferences named in section 3.0 should also be recognized and appropriate corrections made when necessary. The procedures that can be used for this purpose are described in section 9.

3.0 Interferences

Sediments represent diverse matrix types and samples from each location may present its own analytical challenge. Spiked samples and any relevant standard reference material should be processed to aid in determining whether the digestion procedure is appropriate for a specific sediment sample or there are matrix or other effects interfering with the analysis of the resultant sediment digestate.

3.1 Spectral Interferences

Spectral interferences are caused by: (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of simultaneous multi-element instruments must verify the absence of spectral interference from an element in a sample for which there is no instrument detection channel. Potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferants can be assumed.

The magnitude of the interference effects summarized in Table 2 are expressed as analyte concentration equivalents (i.e., false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that Cu is to be determined (at 324.754 ηm) in a sample containing approximately 10 mg/L of V. According to Table 2, 100 mg/L of V would yield a false signal for Cu equivalent to approximately 0.02 mg/L. Therefore, the presence of 10 mg/L of V would result in a false signal for Cu equivalent to approximately 0.002 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, and argon flow rate.

The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.2 Physical Interferences

Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, or by using the standard additions method. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Further, it has been reported that better control of the argon flow rate improves instrument performance. Improved argon flow rate control can be accomplished with the use of mass flow controllers.

3.3 Chemical Interferences

Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, etc.), buffering of the sample, matrix matching, and standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Drying oven, capable of maintaining 30° C.
4. Thermometer, 0 to 200° C.
5. Centrifuge and centrifuge tubes.
6. Inductively coupled argon plasma emission spectrometer.
7. Computer-controlled emission spectrometer with background correction.
8. Radio frequency generator.
9. Argon gas supply, welding grade or better.

4.2 Materials

1. Conical Phillips beakers, 250 mL, or equivalent.
2. Watch glasses.
3. Whatman No. 41 filter paper, or equivalent.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.

3. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
4. Hydrogen peroxide, 30% (H_2O_2). Oxidant should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the H_2O_2 is <MDL, then the acid can be used.
5. Nitric acid (1:1). Add 500 mL concentrated HNO_3 to 400 mL Type II water. Dilute to 1 liter with Type II water.
6. Hydrochloric acid (1:1). Add 500 mL concentrated HCl to 400 mL Type II water. Dilute to 1 liter with Type II water.
7. Standard stock solutions. May be purchased or prepared from ultrahigh purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hr at 105° C, unless otherwise specified.

   CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

8. Barium standard stock solution (100 µg/mL). Dissolve 0.1500 g $BaCl_2$ dried at 250° C for 2 hr in 10 mL Type II water with 1 mL 1:1 HCl. Add 10.0 mL 1:1 HCl. Dilute to 1 liter with Type II water.
9. Cobalt standard stock solution (100 µg/mL). Dissolve 0.1000 g of cobalt metal in a minimum amount of 1:1 HNO_3. Add 10.0 mL 1:1 HCl. Dilute to 1 liter with Type II water.
10. Copper standard stock solution (100 µg/mL). Dissolve 0.1300 g CuO in a minimum amount of 1:1 HNO_3. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
11. Iron standard stock solution (100 µg/mL). Dissolve 0.1400 g Fe_2O_3 in a warm mixture of 20 mL 1:1 HCl and 2 mL of concentrated HNO_3. Cool. Add an additional 5.0 mL of concentrated HNO_3. Dilute to 1 liter with Type II water.
12. Vanadium standard stock solution (100 µg/mL). Dissolve 0.2300 g NH_4VO_3 in a minimum amount of concentrated HNO_3. Heat to increase rate of dissolution. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
13. Mixed calibration standard solutions. Care should be taken when preparing mixed standards for ICP analysis to ensure that the elements in the final mixed standard are compatible and stable together. One set of mixed calibration standards that has been found to be useful is listed in Table 3. Prior to preparing the mixed standards, each stock solution

should be analyzed separately to determine possible spectral interferences or the presence of impurities.

To prepare the mixed calibration standard for copper, combine appropriate volumes of the individual stock solutions indicated in Table 3 in volumetric flask. Add 2 mL 1:1 HNO_3 and 10 mL of 1:1 HCl and dilute to 100 mL with Type II water. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see section 9.9) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should be scanned using a sequential spectrometer to verify the absence of interelement spectral interference in the recommended mixed standard solutions.

TABLE 3. MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo, and Si
IV	Al, Ca, Cr, K, Na, and Ni

NOTE: Premixed standard solutions (traceable to NIST) containing the combined elements as listed in solutions I through IV are available from a number of commercial vendors.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection. A holding time of 6 months is generally cited for this parameter.

Samples may be stored for a longer period of time by air-drying or freezing. However, these samples should not be used for the preparation of sediment elutriates because the process of freezing and thawing the samples may influence the migration potential of sediment-associated constituents.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of samples to be analyzed for copper in sediments.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The oven or hot plate should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

Prepare a calibration blank (see section 9.5.1) and at least three mixed calibration standards in the appropriate concentration range to correlate copper concentrations with the ICP's linear response range. Prepare standards for instrument calibration as instructed in section 5.13. Match the sample matrix and that of the standards as closely as possible.

Calibrate the instrument according to the instrument manufacturer's recommended procedures using typical mixed calibration standard solutions. Flush the system with the calibration blank between each standard. Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.

8.0 Procedure

8.1 Sample Digestion

1. Weigh a 1.00 to 2.00 g aliquot of the homogenized, field-moist sediment to the nearest 0.01 g and quantitatively transfer the sample to a 250 mL conical beaker.
2. Add 10 mL of 1:1 HNO_3, mix the slurry, and cover with a watch glass.
3. Heat the sample to 95° C and reflux for 10 to 15 min without boiling.
4. Allow the sample to cool. Add 5 mL of concentrated HNO_3, replace the watch glass, and reflux for 30 min. Repeat this last step to ensure complete oxidation of the sample. Using a ribbed watch glass, allow the solution to evaporate to 5 mL without boiling, while maintaining a covering of solution over the bottom of the beaker.
5. After the sample has cooled, add 2 mL of Type II water and 3 mL of 30% H_2O_2. Cover the beaker with a watch glass and return the covered beaker to the hot plate for warming and to start the peroxide reaction. Heat until effervescence subsides. Allow the beaker to cool.

   NOTE: Exercise care to ensure that sample is not lost due to excessively vigorous effervescence.

6. 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: The total volume of the 30% H_2O_2 should not exceed 10 mL.

7. Add 5 mL of concentrated HCl and 10 mL of Type II water, return the covered beaker to the hot plate, and reflux for an additional 15 min without boiling. After cooling, dilute to 100 mL with Type II water.
8. Suspended particulates should be removed by filtration, by centrifugation, or by allowing the sample to settle prior to analysis.

   a. Filtration: Filter through Whatman No. 41 filter paper (or equivalent) and dilute to 100 mL with Type II water.

   b. Centrifugation: Centrifugation at 2,000 to 3,000 rpm for 10 min is usually sufficient to clear the supernatant.

9. The diluted sample has an approximate acid concentration of 5.0% (v/v) HCl and 5.0% (v/v) HNO_3. The sample is now ready for analysis.

8.2 Sample Analysis

1. The analyst should follow the instructions provided by the instrument's manufacturer. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 min of operation prior to calibration). For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within instrument linear range where coordination factors are valid. The analyst must: (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.

2. Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5% (or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this conditions.

3. Flush the system with the calibration blank solution for at least 1 min before the analysis of each sample. Analyze samples.

   NOTE: Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for copper in sediments is 5 mg/kg (dry weight).

The detection limit specified in this method is presented to account for typical sediment background contents of chromium in the Great Lakes.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured arsenic concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards 2704 - Buffalo River sediment and 1646 - Estuarine sediment, should be run to monitor the performance of the ICP and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a routine sediment sample collected and homogenized in bulk that has undergone multiple analyses by the analytical laboratory. Alternately, a LCS can be created by spiking a known quantity of the contaminant(s) of concern into a clean sediment, homogenizing the bulk sample, followed by multiple analyses at the analytical laboratory. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

9.5.1 Calibration Blank

The calibration blank is prepared by diluting 2 mL of 1:1 HNO_3 and of 1:1 HCl to 100 mL with Type II water. Prepare a sufficient quantity to flush the system between standards and samples.

The calibration blank should be analyzed prior to routine sample analysis, after every 10 samples, and at the end of the analytical run. The measured concentration in the calibration blank should be less than or equal to the method detection limit.

9.5.2 Reagent Blank

The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - copper, to a 1-2 g sample aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

9.8 Interference Check Sample

The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the method detection limit for each element. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

The interference check sample should be analyzed at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Results should be within ± 20% of the known concentration.

9.9 Calibration Control Sample

A calibration control sample should be prepared in the same acid matrix as the calibration standards at 10 times the method detection limit. This sample should be prepared in accordance with the instructions provided by the supplier. The calibration control sample is used to verify the integrity of the calibration standards on a weekly basis.

9.10 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor

negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

9.10.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the instrumental detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.10.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.

4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 µg/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported in µg/kg with up to three significant figures.

The concentrations determined are to be reported on the basis of the actual weight of the sample. If a dry weight analysis is desired, then the percent solids of the sample must also be provided.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Winge, R.K., V.J. Peterson, and V.A. Fassel. 1979. Inductively coupled plasma-atomic emission spectroscopy: Prominent lines, final report, March 1977 - February 1978, Ames laboratory, Ames, IA. EPA-600/4-79-017. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Table 1. Recommended Wavelengths and Estimated Instrumental Detection Limits.

Element	Wavelength[a] (ηm)	Estimated Detection Limit[b] (µg/L)
Barium	455.403	2
Cobalt	228.616	7
Copper	324.754	6
Iron	259.940	7
Vanadium	292.402	8

a - The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see section 3.1).

b - The estimated instrumental detection limits are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

Table 2. Analyte Concentration Equivalents arising from Interference at the 100 mg/L Level.

Analyte	Wavelength (ηm)	Interferant[a,b] Al	Ca	Cr	Cu	Fe	Mg	Mn	Mo	Tl	V
Barium	455.403	-	-	-	-	-	-	-	-	-	-
Cobalt	228.616	-	-	0.03	-	0.005	-	-	0.03	0.15	-
Copper	324.754	-	-	-	-	0.003	-	-	-	0.05	0.02
Iron	259.940	-	-	-	-	-	-	0.12	-	-	-
Vanadium	292.402	-	-	0.05	-	0.005	-	-	-	0.02	-

a - Dashes indicate that no interference was observed even when interferants were introduced at the following levels:

Al - 1000 mg/L,
Ca - 1000 mg/L,
Cr - 200 mg/L,
Cu - 200 mg/L,
Fe - 1000 mg/L.
Mg - 1000 mg/L,
Mn - 200 mg/L,
Tl - 200 mg/L,
V - 200 mg/L, and

b - The figures recorded as analyte concentrations are not the actual observed concentrations; to obtain those figures, add the listed concentration to the interferant figure.

Table 4. ICP Precision and Accuracy Data[a]

Element	Sample No. 1 True Value (µg/L)	Sample No. 1 Mean Reported Value (µg/L)	Sample No. 1 Mean SD[b] (%)	Sample No. 2 True Value (µg/L)	Sample No. 2 Mean Reported Value (µg/L)	Sample No. 2 Mean SD[b] (%)	Sample No. 3 True Value (µg/L)	Sample No. 3 Mean Reported Value (µg/L)	Sample No. 3 Mean SD[b] (%)
V	750	749	1.8	70	69	2.9	170	169	1.1
Cu	250	235	5.1	11	11	40	70	67	7.9
Fe	600	594	3.0	20	19	15	180	178	6.0
Co	700	512	10	20	20	4.1	120	108	21

a - Not all elements were analyzed by all laboratories.
b - SD = standard deviation.

MERCURY IN SEDIMENTS
(MANUAL CVAA)

1.0 Scope and Application

This method is appropriate for the determination of mercury in sediment samples. All samples must be subjected to acid dissolution prior to analysis.

This procedure is based on SW-846 Method 7471 (EPA, 1986).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

An aliquot of the sediment sample is digested with aqua regia at elevated temperatures. The resultant solution is then treated with potassium permanganate to reduce any mercury that is present to the elemental state.

The sample is attached to a cold vapor atomic absorption apparatus and the elemental mercury is flushed from the sample in a stream of air. The mercury vapor is passed through a cell positioned in the light path of an atomic absorption spectrophotometer. The mercury concentration in the sample is proportional to the absorption of incident radiation with a wavelength of 253.7 ηm.

3.0 Interferences

Potassium permanganate is added during the sample preparation step to eliminate possible interference from sulfide. Concentrations as high as 20 mg/L of sulfide as sodium sulfide do not interfere with the recovery of added inorganic mercury from ASTM Type II water.

Although copper has also been reported to interfere with the analysis of mercury, studies suggest that copper concentrations as high as 10 mg/L had no effect on the recovery of mercury from spiked samples.

Interference from certain volatile organic materials, which may absorb radiation at a wavelength of 253 ηm, is also possible but seldom encountered

(EPA, 1979). A preliminary run without reagents can be performed to identify the presence of and to correct for this matrix effect.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, 3-5 weights covering expected weight range.
3. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
4. Thermometer, 0 to 100° C range.
5. Atomic absorption spectrophotometer. Any atomic absorption unit having an open sample presentation area in which to mount the absorption cell is suitable. Instrument settings recommended by the particular manufacturer should be followed. Instruments designed specifically for the measurement of mercury using the cold vapor technique are commercially available and may be substituted for the atomic absorption spectrophotometer.
6. Mercury hollow cathode lamp or electrodeless discharge lamp.
7. Recorder. Any multi-range variable-speed recorder that is compatible with the UV detection system is suitable.
8. Absorption cell. Standard spectrophotometer cells 10 cm long with quartz end windows may be used. Suitable cells may be constructed from Plexiglas tubing, 2.54 cm O.D. x 11.43 cm. The ends are ground perpendicular to the longitudinal axis, and quartz windows (2.54 cm diameter x 0.16 cm thickness) are cemented in place. The cell is strapped to a burner for support and aligned in the light beam by use of two 5.08 cm x 5.08 cm cards. Holes with a diameter of 2.54 cm are cut in the middle of each card. The cards are then placed over each end of the cell. The cell is then positioned and adjusted vertically and horizontally to give the maximum transmittance.
9. Air pump. Any peristaltic pump capable of delivering 1 L/min air may be used. A Masterflex pump with electronic speed control has been found to be satisfactory.
10. Flowmeter. Capable of measuring an air flow of 1 L/min.

11. Aeration tube. A straight glass frit with a coarse porosity. Tygon tubing is used for passage of the mercury vapor from the sample bottle to the absorption cell and return.
12. Drying tube, 15.2 cm X 1.90 cm diameter tube containing 20 g of magnesium perchlorate.

    NOTE: In place of the magnesium perchlorate drying tube, a small reading lamp with a 60 W bulb may be used to prevent condensation of moisture inside the cell. The lamp is positioned to shine on the absorption cell maintaining the air temperature in the cell about 10° C above ambient.

13. Cold vapor generator.

    a. The apparatus shown in Figure 1 is a closed system. An open system, in which the mercury vapor is passed through the absorption cell only once, may be used in place of the closed system.
    b. Because mercury vapor is potentially toxic, precautions must be taken to avoid inhalation of the vapor. Therefore, a bypass has been included in the analytical apparatus to either vent the mercury vapor into an exhaust hood or to pass the vapor through some absorbing medium such as:

        1. equal volumes of 0.1 M $KMnO_4$ and 10% H_2SO_4,
        2. 0.25% iodine in a 3% KI solution, or
        3. specially treated charcoal that will absorb mercury vapor.

4.2 Materials

1. BOD bottles, 300 mL, or equivalent.
2. Aluminum foil.
3. Volumetric flasks, class A, 100 mL.
4. Graduated cylinders, various sizes up to 100 mL, or equivalent.

5.0 Reagents and Standards

1. ASTM Type II water (ASTM D1193). Water supply should be continually tested to verify that contaminants are not present at levels that will interfere with method performance.

2. Hydrochloric acid (HCl), conc. reagent grade.
3. Nitric acid (HNO_3), conc. reagent grade.
4. Sulfuric acid (H_2SO_4), conc. reagent grade.
5. Aqua regia. Prepare immediately before use by carefully adding three volumes of concentrated HCl to one volume of concentrated HNO_3.
6. Sulfuric acid, 0.5 N. Dilute 14.0 mL of concentrated sulfuric acid to 1.0 liter with ASTM Type II water.
7. 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.

   NOTE: Stannous chloride may be used in place of stannous sulfate.

8. Sodium chloride-hydroxylamine sulfate solution. Dissolve 12 g of sodium chloride and 12 g of hydroxylamine sulfate in ASTM Type II water. Dilute to 100 mL with Type II water.

   NOTE: Hydroxylamine hydrochloride may be used in place of hydroxylamine sulfate.

9. Potassium permanganate, 5% w/v solution ($KMnO_4$). Dissolve 5 g of potassium permanganate in 100 mL of ASTM Type II water.
10. Mercury stock solution. Dissolve 0.1354 g of mercuric chloride in 75 mL of ASTM Type II water. Add 10 mL of concentrated nitric acid and adjust the volume to 100 mL with ASTM Type II water. (1.0 mL = 1.0 mg Hg).
11. Mercury working 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 adding mercury stock solution.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection. A holding time of 28 days is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of samples to be analyzed for arsenic in sediments.

If sediment samples are to be dried prior to analysis, moisture should be driven off at a temperature of 60° C or less to minimize the potential volatilization of mercury.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The hot plate/water bath should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

Calibration curves should be composed of a minimum of a blank and three standards. To calibrate and standardize to atomic absorption spectrophotometer, the following steps should be used to prepare standards and prepare the instrument:

1. Transfer 0.0, 0.5, 1.0, 2.0, 5.0, and 10-mL aliquots of the mercury working standard containing 0-1.0 µg, respectively, of mercury to a series of 300-mL BOD bottles.
2. Add enough ASTM Type II water to each bottle to make a total volume of 10 mL.
3. Add 5 mL of aqua regia and heat 2 min in a water bath at 95° C.
4. Allow the sample to cool.

5. Add 50 mL ASTM Type II water and 15 mL of $KMnO_4$ solution to each bottle and return to the water bath for 30 min.
6. Cool and add 6 mL of sodium chloride-hydroxylamine sulfate solution to reduce the excess permanganate.
7. Add 50 mL of ASTM Type II water.
8. To the first standard, add 5 mL of stannous sulfate solution, and immediately attach the bottle to the aeration apparatus. At this point, the sample is allowed to stand quietly without manual agitation.
9. The circulating pump, which has previously been adjusted to a rate of 1 liter per minute, is allowed to run continuously. The absorbance will increase and reach a maximum within 30 seconds. As soon as the recorder pen levels off, approximately 1 minute, open the bypass valve and continue the aeration until the absorbance returns to a minimum value. Due to the potential toxicity of these vapors, they should be properly vented through a fume hood or absorbing medium.
10. Close the bypass valve, remove the stopper and frit from the BOD bottle, and continue the aeration.
11. Repeat steps 8 through 10 for each of the standards.
12. Construct a standard curve by plotting the recorded absorbance versus the concentration of mercury in the standards.

8.0 Procedure

8.1 Sample Digestion

Digest and oxidize the sediment sample using the procedure specified in either step 8.1.1 or 8.1.2.

8.1.1 Sample Digestion Option 1 - Water Bath Digestion

1. Weigh a 0.2 to 0.5 g aliquot of well mixed, field-moist sediment sample and transfer to the bottom of a BOD bottle.
2. Add 5 mL of ASTM Type II water and 5 mL of aqua regia.
3. Heat 2 min in a water bath at 95° C and allow to cool.
4. Add 50 mL ASTM Type II water and 15 mL potassium permanganate solution to each sample bottle.
5. Mix thoroughly and place in the water bath for 30 min at 95° C.

6. Cool and add 6 mL of sodium chloride-hydroxylamine sulfate to reduce the excess permanganate.

   CAUTION: Do this addition under a vacuum hood since Cl_2 could be evolved.

7. Add 55 mL of ASTM Type II water.
8. Continue as described in step 8.2.

8.1.2 Sample Digestion Option 2 - Autoclave Digestion

1. Weigh a 0.2 g aliquot of well mixed, field-moist sediment sample and transfer to the bottom of a BOD bottle.
2. Add 5 mL of concentrated H_2SO_4 and 2 mL of concentrated HNO_3.
3. Add 5 mL of saturated $KMnO_4$ solution and cover the bottle with a piece of aluminum foil.
4. Autoclave samples at 121° C and 15 psi for 15 minutes and allow to cool.
5. Add 50 mL ASTM Type II water and 15 mL potassium permanganate solution to each sample bottle.
6. Cool and bring the volume of the sample to 100 mL with ASTM Type II water.
7. Add 6 mL of sodium chloride-hydroxylamine sulfate to reduce the excess permanganate.

   CAUTION: Do this addition under a vacuum hood as Cl_2 could be evolved.

8. Purge the dead air space and continue as described in step 8.2.

8.2 Sample Analysis

1. To the first sample, add 5 mL of stannous sulfate solution, and immediately attach the bottle to the aeration apparatus. At this point, the sample is allowed to stand quietly without manual agitation.
2. The circulating pump, which has previously been adjusted to a rate of 1 liter per minute, is allowed to run continuously. The absorbance will increase and reach a maximum within 30 seconds. As soon as the recorder pen levels off, approximately 1 minute, open the bypass valve and continue the aeration until the absorbance returns to a minimum

value. Due to the potential toxicity of these vapors, they should be properly vented through a fume hood or absorbing medium.

3. Close the bypass valve, remove the stopper and frit from the BOD bottle, and continue the aeration.
4. Repeat steps 1 through 3 for each of the samples.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for mercury in sediments is 0.002 mg/kg (dry weight).

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured arsenic concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards 2704 - Buffalo River sediment and 1646 - Estuarine sediment, should be run to monitor the life and performance of the graphite tube and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a routine sediment sample collected and homogenized in bulk that has undergone multiple analyses by the analytical laboratory. Alternately, a LCS can be created by spiking a known quantity of the contaminant(s) of concern into a clean sediment, homogenizing the

bulk sample, followed by multiple analyses at the analytical laboratory. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the instrument detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 15 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - arsenic, to a 1-2 g sample aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 3 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

Samples with a concentration above the highest standard or an absorbance that fall on the plateau of the calibration curve should be diluted and reanalyzed.

10.0 Method Performance

The general range of this method with sediment samples, which is dependent upon sample size, is 0.2 to 5 µg/g (EPA, 1979).

The analysis of replicate sediment samples using the digestion procedure in step 8.1.1 and the cold vapor analytical technique produced the following standard deviations at the indicated levels:

0.29 µg/g ± 0.02, and
0.82 µg/g ± 0.03.

Recoveries of mercury at these levels, added as methyl mercuric chloride, were 97% and 94%, respectively.

The data shown in Table 1 were obtained from records of state and contractor laboratories. The data provide an estimate of the precision that can be attained with the combined sample preparation and analysis method.

11.0 Calculations and Reporting

Calculate metal concentrations by (1) the method of standard additions, or (2) from a calibration curve, or (3) directly from the instrument's concentration readout. All dilution or concentration factors must be taken into account. Concentrations reported for multiphased or wet samples must be appropriately qualified (e.g., 5 µg/g dry weight).

Measure the peak height or absorbance of the sample from the chart and determine the mercury concentration from the standard curve.

Calculate the mercury concentration in the sediment sample using the following calculation:

$$\text{Hg, µg/kg (dry weight)} = \frac{X \times V}{g \times \%S}$$

where:

X = is the mercury concentration in the final sediment digest, µg/L.
V = the final sediment digest volume, L.
g = the weight of wet sediment digested, g.
%S = the percent solids concentration of the field moist sediment sample expressed as a decimal fraction.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Gaskill, A., 1986. Compilation and Evaluation of RCRA Method Performance Data, Work Assignment No. 2, EPA Contract No. 68-01-7075.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Table 1. Method Performance Data (after Gaskill, 1986).

Sample Matrix	Preparation Method	Laboratory Replicates
Emission control dust	unknown	12, 12 µg/g
Wastewater treatment sludge	unknown	0., 0.28 µg/g

NICKEL IN SEDIMENTS
(ICP)

1.0 Scope and Application

Inductively coupled plasma atomic emission spectroscopy (ICP) determines nickel and numerous other elements that are present in solution. In order to apply this technique to sediments, the samples must be digested with nitric acid and hydrogen peroxide prior to analysis.

Detection limits, sensitivity, and optimum concentration ranges for nickel, and other metals stable in a mixed standard solution with nickel, will vary with the sample aliquot size, the spectrophotometer being used, and possible matrix interferences. Based on the estimated detection limit of 15 µg/L for nickel in aqueous samples (Table 1), a sample size of 2 g, and a final digestate volume of 100 mL, the estimated detection limit for sediment samples is 750 µg/kg.

The method of standard addition (MSA) shall be used for the analysis of all sample digests unless either serial dilution or matrix spike addition demonstrates that it is not required (see section 9).

This method should only be used by spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

This procedure is based on SW-846 Methods 3050 (for digestion) and 6010 for quantitation (EPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

A well-mixed, representative 1 to 2 g (wet weight) sample of sediment is digested in nitric acid and hydrogen peroxide. The digestate is then refluxed using hydrochloric acid and diluted to volume.

NOTE: A separate sample shall be dried for a total solids determination in order to express the results on a dry-weight basis.

This instrumental method measures light emitted by nickel in the final sediment digest by optical spectrometry. The digested samples are nebulized and the resulting aerosol is transported to a plasma torch. Nickel-specific atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

The possibility of additional interferences named in section 3.0 should also be recognized and appropriate corrections made when necessary. The procedures that can be used for this purpose are described in section 9.

3.0 Interferences

Sediments represent diverse matrix types and samples from each location may present its own analytical challenge. Spiked samples and any relevant standard reference material should be processed to aid in determining whether the digestion procedure is appropriate for a specific sediment sample or there are matrix or other effects interfering with the analysis of the resultant sediment digestate.

3.1 Spectral Interferences

Spectral interferences are caused by: (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of simultaneous multi-element instruments must verify the absence of spectral interference from an element in a sample for which there is no instrument detection channel. Potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferants can be assumed.

The magnitude of the interference effects summarized in Table 2 are expressed as analyte concentration equivalents (i.e., false analyte concentrations) arising from 100 mg/L of the interference element. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, and argon flow rate.

The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.2 Physical Interferences

Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, or by using the standard additions method. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Further, it has been reported that better control of the argon flow rate improves instrument performance. Improved argon flow rate control can be accomplished with the use of mass flow controllers.

3.3 Chemical Interferences

Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant

with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, etc.), buffering of the sample, matrix matching, and standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Drying oven, capable of maintaining 30° C.
4. Thermometer, 0 to 200° C.
5. Centrifuge and centrifuge tubes.
6. Inductively coupled argon plasma emission spectrometer.
7. Computer-controlled emission spectrometer with background correction.
8. Radio frequency generator.
9. Argon gas supply, welding grade or better.

4.2 Materials

1. Conical Phillips beakers, 250 mL, or equivalent.
2. Watch glasses.
3. Whatman No. 41 filter paper, or equivalent.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
3. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.

4. Hydrogen peroxide, 30% (H_2O_2). Oxidant should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the H_2O_2 is <MDL, then the acid can be used.
5. Nitric acid (1:1). Add 500 mL concentrated HNO_3 to 400 mL Type II water. Dilute to 1 liter with Type II water.
6. Hydrochloric acid (1:1). Add 500 mL concentrated HCl to 400 mL Type II water. Dilute to 1 liter with Type II water.
7. Standard stock solutions. May be purchased or prepared from ultrahigh purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hr at 105° C, unless otherwise specified.

   CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

8. Aluminum standard stock solution (100 µg/mL). Dissolve 0.1000 g of aluminum metal in an acid mixture of 4 mL of 1:1 HCl and 1 mL of concentrated HNO_3 in a beaker. Warm gently to effect solution. When solution is complete, transfer quantitatively to a liter flask, add an additional 10 mL of 1:1 HCl. Dilute to 1 liter with Type II water.
9. Calcium standard stock solution (100 µg/mL). Suspend 0.2500 g $CaCO_3$ dried at 180° C for 1 hr before weighing in Type II water and dissolve cautiously with a minimum amount of 1:1 HNO_3. Add 10.0 mL of concentrated HNO_3. Dilute to 1 liter with Type II water.
10. Chromium standard stock solution (100 µg/mL). Dissolve 0.1900 g CrO_3 in Type II water. When solution is complete, acidify with 10 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
11. Potassium standard stock solution (100 µg/mL). Dissolve 0.1900 g KCl dried at 110° C in Type II water. Dilute to 1 liter with Type II water.
12. Sodium standard stock solution (100 µg/mL). Dissolve 0.2500 g NaCl in Type II water. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
13. Nickel standard stock solution (100 µg/mL). Dissolve 0.1000 g of nickel metal in 10.0 mL hot concentrated HNO_3. Cool. Dilute to 1 liter with Type II water.
14. Mixed calibration standard solutions. Care should be taken when preparing mixed standards for ICP analysis to ensure that the elements in the final mixed standard are compatible and stable together. One set of mixed calibration standards that has been found to be useful is listed in Table 3. Prior to preparing the mixed standards, each stock solution

should be analyzed separately to determine possible spectral interferences or the presence of impurities.

To prepare the mixed calibration standard for nickel, combine appropriate volumes of the individual stock solutions indicated in Table 3 in volumetric flask. Add 2 mL 1:1 HNO_3 and 10 mL of 1:1 HCl and dilute to 100 mL with Type II water. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see section 9.9) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should be scanned using a sequential spectrometer to verify the absence of interelement spectral interference in the recommended mixed standard solutions.

TABLE 3. MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo, and Si
IV	Al, Ca, Cr, K, Na, and Ni

NOTE: Premixed standard solutions (traceable to NIST) containing the combined elements as listed in solutions I through IV are available from a number of commercial vendors.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection. A holding time of 6 months is generally cited for this parameter.

Samples may be stored for a longer period of time by air-drying or freezing. However, these samples should not be used for the preparation of sediment elutriates because the process of freezing and thawing the samples may influence the migration potential of sediment-associated constituents.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of samples to be analyzed for nickel in sediments.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The oven or hot plate should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

Prepare a calibration blank (see section 9.5.1) and at least three mixed calibration standards in the appropriate concentration range to correlate nickel concentrations with the ICP's linear response range. Prepare standards for instrument calibration as instructed in section 5.14. Match the sample matrix and that of the standards as closely as possible.

Calibrate the instrument according to the instrument manufacturer's recommended procedures using typical mixed calibration standard solutions. Flush the system with the calibration blank between each standard. Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.

8.0 Procedure

8.1 Sample Digestion

1. Weigh a 1.00 to 2.00 g aliquot of the homogenized, field-moist sediment to the nearest 0.01 g and quantitatively transfer the sample to a 250 mL conical beaker.
2. Add 10 mL of 1:1 HNO_3, mix the slurry, and cover with a watch glass.
3. Heat the sample to 95° C and reflux for 10 to 15 min without boiling.
4. Allow the sample to cool. Add 5 mL of concentrated HNO_3, replace the watch glass, and reflux for 30 min. Repeat this last step to ensure complete oxidation of the sample. Using a ribbed watch glass, allow the solution to evaporate to 5 mL without boiling, while maintaining a covering of solution over the bottom of the beaker.
5. After the sample has cooled, add 2 mL of Type II water and 3 mL of 30% H_2O_2. Cover the beaker with a watch glass and return the covered beaker to the hot plate for warming and to start the peroxide reaction. Heat until effervescence subsides. Allow the beaker to cool.

   NOTE: Exercise care to ensure that sample is not lost due to excessively vigorous effervescence.

6. 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: The total volume of the 30% H_2O_2 should not exceed 10 mL.

7. Add 5 mL of concentrated HCl and 10 mL of Type II water, return the covered beaker to the hot plate, and reflux for an additional 15 min without boiling. After cooling, dilute to 100 mL with Type II water.
8. Suspended particulates should be removed by filtration, by centrifugation, or by allowing the sample to settle prior to analysis.

   a. Filtration: Filter through Whatman No. 41 filter paper (or equivalent) and dilute to 100 mL with Type II water.

   b. Centrifugation: Centrifugation at 2,000 to 3,000 rpm for 10 min is usually sufficient to clear the supernatant.

9. The diluted sample has an approximate acid concentration of 5.0% (v/v) HCl and 5.0% (v/v) HNO_3. The sample is now ready for analysis.

8.2 Sample Analysis

1. The analyst should follow the instructions provided by the instrument's manufacturer. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 min of operation prior to calibration). For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within instrument linear range where coordination factors are valid. The analyst must: (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.

2. Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5% (or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this conditions.

3. Flush the system with the calibration blank solution for at least 1 min before the analysis of each sample. Analyze samples.

   NOTE: Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for nickel in sediments is 15 mg/kg (dry weight).

The detection limit specified in this method is presented to account for typical sediment background contents of chromium in the Great Lakes.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured arsenic concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards 2704 - Buffalo River sediment and 1646 - Estuarine sediment, should be run to monitor the performance of the ICP and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a routine sediment sample collected and homogenized in bulk that has undergone multiple analyses by the analytical laboratory. Alternately, a LCS can be created by spiking a known quantity of the contaminant(s) of concern into a clean sediment, homogenizing the bulk sample, followed by multiple analyses at the analytical laboratory. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

9.5.1 Calibration Blank

The calibration blank is prepared by diluting 2 mL of 1:1 HNO_3 and of 1:1 HCl to 100 mL with Type II water. Prepare a sufficient quantity to flush the system between standards and samples.

The calibration blank should be analyzed prior to routine sample analysis, after every 10 samples, and at the end of the analytical run. The measured concentration in the calibration blank should be less than or equal to the method detection limit.

9.5.2 Reagent Blank

The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - nickel, to a 1-2 g sample aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

9.8 Interference Check Sample

The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the method detection limit for each element. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

The interference check sample should be analyzed at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Results should be within ± 20% of the known concentration.

9.9 Calibration Control Sample

A calibration control sample should be prepared in the same acid matrix as the calibration standards at 10 times the method detection limit. This sample should be prepared in accordance with the instructions provided by the supplier. The calibration control sample is used to verify the integrity of the calibration standards on a weekly basis.

9.10 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor

negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

9.10.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the instrumental detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.10.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.

4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 µg/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported in µg/kg with up to three significant figures.

The concentrations determined are to be reported on the basis of the actual weight of the sample. If a dry weight analysis is desired, then the percent solids of the sample must also be provided.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Winge, R.K., V.J. Peterson, and V.A. Fassel. 1979. Inductively coupled plasma-atomic emission spectroscopy: Prominent lines, final report, March 1977 - February 1978, Ames laboratory, Ames, IA. EPA-600/4-79-017. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Table 1. Recommended Wavelengths and Estimated Instrumental Detection Limits.

Element	Wavelength[a] (ηm)	Estimated Detection Limit[b] (µg/L)
Aluminum	308.215	45
Chromium	267.716	7
Copper	324.754	6
Nickel	231.604	15
Potassium	766.491	See footnote c
Sodium	588.995	29

a - The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see section 3.1).
b - The estimated instrumental detection limits are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.
c - Highly dependent on operating conditions and plasma position.

Table 2. Analyte Concentration Equivalents arising from Interference at the 100 mg/L Level.

Analyte	Wavelength (ηm)	Interferant[a,b] Al	Ca	Cr	Cu	Fe	Mg	Mn	Mo	Tl	V
Aluminum	308.215	-	-	-	-	-	-	0.21	-	-	1.4
Calcium	317.933	-	-	0.08	-	0.01	0.01	0.04	-	0.03	0.03
Chromium	267.716	-	-	-	-	0.003	-	0.04	-	-	0.04
Nickel	231.604	-	-	-	-	-	-	-	-	-	-
Sodium	588.995	0.30	-	-	-	-	-	-	-	-	-

a - Dashes indicate that no interference was observed even when interferants were introduced at the following levels:

Al - 1000 mg/L,	Mg - 1000 mg/L,
Ca - 1000 mg/L,	Mn - 200 mg/L,
Cr - 200 mg/L,	Tl - 200 mg/L,
Cu - 200 mg/L,	V - 200 mg/L, and
Fe - 1000 mg/L.	

b - The figures recorded as analyte concentrations are not the actual observed concentrations; to obtain those figures, add the listed concentration to the interferant figure.

Table 4. ICP Precision and Accuracy Data[a]

Element	Sample No. 1 True Value (µg/L)	Sample No. 1 Mean Reported Value (µg/L)	Sample No. 1 Mean SD[b] (%)	Sample No. 2 True Value (µg/L)	Sample No. 2 Mean Reported Value (µg/L)	Sample No. 2 Mean SD[b] (%)	Sample No. 3 True Value (µg/L)	Sample No. 3 Mean Reported Value (µg/L)	Sample No. 3 Mean SD[b] (%)
Cr	150	149	3.8	10	10	18	50	50	3.3
Al	700	695	5.6	60	62	33	160	161	13
Ni	250	245	5.8	30	28	11	60	55	14

a - Not all elements were analyzed by all laboratories.
b - SD = standard deviation.

LEAD IN SEDIMENTS
(ICP)

1.0 Scope and Application

Inductively coupled plasma atomic emission spectroscopy (ICP) determines lead and numerous other elements that are present in solution. In order to apply this technique to sediments, the samples must be digested with nitric acid and hydrogen peroxide prior to analysis.

Detection limits, sensitivity, and optimum concentration ranges for lead, and other metals stable in a mixed standard solution with lead, will vary with the sample aliquot size, the spectrophotometer being used, and possible matrix interferences. Based on the estimated detection limit of 42 µg/L for lead in aqueous samples (Table 1), a sample size of 2 g, and a final digestate volume of 100 mL, the estimated detection limit for sediment samples is 2100 µg/kg.

The method of standard addition (MSA) shall be used for the analysis of all sample digests unless either serial dilution or matrix spike addition demonstrates that it is not required (see section 9).

This method should only be used by spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

This procedure is based on SW-846 Methods 3050 (for digestion) and 6010 for quantitation (EPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

A well-mixed, representative 1 to 2 g (wet weight) sample of sediment is digested in nitric acid and hydrogen peroxide. The digestate is then refluxed using hydrochloric acid and diluted to volume.

NOTE: A separate sample shall be dried for a total solids determination in order to express the results on a dry-weight basis.

This instrumental method measures light emitted by lead in the final sediment digest by optical spectrometry. The digested samples are nebulized and the resulting aerosol is transported to a plasma torch. Lead-specific atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

The possibility of additional interferences named in section 3.0 should also be recognized and appropriate corrections made when necessary. The procedures that can be used for this purpose are described in section 9.

3.0 Interferences

Sediments represent diverse matrix types and samples from each location may present its own analytical challenge. Spiked samples and any relevant standard reference material should be processed to aid in determining whether the digestion procedure is appropriate for a specific sediment sample or there are matrix or other effects interfering with the analysis of the resultant sediment digestate.

3.1 Spectral Interferences

Spectral interferences are caused by: (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of simultaneous multi-element instruments must verify the absence of spectral interference from an element in a sample for which there is no instrument detection channel. Potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferants can be assumed.

The magnitude of the interference effects summarized in Table 2 are expressed as analyte concentration equivalents (i.e., false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that Pb is to be determined (at 220.353 ηm) in a sample containing approximately 10 mg/L of Al. According to Table 2, 100 mg/L of Al would yield a false signal for Pb equivalent to approximately 0.17 mg/L. Therefore, the presence of 10 mg/L of Al would result in a false signal for Pb equivalent to approximately 0.017 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, and argon flow rate.

The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.2 Physical Interferences

Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, or by using the standard additions method. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Further, it has been reported that better control of the argon flow rate improves instrument performance. Improved argon flow rate control can be accomplished with the use of mass flow controllers.

3.3 Chemical Interferences

Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, etc.), buffering of the sample, matrix matching, and standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Drying oven, capable of maintaining 30° C.
4. Thermometer, 0 to 200° C.
5. Centrifuge and centrifuge tubes.
6. Inductively coupled argon plasma emission spectrometer.
7. Computer-controlled emission spectrometer with background correction.
8. Radio frequency generator.
9. Argon gas supply, welding grade or better.

4.2 Materials

1. Conical Phillips beakers, 250 mL, or equivalent.
2. Watch glasses.
3. Whatman No. 41 filter paper, or equivalent.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.

3. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
4. Hydrogen peroxide, 30% (H_2O_2). Oxidant should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the H_2O_2 is <MDL, then the acid can be used.
5. Nitric acid (1:1). Add 500 mL concentrated HNO_3 to 400 mL Type II water. Dilute to 1 liter with Type II water.
6. Hydrochloric acid (1:1). Add 500 mL concentrated HCl to 400 mL Type II water. Dilute to 1 liter with Type II water.
7. Standard stock solutions. May be purchased or prepared from ultrahigh purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hr at 105° C, unless otherwise specified.

   CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

8. Beryllium standard stock solution (100 µg/mL). Dissolve 1.970 g $BeSO_4 \cdot 4H_2O$ (analytical reagent grade, undried), in 100 mL of Type II water, add 10.0 ml concentrated HNO_3. Dilute to 1 liter with Type II water.
9. Cadmium standard stock solution (100 µg/mL). Dissolve 0.1100 g CdO (analytical reagent grade) in a minimum amount of 1:1 HNO_3. Heat to increase rate of dissolution. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
10. Lead standard stock solution (100 µg/mL). Dissolve 0.1600 g $Pb(NO_3)_2$ (analytical reagent grade) in a minimum amount of 1:1 HNO_3. Add 10 mL 1:1 HNO_3. Add 10 mL 1:1 HNO_3. Dilute to 1 liter with Type II water.
11. Manganese standard stock solution (100 µg/mL). Dissolve 0.1000 g of manganese metal, in an acid mixture of 10 mL concentrated HCl and 1 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
12. Selenium standard stock solution (100 µg/mL). Dissolve 0.1700 g H_2SeO_3 (analytical reagent grade, undried) in 100 mL of Type II water. Dilute to 1 liter with Type II water.
13. Zinc standard stock solution (100 µg/mL). Dissolve 0.1200 g ZnO in a minimum amount of 1:1 HNO_3. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
14. Mixed calibration standard solutions. Care should be taken when preparing mixed standards for ICP analysis to ensure that the elements

in the final mixed standard are compatible and stable together. One set of mixed calibration standards that has been found to be useful is listed in Table 3. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interferences or the presence of impurities.

To prepare the mixed calibration standard for lead, combine appropriate volumes of the individual stock solutions indicated in Table 3 in volumetric flask. Add 2 mL 1:1 HNO_3 and 10 mL of 1:1 HCl and dilute to 100 mL with Type II water. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see section 9.9) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should be scanned using a sequential spectrometer to verify the absence of interelement spectral interference in the recommended mixed standard solutions.

TABLE 3. MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo, and Si
IV	Al, Ca, Cr, K, Na, and Ni

NOTE: Premixed standard solutions (traceable to NIST) containing the combined elements as listed in solutions I through IV are available from a number of commercial vendors.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection. A holding time of 6 months is generally cited for this parameter. Samples may be stored for a longer period of time by air-drying or freezing. However, these samples should not be used for the preparation of sediment elutriates because the process of freezing and thawing the samples may influence the migration potential of sediment-associated constituents.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of samples to be analyzed for lead in sediments.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The oven or hot plate should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

Prepare a calibration blank (see section 9.5.1) and at least three mixed calibration standards in the appropriate concentration range to correlate lead concentrations with the ICP's linear response range. Prepare standards for instrument calibration as instructed in section 5.14. Match the sample matrix and that of the standards as closely as possible.

Calibrate the instrument according to the instrument manufacturer's recommended procedures using typical mixed calibration standard solutions. Flush the system with the calibration blank between each standard. Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.

8.0 Procedure

8.1 Sample Digestion

1. Weigh a 1.00 to 2.00 g aliquot of the homogenized, field-moist sediment to the nearest 0.01 g and quantitatively transfer the sample to a 250 mL conical beaker.
2. Add 10 mL of 1:1 HNO_3, mix the slurry, and cover with a watch glass.
3. Heat the sample to 95° C and reflux for 10 to 15 min without boiling.
4. Allow the sample to cool. Add 5 mL of concentrated HNO_3, replace the watch glass, and reflux for 30 min. Repeat this last step to ensure complete oxidation of the sample. Using a ribbed watch glass, allow the solution to evaporate to 5 mL without boiling, while maintaining a covering of solution over the bottom of the beaker.
5. After the sample has cooled, add 2 mL of Type II water and 3 mL of 30% H_2O_2. Cover the beaker with a watch glass and return the covered beaker to the hot plate for warming and to start the peroxide reaction. Heat until effervescence subsides. Allow the beaker to cool.

   NOTE: Exercise care to ensure that sample is not lost due to excessively vigorous effervescence.

6. 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: The total volume of the 30% H_2O_2 should not exceed 10 mL.

7. Add 5 mL of concentrated HCl and 10 mL of Type II water, return the covered beaker to the hot plate, and reflux for an additional 15 min without boiling. After cooling, dilute to 100 mL with Type II water.
8. Suspended particulates should be removed by filtration, by centrifugation, or by allowing the sample to settle prior to analysis.

   a. Filtration: Filter through Whatman No. 41 filter paper (or equivalent) and dilute to 100 mL with Type II water.

   b. Centrifugation: Centrifugation at 2,000 to 3,000 rpm for 10 min is usually sufficient to clear the supernatant.

9. The diluted sample has an approximate acid concentration of 5.0% (v/v) HCl and 5.0% (v/v) HNO_3. The sample is now ready for analysis.

8.2 Sample Analysis

1. The analyst should follow the instructions provided by the instrument's manufacturer. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 min of operation prior to calibration). For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within instrument linear range where coordination factors are valid. The analyst must: (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.

2. Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5% (or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this conditions.

3. Flush the system with the calibration blank solution for at least 1 min before the analysis of each sample. Analyze samples.

   NOTE: Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for lead in sediments is 10 mg/kg (dry weight).

The detection limit specified in this method is presented to account for typical sediment background contents of chromium in the Great Lakes.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured arsenic concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards 2704 - Buffalo River sediment and 1646 - Estuarine sediment, should be run to monitor the performance of the ICP and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a routine sediment sample collected and homogenized in bulk that has undergone multiple analyses by the analytical laboratory. Alternately, a LCS can be created by spiking a known quantity of the contaminant(s) of concern into a clean sediment, homogenizing the bulk sample, followed by multiple analyses at the analytical laboratory. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

9.5.1 Calibration Blank

The calibration blank is prepared by diluting 2 mL of 1:1 HNO_3 and of 1:1 HCl to 100 mL with Type II water. Prepare a sufficient quantity to flush the system between standards and samples.

The calibration blank should be analyzed prior to routine sample analysis, after every 10 samples, and at the end of the analytical run. The measured concentration in the calibration blank should be less than or equal to the method detection limit.

9.5.2 Reagent Blank

The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - lead, to a 1-2 g sample aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

9.8 Interference Check Sample

The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the method detection limit for each element. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

The interference check sample should be analyzed at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Results should be within ± 20% of the known concentration.

9.9 Calibration Control Sample

A calibration control sample should be prepared in the same acid matrix as the calibration standards at 10 times the method detection limit. This sample should be prepared in accordance with the instructions provided by the supplier. The calibration control sample is used to verify the integrity of the calibration standards on a weekly basis.

9.10 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor

negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

9.10.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the instrumental detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.10.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.

4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 µg/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported in µg/kg with up to three significant figures.

The concentrations determined are to be reported on the basis of the actual weight of the sample. If a dry weight analysis is desired, then the percent solids of the sample must also be provided.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Winge, R.K., V.J. Peterson, and V.A. Fassel. 1979. Inductively coupled plasma-atomic emission spectroscopy: Prominent lines, final report, March 1977 - February 1978, Ames laboratory, Ames, IA. EPA-600/4-79-017. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Table 1. Recommended Wavelengths and Estimated Instrumental Detection Limits.

Element	Wavelength[a] (ηm)	Estimated Detection Limit[b] (µg/L)
Beryllium	313.042	0.3
Cadmium	226.502	4
Lead	220.353	42
Manganese	257.610	2
Selenium	196.026	75
Zinc	213.856	2

a - The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see section 3.1).
b - The estimated instrumental detection limits are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

Table 2. Analyte Concentration Equivalents arising from Interference at the 100 mg/L Level.

Analyte	Wavelength (ηm)	Interferant[a,b] Al	Ca	Cr	Cu	Fe	Mg	Mn	Mi	Tl	V
Beryllium	313.042	-	-	-	-	-	-	-	-	0.04	0.05
Cadmium	226.502	-	-	-	-	0.03	-	-	0.02	-	-
Lead	220.353	0.17	-	-	-	-	-	-	-	-	-
Manganese	257.610	0.005	-	0.01	-	0.002	0.002	-	-	-	-
Selenium	196.026	0.23	-	-	-	0.09	-	-	-	-	-
Zinc	213.856	-	-	-	0.14	-	-	-	0.29	-	-

a - Dashes indicate that no interference was observed even when interferants were introduced at the following levels:

Al - 1000 mg/L,
Ca - 1000 mg/L,
Cr - 200 mg/L,
Cu - 200 mg/L,
Fe - 1000 mg/L.
Mg - 1000 mg/L,
Mn - 200 mg/L,
Tl - 200 mg/L,
V - 200 mg/L, and

b - The figures recorded as analyte concentrations are not the actual observed concentrations. To obtain those figures, add the listed concentration to the interferant figure.

Table 4. ICP Precision and Accuracy Data[a]

Element	Sample No. 1 True Value (µg/L)	Sample No. 1 Mean Reported Value (µg/L)	Sample No. 1 Mean SD[b] (%)	Sample No. 2 True Value (µg/L)	Sample No. 2 Mean Reported Value (µg/L)	Sample No. 2 Mean SD[b] (%)	Sample No. 3 True Value (µg/L)	Sample No. 3 Mean Reported Value (µg/L)	Sample No. 3 Mean SD[b] (%)
Be	750	733	6.2	20	20	9.8	180	176	5.2
Mn	350	345	2.7	15	15	6.7	100	99	3.3
Cd	50	48	12	2.5	2.9	16	14	13	16
Pb	250	236	16	24	30	32	80	80	14
Zn	200	201	5.6	16	19	45	80	82	9.4
Se[c]	40	32	21.9	6	8.5	42	1`0	8.5	8.3

a - Not all elements were analyzed by all laboratories.
b - SD = standard deviation.
c - Results for Se are from two laboratories.

ZINC IN SEDIMENTS
(ICP)

1.0 Scope and Application

Inductively coupled plasma atomic emission spectroscopy (ICP) determines zinc and numerous other elements that are present in solution. In order to apply this technique to sediments, the samples must be digested with nitric acid and hydrogen peroxide prior to analysis.

Detection limits, sensitivity, and optimum concentration ranges for zinc, and other metals stable in a mixed standard solution with zinc, will vary with the sample aliquot size, the spectrophotometer being used, and possible matrix interferences. Based on the estimated detection limit of 2 µg/L for zinc in aqueous samples (Table 1), a sample size of 2 g, and a final digestate volume of 100 mL, the estimated detection limit for sediment samples is 100 µg/kg.

The method of standard addition (MSA) shall be used for the analysis of all sample digests unless either serial dilution or matrix spike addition demonstrates that it is not required (see section 9).

This method should only be used by spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

This procedure is based on SW-846 Methods 3050 (for digestion) and 6010 for quantitation (EPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

A well-mixed, representative 1 to 2 g (wet weight) sample of sediment is digested in nitric acid and hydrogen peroxide. The digestate is then refluxed using hydrochloric acid and diluted to volume.

NOTE: A separate sample shall be dried for a total solids determination in order to express the results on a dry-weight basis.

This instrumental method measures light emitted by zinc in the final sediment digest by optical spectrometry. The digested samples are nebulized and the resulting aerosol is transported to a plasma torch. Zinc-specific atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

The possibility of additional interferences named in section 3.0 should also be recognized and appropriate corrections made when necessary. The procedures that can be used for this purpose are described in section 9.

3.0 Interferences

Sediments represent diverse matrix types and samples from each location may present its own analytical challenge. Spiked samples and any relevant standard reference material should be processed to aid in determining whether the digestion procedure is appropriate for a specific sediment sample or there are matrix or other effects interfering with the analysis of the resultant sediment digestate.

3.1 Spectral Interferences

Spectral interferences are caused by: (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of simultaneous multi-element instruments must verify the absence of spectral interference from an element in a sample for which there is no instrument detection channel. Potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferants can be assumed.

The magnitude of the interference effects summarized in Table 2 are expressed as analyte concentration equivalents (i.e., false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that Zn is to be determined (at 213.856 ηm) in a sample containing approximately 10 mg/L of Cu. According to Table 2, 100 mg/L of Cu would yield a false signal for Zn equivalent to approximately 0.14 mg/L. Therefore, the presence of 10 mg/L of Cu would result in a false signal for Zn equivalent to approximately 0.014 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, and argon flow rate.

The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.2 Physical Interferences

Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, or by using the standard additions method. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Further, it has been reported that better control of the argon flow rate improves instrument performance. Improved argon flow rate control can be accomplished with the use of mass flow controllers.

3.3 Chemical Interferences

Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, etc.), buffering of the sample, matrix matching, and standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Drying oven, capable of maintaining 30° C.
4. Thermometer, 0 to 200° C.
5. Centrifuge and centrifuge tubes.
6. Inductively coupled argon plasma emission spectrometer.
7. Computer-controlled emission spectrometer with background correction.
8. Radio frequency generator.
9. Argon gas supply, welding grade or better.

4.2 Materials

1. Conical Phillips beakers, 250 mL, or equivalent.
2. Watch glasses.
3. Whatman No. 41 filter paper, or equivalent.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.

3. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
4. Hydrogen peroxide, 30% (H_2O_2). Oxidant should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the H_2O_2 is <MDL, then the acid can be used.
5. Nitric acid (1:1). Add 500 mL concentrated HNO_3 to 400 mL Type II water. Dilute to 1 liter with Type II water.
6. Hydrochloric acid (1:1). Add 500 mL concentrated HCl to 400 mL Type II water. Dilute to 1 liter with Type II water.
7. Standard stock solutions. May be purchased or prepared from ultrahigh purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hr at 105° C, unless otherwise specified.

   CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

8. Beryllium standard stock solution (100 µg/mL). Dissolve 1.970 g $BeSO_4 \cdot 4H_2O$ (analytical reagent grade, undried), in 100 mL of Type II water, add 10.0 ml concentrated HNO_3. Dilute to 1 liter with Type II water.
9. Cadmium standard stock solution (100 µg/mL). Dissolve 0.1100 g CdO (analytical reagent grade) in a minimum amount of 1:1 HNO_3. Heat to increase rate of dissolution. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
10. Lead standard stock solution (100 µg/mL). Dissolve 0.1600 g $Pb(NO_3)_2$ (analytical reagent grade) in a minimum amount of 1:1 HNO_3. Add 10 mL 1:1 HNO_3. Add 10 mL 1:1 HNO_3. Dilute to 1 liter with Type II water.
11. Manganese standard stock solution (100 µg/mL). Dissolve 0.1000 g of manganese metal, in an acid mixture of 10 mL concentrated HCl and 1 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
12. Selenium standard stock solution (100 µg/mL). Dissolve 0.1700 g H_2SeO_3 (analytical reagent grade, undried) in 100 mL of Type II water. Dilute to 1 liter with Type II water.
13. Zinc standard stock solution (100 µg/mL). Dissolve 0.1200 g ZnO in a minimum amount of 1:1 HNO_3. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
14. Mixed calibration standard solutions. Care should be taken when preparing mixed standards for ICP analysis to ensure that the elements

in the final mixed standard are compatible and stable together. One set of mixed calibration standards that has been found to be useful is listed in Table 3. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interferences or the presence of impurities.

To prepare the mixed calibration standard for zinc, combine appropriate volumes of the individual stock solutions indicated in Table 3 in volumetric flask. Add 2 mL 1:1 HNO_3 and 10 mL of 1:1 HCl and dilute to 100 mL with Type II water. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see section 9.9) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should be scanned using a sequential spectrometer to verify the absence of interelement spectral interference in the recommended mixed standard solutions.

TABLE 3. MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo, and Si
IV	Al, Ca, Cr, K, Na, and Ni

NOTE: Premixed standard solutions (traceable to NIST) containing the combined elements as listed in solutions I through IV are available from a number of commercial vendors.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection. A holding time of 6 months is generally cited for this parameter. Samples may be stored for a longer period of time by air-drying or freezing. However, these samples should not be used for the preparation of sediment elutriates because the process of freezing and thawing the samples may influence the migration potential of sediment-associated constituents.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of samples to be analyzed for zinc in sediments.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The oven or hot plate should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

Prepare a calibration blank (see section 9.5.1) and at least three mixed calibration standards in the appropriate concentration range to correlate zinc concentrations with the ICP's linear response range. Prepare standards for instrument calibration as instructed in section 5.14. Match the sample matrix and that of the standards as closely as possible.

Calibrate the instrument according to the instrument manufacturer's recommended procedures using typical mixed calibration standard solutions. Flush the system with the calibration blank between each standard. Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.

8.0 Procedure

8.1 Sample Digestion

1. Weigh a 1.00 to 2.00 g aliquot of the homogenized, field-moist sediment to the nearest 0.01 g and quantitatively transfer the sample to a 250 mL conical beaker.
2. Add 10 mL of 1:1 HNO_3, mix the slurry, and cover with a watch glass.
3. Heat the sample to 95° C and reflux for 10 to 15 min without boiling.
4. Allow the sample to cool. Add 5 mL of concentrated HNO_3, replace the watch glass, and reflux for 30 min. Repeat this last step to ensure complete oxidation of the sample. Using a ribbed watch glass, allow the solution to evaporate to 5 mL without boiling, while maintaining a covering of solution over the bottom of the beaker.
5. After the sample has cooled, add 2 mL of Type II water and 3 mL of 30% H_2O_2. Cover the beaker with a watch glass and return the covered beaker to the hot plate for warming and to start the peroxide reaction. Heat until effervescence subsides. Allow the beaker to cool.

   NOTE: Exercise care to ensure that sample is not lost due to excessively vigorous effervescence.

6. 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: The total volume of the 30% H_2O_2 should not exceed 10 mL.

7. Add 5 mL of concentrated HCl and 10 mL of Type II water, return the covered beaker to the hot plate, and reflux for an additional 15 min without boiling. After cooling, dilute to 100 mL with Type II water.
8. Suspended particulates should be removed by filtration, by centrifugation, or by allowing the sample to settle prior to analysis.

   a. Filtration: Filter through Whatman No. 41 filter paper (or equivalent) and dilute to 100 mL with Type II water.

   b. Centrifugation: Centrifugation at 2,000 to 3,000 rpm for 10 min is usually sufficient to clear the supernatant.

9. The diluted sample has an approximate acid concentration of 5.0% (v/v) HCl and 5.0% (v/v) HNO_3. The sample is now ready for analysis.

8.2 Sample Analysis

1. The analyst should follow the instructions provided by the instrument's manufacturer. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 min of operation prior to calibration). For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within instrument linear range where coordination factors are valid. The analyst must: (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.

2. Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5% (or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this conditions.

3. Flush the system with the calibration blank solution for at least 1 min before the analysis of each sample. Analyze samples.

   NOTE: Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for zinc in sediments is 30 mg/kg (dry weight).

The detection limit specified in this method is presented to account for typical sediment background contents of chromium in the Great Lakes.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured arsenic concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards 2704 - Buffalo River sediment and 1646 - Estuarine sediment, should be run to monitor the performance of the ICP and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a routine sediment sample collected and homogenized in bulk that has undergone multiple analyses by the analytical laboratory. Alternately, a LCS can be created by spiking a known quantity of the contaminant(s) of concern into a clean sediment, homogenizing the bulk sample, followed by multiple analyses at the analytical laboratory. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

9.5.1 Calibration Blank

The calibration blank is prepared by diluting 2 mL of 1:1 HNO_3 and of 1:1 HCl to 100 mL with Type II water. Prepare a sufficient quantity to flush the system between standards and samples.

The calibration blank should be analyzed prior to routine sample analysis, after every 10 samples, and at the end of the analytical run. The measured concentration in the calibration blank should be less than or equal to the method detection limit.

9.5.2 Reagent Blank

The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - zinc, to a 1-2 g sample aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

9.8 Interference Check Sample

The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the method detection limit for each element. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

The interference check sample should be analyzed at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Results should be within ± 20% of the known concentration.

9.9 Calibration Control Sample

A calibration control sample should be prepared in the same acid matrix as the calibration standards at 10 times the method detection limit. This sample should be prepared in accordance with the instructions provided by the supplier. The calibration control sample is used to verify the integrity of the calibration standards on a weekly basis.

9.10 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor

negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

9.10.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the instrumental detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.10.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.

4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 µg/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported in µg/kg with up to three significant figures.

The concentrations determined are to be reported on the basis of the actual weight of the sample. If a dry weight analysis is desired, then the percent solids of the sample must also be provided.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Winge, R.K., V.J. Peterson, and V.A. Fassel. 1979. Inductively coupled plasma-atomic emission spectroscopy: Prominent lines, final report, March 1977 - February 1978, Ames laboratory, Ames, IA. EPA-600/4-79-017. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Table 1. Recommended Wavelengths and Estimated Instrumental Detection Limits.

Element	Wavelength[a] (ηm)	Estimated Detection Limit[b] (µg/L)
Beryllium	313.042	0.3
Cadmium	226.502	4
Lead	220.353	42
Manganese	257.610	2
Selenium	196.026	75
Zinc	213.856	2

a - The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see section 3.1).

b - The estimated instrumental detection limits are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

Table 2. Analyte Concentration Equivalents arising from Interference at the 100 mg/L Level.

Analyte	Wavelength (ηm)	Interferant[a,b] Al	Ca	Cr	Cu	Fe	Mg	Mn	Mi	Tl	V
Beryllium	313.042	-	-	-	-	-	-	-	-	0.04	0.05
Cadmium	226.502	-	-	-	-	0.03	-	-	0.02	-	-
Lead	220.353	0.17	-	-	-	-	-	-	-	-	-
Manganese	257.610	0.005	-	0.01	-	0.002	0.002	-	-	-	-
Selenium	196.026	0.23	-	-	-	0.09	-	-	-	-	-
Zinc	213.856	-	-	-	0.14	-	-	-	0.29	-	-

a - Dashes indicate that no interference was observed even when interferants were introduced at the following levels:

Al - 1000 mg/L,
Ca - 1000 mg/L,
Cr - 200 mg/L,
Cu - 200 mg/L,
Fe - 1000 mg/L.
Mg - 1000 mg/L,
Mn - 200 mg/L,
Tl - 200 mg/L,
V - 200 mg/L, and

b - The figures recorded as analyte concentrations are not the actual observed concentrations. To obtain those figures, add the listed concentration to the interferant figure.

Table 4. ICP Precision and Accuracy Data[a]

Element	Sample No. 1 True Value (µg/L)	Sample No. 1 Mean Reported Value (µg/L)	Sample No. 1 Mean SD[b] (%)	Sample No. 2 True Value (µg/L)	Sample No. 2 Mean Reported Value (µg/L)	Sample No. 2 Mean SD[b] (%)	Sample No. 3 True Value (µg/L)	Sample No. 3 Mean Reported Value (µg/L)	Sample No. 3 Mean SD[b] (%)
Be	750	733	6.2	20	20	9.8	180	176	5.2
Mn	350	345	2.7	15	15	6.7	100	99	3.3
Cd	50	48	12	2.5	2.9	16	14	13	16
Pb	250	236	16	24	30	32	80	80	14
Zn	200	201	5.6	16	19	45	80	82	9.4
Se[c]	40	32	21.9	6	8.5	42	1`0	8.5	8.3

a - Not all elements were analyzed by all laboratories.
b - SD = standard deviation.
c - Results for Se are from two laboratories.

TOTAL ORGANIC CARBON IN SEDIMENTS

1.0 Scope and Application

This method is applicable to the determination of total organic carbon (TOC) content in sediment samples. TOC is often used as a surrogate indicator for the presence of organic pollutants.

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Inorganic carbonates are removed from a well mixed aliquot of sediment by acidification with phosphoric acid. After carbonate removal, the sample is dried and ground to pass through a 80-mesh sieve or finer. An aliquot is then oxidized at temperatures greater than 1,000° C with catalysts as specified by the instrument manufacturer. The evolved CO_2 is determined by thermal conductivity (TC) or infrared (IR) spectroscopy.

3.0 Interferences

Non-representative particulates such as leaves, sticks, fish and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result.

Carbonate and bicarbonate carbon represent an interference and must therefore, be removed prior to sample analysis. Incomplete removal of carbonate or bicarbonates will lead to total organic carbon contents that are biased high.

Removal of inorganic carbonates by acidification may lead to the loss of volatile organic compounds/substances from the sample. Additionally, drying the sample may lead to the loss of volatile organic compounds/substances.

Ambient CO_2 not associated with the sample present possible gaseous interferences. Care must be taken with the blank to hold CO_2 below the method detection limit. The use of high purity carrier gas or helium helps reduce CO_2.

Sediment residue can accumulate at the top of the combustion column. The column should be cleaned if sufficient residue accumulates to affect analytical results.

4.0 Apparatus and Materials

4.1 Apparatus

NOTE: This list is generic for total carbon analysis. The specific requirements will vary with the instrument. Some additional apparatus may be required; other equipment may not be needed.

1. Analytical balance, capable of weighing to ± 0.001 mg (± 1 µg).
2. Analytical balance calibration weights, 3-5 weights covering expected weight range.
3. Brass sieve, 80-mesh or finer.
4. Carbon analyzer with infrared detector.
5. Convection oven.
6. Desiccator and desiccant.
7. Mortar and pestle, agate or porcelain.
8. Thermometer, 0 to 200° C range.

4.2 Materials

1. Absorbents (as needed).
2. Carrier gases with in-line filter (as needed).
3. Catalysts and combustion accelerators (as needed).
4. Combustion vehicles. Vials, crucibles, boats, or tin sample capsules.
5. Evaporating dishes, porcelain, 90 mm, 100 mL capacity. (aluminum, Vycor, or platinum weighing dishes may be substituted and smaller size dishes may be used, if required.)
6. Oxygen with in-line filter (high purity; >99.5% @ 30 psi).

5.0 Reagents and Standards

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated phosphoric acid, reagent grade (H_3PO_4).

3. Phosphoric acid, 10%. Add 100 mL concentrated H_3PO_4 to 700 mL Type II water. Dilute to 1 liter with Type II water.

6.0 Sample Handling and Preservation

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection. A holding time of 28 days is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C) to minimize decomposition of organics between sample collection and sample analysis.

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of samples to be analyzed for total organic carbon in sediments.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically to ensure that they are measuring temperature accurately.

The oven should be monitored to ensure that temperature fluctuation does not exceed ± 5° C.

Follow the instrument manufacturer's instructions regarding calibration and standardization. In general, the instrument should be calibrated at least once per day or once per batch of samples, whichever is more frequent. Use either NIST reference materials or standards supplied by the manufacturer and approved by the laboratory or QA manager. The concentration range of the standards must be representative of the C concentrations in the sediment samples.

In general, the calibration procedure for carbon analyzers is as follows:

a. Analyze 3 blank samples to determine instrument stability.
b. If a stable baseline is obtained, run 3 to 5 conditioning analyses.

   NOTE: Acetonitrile (C_2H_3N) is commonly used as the conditioner sample.

c. Analyze 3 to 5 samples of a known standard.
d. Analyze a blank sample to check for analyte memory effects.

8.0 Procedure

8.1 Sample Preparation

1. Weigh a 5 g aliquot of the homogenized, field-moist sediment to the nearest gram and transfer the sample to a 100 mL evaporating dish.
2. Dry the sample overnight at 60° C.
3. Break up dried sediment pellet with a spatula and add several drops of 10% phosphoric acid.
4. Continue adding 10% phosphoric acid until all effervescence is completed.

   NOTE: Do not add too much 10% phosphoric acid in any given increment since this may cause loss of sample due to frothing.

5. Add 2 mL of 10% phosphoric acid, stir the sample, and allow to sit covered for 4 hours.

   NOTE: The sample should be stirred every hour.

6. Dry the sample overnight at 60° C.
7. Using a mortar and pestle, grind sample such that the whole sample passes through a 80-mesh sieve or finer.
8. Store ground sample in desiccator until ready for analysis.

8.2 Sample Analysis

1. Weigh approximately 100 mg of dried homogenized sediment into an appropriate tared combustion vehicle. Record weight of sample.

   NOTE: If high organic carbon contents are suspected for a given sample, sample size may have to be reduced to approximately 20 mg of dried sediment.

2. Perform analysis as recommended by instrument manufacturer.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for total organic carbon in sediments is 0.1%.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured total organic carbon concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards 2704 - Buffalo River sediment and 1646 - Estuarine sediment, should be run to monitor the performance of the carbon analyzer and to assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a routine sediment sample collected and homogenized in bulk that has undergone multiple analyses by the analytical laboratory. Alternately, a LCS can be created by spiking a known quantity of the contaminant(s) of concern into a clean sediment, homogenizing the bulk sample, followed by multiple analyses at the analytical laboratory. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

A minimum of one reagent blank (an empty combustion vehicle containing any combustion catalysts/accelerators used during routine sample analysis) per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

10.0 Method Performance

In a study involving a quality control check sample for soils, for 41 observations, the mean, standard deviation, and coefficient of variation for total carbon content was 11.38, 0.062, and 5.5%, respectively (USDA-SCS, 1992).

11.0 Calculations and Reporting

Calculations should be performed following instrument manufacturer's instructions.

Total organic carbon should be reported on a weight percent basis.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. Department of Agriculture - Soil Conservation Service. 1992. Soil Survey Laboratory Methods Manual. Soil Survey Investigations Report No. 42. Version 2.0. National Soil Survey Laboratory, Lincoln, NE.

TOTAL PETROLEUM HYDROCARBONS IN SEDIMENTS (SPECTROPHOTOMETRIC, INFRARED)

1.0 Scope and Application

This method is appropriate for the determination of fluorocarbon-113 extractable petroleum hydrocarbons from sediment samples. It should be noted that this method will change upon identification and approval of an environmentally friendly solvent.

This method can be used when relatively polar, heavy petroleum fractions are present, or when the levels of non-volatile greases challenge the solubility limit of the solvent.

The method is not recommended for measurement of low-boiling fractions that volatilize at temperatures below 70° C.

This method is based on a combination of EPA SW-846 Method 9071 (USEPA, 1986) and EPA Method 418.1 (USEPA, 1983).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

A 20 g sample of wet sediment, with a known dry-solids content, is acidified to pH 2 with hydrochloric acid. Magnesium sulfate monohydrate, which will combine with 75% of its own weight in water, is then added to dry the sample.

After drying, petroleum hydrocarbons are extracted from the sample using Fluorocarbon 113. Infrared analysis of the extract at 2930 cm^{-1} is performed and total petroleum hydrocarbon contents are determined by direct comparison with standards.

3.0 Interferences

Total petroleum hydrocarbons (TPHs) are operationally defined by the extraction procedure and the analytical technique.

The method is not considered applicable to light hydrocarbons that volatilize below 70° C. Also, some crude oils and heavy fuel oils that are not soluble in fluorocarbon-113 will have low recoveries.

The rate and time of extraction in the Soxhlet apparatus should be strictly controlled because of varying solubilities of different greases.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Infrared spectrophotometer, scanning or fixed wavelength, for measurement around 2930 cm^{-1}.
4. Magnetic stirrer, with Teflon coated stirring bars.
5. Mortar and pestle.
6. Soxhlet extraction apparatus.
7. Vacuum pump or other source of vacuum.

4.2 Materials

1. Separatory funnel with Teflon stopcock, 2000 mL.
2. Beakers, glass, 150 mL.
3. Cells, 10 mm, 50 mm, and 100 mm pathlength, sodium chloride or infrared grade glass.
4. Extraction thimbles, paper.
5. Glass bottles with stoppers, 50 mL.
6. Glass wool or beads.
7. Volumetric flasks, class A, 200 mL.
8. Volumetric flasks, class A, 100 mL.
9. Whatman filter paper No. 40, 11 cm.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.

2. Fluorocarbon-113 (1,1,2-trichloro-1,2,2-trifluoroethane), boiling point 47° C.

   NOTE: The solvent should leave no measurable residue on evaporation. Redistill if necessary.

3. Concentrated hydrochloric acid (HCl), reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
4. Hydrochloric acid (HCl), 1:1. Mix equal volumes of concentrated HCl and Type II water.
5. Magnesium sulfate monohydrate ($MgSO_4 \cdot H_2O$). Prepare $MgSO_4 \cdot H_2O$ by spreading a thin layer in a dish and drying in an oven at 150° C overnight.
6. 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.
7. Sodium sulfate (Na_2SO_4), anhydrous crystal.
8. Reference oil used for calibration mixtures. Pipet 15.0 mL n-hexadecane ($C_{16}H_{34}$), 15.0 mL isooctane (C_8H_{18}), and 10.0 mL chlorobenzene (C_6H_5Cl) into a 50 mL glass-stoppered bottle. Maintain the integrity of the mixture by keeping stoppered except when withdrawing aliquots.
9. Reference oil stock solution. Pipet 1.0 mL reference oil into a tared 200 mL volumetric flask and immediately stopper. Weigh and dilute to volume with fluorocarbon-113.
10. Reference oil working standards. Pipet appropriate volumes of stock standard into 100 mL volumetric flasks according to the cell pathlength to be used. Dilute to volume with fluorocarbon-113. Calculate concentration of standards from the stock standard.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

It is recommended that only field-moist samples be used in the TPH analysis.

A holding time of 28 days after sample collection is generally cited for this parameter.

The sample should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Sample aliquots to be analyzed for TPH should be collected and stored in glass bottles.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Select appropriate working standards and cell pathlength based on the expected total petroleum hydrocarbon concentration in the final sediment extract. The following information is presented as a guide for selecting a suitable cell pathlength:

Pathlength	Range
10 mm	2-40 mg
50 mm	0.5-8 mg
100 mm	0.1-4 mg

Calibrate the instrument for the appropriate cells using a series of working standards. It is not necessary to add silica gel to the standards.

Scan the standards from 3200 to 2700 cm^{-1} using a scanning infrared spectrophotometer. Fluorocarbon-113 should be used in the reference beam of a dual beam instrument or to zero a single beam instrument. The absorbance of the 2930 cm^{-1} peak should be used to construct a standard curve.

8.0 Procedure

1. Weigh out 20 ± 0.5 g of the homogenized, field-moist sediment and place in a 150 mL beaker.

   NOTE: The dry-solids content of the sediment should be determined on a separate sample aliquot.

2. Acidify the sample to pH 2 with approximately 0.3 mL concentrated HCl.
3. Add 25 g prepared $MgSO_4 \cdot H_2O$ to the acidified sample and stir to create a smooth paste.
4. Spread the paste on the sides of the beaker to facilitate drying. Let the paste stand 15-30 minutes or until the material has solidified.
5. Transfer the solids to a mortar and grind to a fine powder.
6. Add the powder to a paper extraction thimble.
7. Wipe both the beaker and the mortar with pieces of filter paper moistened with solvent and add the paper to the thimble.
8. Fill the thimble with glass wool (or glass beads).
9. Place the thimble in a Soxhlet apparatus and extract using fluorocarbon-113 at a rate of 20 cycles/hour for 4 hours.
10. Using grease-free cotton, filter the extract into a volumetric flask. Dilute to volume with fluorocarbon-113.

    NOTE: If the final filtrate is turbid, refilter into a clean flask.

    NOTE: If an emulsion forms, it can be broken by filtering the extract through 1 g sodium sulfate in a filter paper cone. Additional 1 g portions of sodium sulfate can be used as required.

11. Discard about 5-10 mL solution from the volumetric flask. Add 3 g silica gel and a stirring bar.
12. Stopper the volumetric flask and stir the solution for a minimum of 5 min on a magnetic stirrer.
13. After the silica gel has settled in the sample extract, fill a clean cell with solution and determine the absorbance of the extract.

    NOTE: If the absorbance exceeds 0.8, prepare an appropriate dilution and reanalyze the sample.

NOTE: The possibility that the absorptive capacity of the silica gel has been exceeded can be tested at this point by adding another 3.0 g silica gel to the extract and repeating the treatment and determination.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for TPH in sediments is 5000 µg/kg.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured TPH concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a routine sediment sample collected and homogenized in bulk that has undergone multiple analyses by the analytical laboratory. Alternately, a LCS can be created by spiking a known quantity of the contaminant(s) of concern into a clean sediment, homogenizing the bulk sample, followed by multiple analyses at the analytical laboratory. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the instrument detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte (i.e., reference oil), in this case - TPH, to the 20 g aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

10.0 Method Performance

The analysis of six replicate sludge samples extracted with this method and analyzed in a single laboratory produced a standard deviation of 4.6%.

11.0 Calculations and Reporting

Determine the concentration of petroleum hydrocarbons in the extract by comparing the response against the calibration plot. The concentration of total petroleum hydrocarbons in the original sediment sample can then be calculated as follows:

$$\text{TPH, mg/kg (wet weight)} = \frac{X \times Y \times 1000}{g}$$

where:
X = the concentration of total petroleum hydrocarbons in the final sediment extract, mg/L.
Y = volume of final sediment extract, L.
g = wet weight of sediment extracted, g.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Blum, K.A. and M.J. Taras. 1968. Determination of Emulsifying Oil in Industrial Wastewater" JWPCF Research Supplement 40:R404.

U.S. Environmental Protection Agency. 1983. Methods for the Chemical Analysis of Water and Wastes. EPA-600/4-79-020. U.S. Environmental Protection Agency Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

U.S. Environmental Protection Agency. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

PHENOLICS IN SEDIMENTS
(COLORIMETRIC, AUTOMATED 4-AAP)

1.0 Scope and Application

This method is applicable to the determination of phenolic compounds in sediment samples. The minimum detectable concentration of phenol in the final sediment distillate is 2 µg/L and the working range of the method is 2 to 500 µg/L when using phenol as a standard. The useful range of the procedure can be extended by modifying the sample size or diluting the final sediment distillate prior to analysis.

This method is based on SW-846 Method 9066 (EPA, 1986).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Phenolic compounds are separated from the sediment sample matrix by distillation under acidic conditions (pH <4.0). The phenolic compounds in the distillate are then reacted with alkaline ferricyanide ($K_3Fe(CN)_6$) and 4-amino-antipyrine (4-AAP) to form a red complex which is measured at 505 or 520 ηm.

3.0 Interferences

Color and turbidity in the original sample can interfere with this colorimetric procedure. Color interference is eliminated by distilling the phenolic compounds from the original sample prior to analysis. Turbidity is removed by sample filtration prior to analysis.

Oxidizing agents, such as chlorine, detected by the liberation of iodine upon acidification in the presence of potassium iodide, are removed immediately after sampling by the addition of an excess of ferrous ammonium sulfate (see section 5.0, item 3). If chlorine is not removed, the phenolic compounds may be partially oxidized and the sample results may be biased low.

Background contamination from plastic tubing and sample containers is eliminated by filling the wash receptacle by siphon (using Kel-F tubing) and using glass tubes for the samples and standards.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Distillation apparatus, all glass, consisting of a 1 liter pyrex distillation flask and a Graham condenser.
4. pH meter.
5. Automated continuous-flow analytical instrument with:
   a. sampler equipped with continuous mixer,
   b. manifold,
   c. proportioning pump II or III,
   d. heating bath with distillation coil,
   e. distillation head,
   f. colorimeter equipped with a 50 mm flowcell and 505 or 520 ηm filter, and
   g. recorder.

4.2 Materials

1. Volumetric flasks, class A, 1 L.
2. Volumetric flasks, class A, 100 mL.
3. Whatman filter paper no. 12.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. 4-Aminoantipyrine (4-AAP). Dissolve 0.65 g of 4-aminoantipyrine in 800 mL of Type II water. Dilute to 1 liter with Type II water. Prepare fresh daily.
3. Ferrous ammonium sulfate ($FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$. Dissolve 1.1 g ferrous ammonium sulfate in 500 mL of Type II water containing 1 mL

concentrated H_2SO_4. Dilute to 1 liter with freshly boiled and cooled Type II water.

4. Buffered potassium ferricyanide ($K_3Fe(CN)_6$). Dissolve 2.0 g potassium ferricyanide ($K_3Fe(CN)_6$), 3.1 g boric acid (H_3BO_3), and 3.75 g potassium chloride (KCl) in 800 mL of Type II water. Adjust to pH of 10.3 with 1 N sodium hydroxide. Dilute to 1 liter with Type II water. Add 0.5 mL of Brij-35 (available from Technicon). Prepare fresh weekly.

   NOTE: Brij-35 is a wetting agent and is a proprietary Technicon product.

5. Sodium hydroxide (NaOH), 1 N. Dissolve 40 g NaOH in 500 mL of Type II water. Cool. Dilute to 1 liter with Type II water.
6. Concentrated sulfuric acid (H_2SO_4), reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
7. Sulfuric acid (H_2SO_4), 1 N. Add 28 mL concentrated H_2SO_4 to 900 mL of Type II water. Dilute to 1 liter with Type II water.
8. Phenol stock solution. Dissolve 1.00 g phenol (C_6H_5OH) in 500 mL of Type II water. Dilute to 1 liter with Type II water. Add 0.5 mL concentrated H_2SO_4 as preservative (1.0 mg/mL phenol).

   CAUTION: This solution is toxic.

9. Phenol standard solution A. Dilute 10.0 mL of phenol stock solution to 1 liter with Type II water (0.01 mg/mL phenol).
10. Phenol standard solution B. Dilute 100.0 mL of phenol standard solution A to 1 liter with Type II water (0.001 mg/mL phenol).
11. Phenol standard solution C. Dilute 100.00 mL of phenol standard solution B to 1 liter with Type II water (0.0001 mg/mL phenol).

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

It is recommended that only field-moist samples be used in the phenol analysis. This recommendation is based on the fact that dried samples may lose phenol by biological degradation. Also, both air-dried and frozen samples may lose phenol during the drying and/or freezing cycles.

A holding time of 28 days after sample collection is generally cited for this parameter.

The sample should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Sample aliquots to be analyzed for phenolic compounds should be collected and stored in glass bottles.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Calibration curves must be composed of a minimum of a blank and three standards. A separate calibration curve should be prepared for every hour of continuous sample analysis.

Using standard solution A, B, or C, prepare the following standards in 100 mL volumetric flasks:

Standard Solution (mL)	Concentration (µg/L)
Solution C	
1.0	1.0
2.0	2.0
3.0	3.0
5.0	5.0
Solution B	
1.0	10.0
2.0	20.0
5.0	50.0
10.0	100.0
Solution A	
2.0	200.0
3.0	300.0
5.0	500.0

Each standard should be preserved by adding 2 drops of concentrated H_2SO_4 to 100.0 mL.

Prepare a linear standard curve by plotting peak heights of standards against concentration values.

8.0 Procedure

1. Place 550 mL of Type II water into a 1-L pyrex distillation flask.
2. Quantitatively transfer a 10 to 50 g aliquot of the sample to be analyzed to the distillation flask.

   NOTE: The amount of sediment used should not contain more than 50 µg phenolic compounds.

3. Adjust the pH of the sample to approximately 4 with the addition of 1 N sulfuric acid.
4. Add a few boiling stones.
5. Attach the condenser and distill over 500 mL of distillate.

   NOTE: If the sample distillate is turbid, it should be filtered through a prewashed membrane filter prior to analysis.

   NOTE: If oil is present in the final distillate, filter the sample through two thicknesses of dry No. 12 Whatman filter paper to remove the oil.

6. Set up a AutoAnalyzer manifold with the following flow rates:

Air	0.32 mL/min.
Sample	2.00 mL/min.
Distilling solution	0.42 mL/min.
Waste from still	0.42 mL/min.
Air	0.32 mL/min.
Resample waste	1.00 mL/min.
Resample	1.2 mL/min.
4-AAP	0.23 mL/min.
Buffered potassium ferricyanide	0.23 mL/min.
Waste from F/C	1.0 mL/min.

7. Fill the wash receptacle by siphon. Use Kel-F tubing with a fast flow (1 liter/hr).
8. Allow colorimeter and recorder to warm up for 30 min.
9. Run a baseline with all reagents feeding Type II water through the sample line.

   NOTE: Use polyethylene tubing for sample line.

   NOTE: When new tubing is used, about 2 hours may be required to flush residual phenol from the tubing and obtain a stable baseline.

10. Place appropriate standards in the sampler in order of decreasing concentration.
11. Complete loading of the sampler tray with unknown and quality assurance/quality control samples in glass tubes.
12. Run with sensitivity setting at full scale or 500.
13. When the baseline becomes steady, switch sample from Type II water to samples and begin analysis.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for phenolics in sediments is 1000 µg/kg.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured phenolic concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per

analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a routine sediment sample collected and homogenized in bulk that has undergone multiple analyses by the analytical laboratory. Alternately, a LCS can be created by spiking a known quantity of the contaminant(s) of concern into a clean sediment, homogenizing the bulk sample, followed by multiple analyses at the analytical laboratory. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the instrument detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - phenolics, to the 10 to 50 g aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision

between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

10.0 Method Performance

Precision and accuracy information are not available at this time.

11.0 Calculations and Reporting

The concentration of phenolic compounds in the original sediment sample can be calculated as follows:

$$\text{Phenol, µg/kg (wet weight)} = \frac{A \times B \times 1000}{g}$$

where:
A = phenol concentration in distillate, µg/L
B = total volume of final distillate, L (0.5 L as written)
g = wet weight of sediment sample, g.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. Method 510. 14th Edition, APHA, New York, New York. p. 574.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Gales, M.E., and R. L. Booth. 1976. Automated 4-AAP Phenolic Method. AWWA 68:540.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

TOTAL POLYCHLORINATED BIPHENYLS (PCBs) AND PESTICIDES IN SEDIMENTS

1.0 Scope and Application

This method is suitable for the determination of chlorinated pesticides and PCB congeners in sediment samples. Table 1 presents the PCB congeners most commonly found in the environment while Table 2 list the pesticides of concern in the Great Lakes. All these compounds may be determined using this method.

This procedure is based on a National Oceanic and Atmospheric Administration (NOAA) method for the determination of Extractable Toxic Organic Compounds in marine sediments (NOAA, 1985).

The extracts produced from this method (sections 8.1 through 8.6) can be used in the determination of PCBs, pesticides, and polynuclear aromatic hydrocarbons (PAHs).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

The sample is extracted with methylene chloride (CH_2Cl_2) and sodium sulfate (Na_2SO_4). The resultant extract is cleaned-up with silica gel and alumina. Additional clean-up steps to remove biological macromolecules are performed using Sephadex LH-20. PCB congeners and pesticides are then quantified using a glass capillary column to resolve all congeners and gas chromatography/electron capture detector (GC/ECD). The concentrations of 20 congeners (Table 3) will be summed to determine the total PCB content in the sediment.

The same extract used to analyze for PCBs and pesticides can be used to analyze polynuclear aromatic hydrocarbons (PAHs) using gas chromatography/mass spectrometry (GC/MS). The method for PAH determination is provided in this methods manual.

Table 1. PCB Congeners Commonly Identified in the Great Lakes.

BZ#	Structure	BZ#	Structure
1	2-chlorobiphenyl	105	2,3,3',4,4'-pentachlorobiphenyl
3	4-chlorobiphenyl	107	2,3,3',4',5-pentachlorobiphenyl
4	2,2'-dichlorobiphenyl	115	2,3,4,4',6-pentachlorobiphenyl
5	2,3-dichlorobiphenyl	119	2,3',4,4',6-pentachlorobiphenyl
6	2,3'-dichlorobiphenyl	122	2',3,3',4,5-pentachlorobiphenyl
9	2,5-dichlorobiphenyl	123	2',3,4,4',5-pentachlorobiphenyl
12	3,4-dichlorobiphenyl	128	2,2',3,3',4,4'-hexachlorobiphenyl
15	4,4'-dichlorobiphenyl	129	2,2',3,3',4,5-hexachlorobiphenyl
16	2,2',3-trichlorobiphenyl	136	2,2',3,3',6,6'-hexachlorobiphenyl
18	2,2',5-trichlorobiphenyl	137	2,2',3,4,4',5-hexachlorobiphenyl
19	2,2',6-trichlorobiphenyl	138	2,2',3,4,4',5'-hexachlorobiphenyl
22	2,3,4'-trichlorobiphenyl	141	2,2',3,4,5,5'-hexachlorobiphenyl
25	2,3',4-trichlorobiphenyl	149	2,2',3,4',5',6-hexachlorobiphenyl
26	2,3',5-trichlorobiphenyl	151	2,2',3,5,5',6-hexachlorobiphenyl
27	2,3',6-trichlorobiphenyl	153	2,2',4,4',5,5'-hexachlorobiphenyl
28	2,4,4'-trichlorobiphenyl	157	2,3,3',4,4',5'-hexachlorobiphenyl
29	2,4,5-trichlorobiphenyl	158	2,3,3',4,4',6-hexachlorobiphenyl
31	2,4',5-trichlorobiphenyl	167	2,3',4,4',5,5'-hexachlorobiphenyl
37	3,4,4'-trichlorobiphenyl	170	2,2',3,3',4,4',5-heptachlorobiphenyl
40	2,2',3,3'-tetrachlorobiphenyl	171	2,2',3,3',4,4',6-heptachlorobiphenyl
41	2,2',3,4-tetrachlorobiphenyl	177	2,2',3,3',4,5,6-heptachlorobiphenyl
44	2,2',3,5'-tetrachlorobiphenyl	180	2,2',3,4,4',5,5'-heptachlorobiphenyl
47	2,2',4,4'-tetrachlorobiphenyl	183	2,2',3,4,4',5',6-heptachlorobiphenyl
49	2,2',4,5'-tetrachlorobiphenyl	185	2,2',3,4,5,5,6'-heptachlorobiphenyl
52	2,2',5,5'-tetrachlorobiphenyl	187	2,2',3,4',5,5',6-heptachlorobiphenyl
53	2,2',5,6'-tetrachlorobiphenyl	189	2,3,3',4,4',5,5'-heptachlorobiphenyl
56	2,3,3',4'-tetrachlorobiphenyl	190	2,3,3',4,4',5,6-heptachlorobiphenyl
66	2,3,4,4'-tetrachlorobiphenyl	191	2,3,3',4,4',5',6-heptachlorobiphenyl
70	2,3',4',5-tetrachlorobiphenyl	193	2,3,3',4',5,5',6-heptachlorobiphenyl
75	2,4,4',6-tetrachlorobiphenyl	194	2,2',3,3',4,4',5,5'-octachlorobiphenyl
77	3,3',4,4'-tetrachlorobiphenyl	195	2,2',3,3',4,4',5,6-octachlorobiphenyl
82	2,2',3,3',4-pentachlorobiphenyl	196	2,2',3,3',4,4',5',6-octachlorobiphenyl
83	2,2',3,3',5-pentachlorobiphenyl	198	2,2',3,3',4,5,5',6-octachlorobiphenyl
84	2,2',3,3',6-pentachlorobiphenyl	199	2,2',3,3',4,5,6,6'-octachlorobiphenyl
85	2,2',3,4,4'-pentachlorobiphenyl	200	2,2',3,3',4,5',6,6'-octachlorobiphenyl
87	2,2',3,4,5'-pentachlorobiphenyl	201	2,2',3,3',4',5,5',6-octachlorobiphenyl
91	2,2',3,4',6-pentachlorobiphenyl	202	2,2',3,3',5,5',6,6'-octachlorobiphenyl
92	2,2',3,5,5'-pentachlorobiphenyl	205	2,3,3',4,4',5,5',6-octachlorobiphenyl
95	2,2',3,5',6-pentachlorobiphenyl	206	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl
97	2,2',3',4,5-pentachlorobiphenyl	207	2,2',3,3',4,4',5,6,6'-nonachlorobiphenyl
99	2,2',4,4',5-pentachlorobiphenyl	208	2,2',3,3',4,5,5',6,6'-nonachlorobiphenyl
101	2,2',4,5,5'-pentachlorobiphenyl		

Table 2. Pesticides of Concern in the Great Lakes.

aldrin	trans-nonachlor
α-chlordane	o,p'-DDE
dieldrin	p,p'-DDE
heptachlor	o,p'-DDD
heptachlor epoxide	p,p'-DDD
hexachlorobenzene	o,p'-DDT
lindane (γ-BHC)	p,p'-DDT
mirex	

Table 3. Twenty PCB Congeners to be Summed to Determine Total PCB Content[a].

BZ#	Structure	BZ#	Structure
8	2,4'-dichlorobiphenyl	126	3,3',4,4',5-pentachlorobiphenyl
18	2,2',5-trichlorobiphenyl	128	2,2',3,3',4,4'-hexachlorobiphenyl
28	2,4,4'-trichlorobiphenyl	138	2,2',3,4,4',5'-hexachlorobiphenyl
44	2,2',3,5'-tetrachlorobiphenyl	153	2,2',4,4',5,5'-hexachlorobiphenyl
52	2,2',5,5'-tetrachlorobiphenyl	169	3,3',4,4',5,5'-hexachlorobiphenyl
66	2,3,4,4'-tetrachlorobiphenyl	170	2,2',3,3',4,4',5-heptachlorobiphenyl
77	3,3',4,4'-tetrachlorobiphenyl	180	2,2',3,4,4',5,5'-heptachlorobiphenyl
101	2,2',4,5,5'-pentachlorobiphenyl	187	2,2',3,4',5,5',6-heptachlorobiphenyl
105	2,3,3',4,4'-pentachlorobiphenyl	206	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl
118	2,3',4,4',5-pentachlorobiphenyl	209	2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl

a = The selected congeners are a combination of those presented in the *Inland Testing Manual* (USEPA/USACE, 1998) and NOAA method (NOAA, 1985).

3.0 Interferences

Interferences by phthalate esters can pose a major problem in pesticide determinations when using the electron capture detector. These compounds generally appear in the chromatogram as large late-eluting peaks. Common flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalates can best be minimized by avoiding contact with any plastic materials. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination.

Elemental sulfur contained in some bottom sediment extracts is also a major interference. This method removes S by the addition of elemental copper.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.001 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Centrifuge, capable of holding 250 mL centrifuge tubes and maintaining speeds of 1500 rpm.
4. Desiccator and desiccant. Desiccants generally used include: anhydrous calcium sulfate, silica gel, or phosphorus pentoxide. Indicating desiccants are preferable since they show when the desiccant needs to be changed or regenerated.
5. Gas chromatograph (GC) including:
   a. dual capillary column inlet system,
   b. autosampler,
   c. cartridge tape unit, and
   d. electron capture detector (ECD), two are needed.
6. Modified Kontes tube heater (block contains: Al inserts fitted to the 0.7 mL line of the tube tip and an Al-foil shroud.
7. Molecular sieve traps (for gas cylinder)

   NOTE: One suggested source for the molecular sieve traps is Hydro-Purge model ASC-l, Coast Engineering Laboratory, Gardena, California.

8. Oxygen traps.
9. UV light source.
10. Water bath, capable of maintaining a temperature of 80 ± 2° C.

4.2 Materials

1. Beakers, 250 mL, or equivalent.
2. Centrifuge tubes, 250 mL, amber, with Teflon™ caps.
3. Chromatography column with reservoir 250 mL, 19 mm ID, 30 cm.

4. Erlenmeyer flask, 500 mL, with stopper.
5. Erlenmeyer flask, 1 L, with stopper.
6. Funnel, curved-stem (curve must be glassblown).
7. Funnel, 200 mm OD, long-stem.
8. Funnel, powder.
9. GC column, silicon-coated fused silica capillary, DB-5, 30 m x 0.25 mm I.D.
10. GC column, silicon-coated fused silica capillary, DB-17HT, 30 m × 0.25 mm I.D.
11. Graduated cylinder, 500 mL.
12. Graduated cylinder, 100 mL.
13. Graduated cylinder, 50 mL.
14. Kontes concentrator tube, 25 mL, with stopper.
15. Snyder column, 3-ball.
16. Syringe, 2000 µL.
17. Syringe, 800 µL.
18. Syringe, 400 µL.
19. Syringe, 200 µL.
20. Syringe, 100 µL.
21. Syringe, 50 µL.
22. Syringe, 10 µL.
23. Teflon wash-bottle, 500 mL (to be filled with CH_2Cl_2).
24. Transfer pipets (Pasteur style) with rubber bulbs.
26. GC vials, 2 mL.
27. GC vials, 100 µL, conical.
28. Volumetric flask, class A, 10 mL.
29. Volumetric pipet, 50 mL.

5.0 Reagents

1. Alumina, 80-200 mesh. Alumina should be activated at 120° C for 2 hr and then cooled to room temperature in a desiccator just before weighing and use.
2. Azulene, reagent grade ($C_{15}H_{18}$).
3. Copper, reagent grade, fine granular. Copper should be activated < 1 hr before use. To activate copper, cover with concentrated. HCl and stir with a glass rod. Allow to stand for 5 min followed by washing twice with CH_3OH and then 3 times with CH_2Cl_2. Leave copper covered with CH_2Cl_2 to avoid contact with air.
4. Helium, grade 4.5 (purified, ≥99.995 %).

5. Hexane, high purity (C_6H_{14}). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
6. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
7. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
8. Methanol, high purity (CH_3OH). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
9. Methylene chloride (dichloromethane), high purity (CH_2Cl_2). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
10. Pentane, high purity (C_5H_{12}). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
11. Perylene, reagent grade ($C_{20}H_{12}$).
12. Sand, Ottawa, MCB, kiln-dried, 30-40 mesh. Sand should be acid-washed (steeped in *aqua regia* (ACS grades $HN0_3$:HCl, 1:3, v:v) overnight, then washed three times each with H_2O, CH_3OH and CH_2Cl_2, dried, and stored at 120° C.
13. Sephadex LH-20, size-exclusion gel. Sephadex LH-20 should be swelled overnight in 6:4:3 solvent.
14. Silica gel, Davison Type 923 or Amicon No. 84080. Silica should be activated at 700° C for 18 hr, stored at 170° C, and cooled to room temperature in a desiccator just before weighing and use.
15. Sodium sulfate, reagent grade, anhydrous granular (Na_2S0_4). Sodium sulfate should be CH_2Cl_2 washed, dried, stored at 120° C, and cooled to room temperature in a desiccator before weighing and use.
16. PCB/pesticide standard stock solution (100 µg/mL). From commercially available neat PCB and pesticide standards, weigh

1.00 mg of each congener and pesticide and dissolve 5 mL hexane. Dilute to 10.0 mL with hexane.

NOTE: PCB congener standards may also be purchased commercially at concentrations of 100 µg/mL.

17. PCB/pesticide primary dilution standard solution (1 µg/mL). Accurately measure a 100 µL aliquot of the PCB and pesticide standard stock solution and dilute to 10.0 mL of hexane.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection. A holding time of 7 days until extraction and 40 days from extraction to analysis is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

NOTE: Samples can be frozen to extend the holding time for up to 1 year.

All sample containers must be prewashed with detergents, acids, and Type II water. Glass containers should be used for the storage of samples to be analyzed for PCBs in sediments. All glassware and materials contacting the solvents should be washed with CH_2Cl_2 three times prior to use.

An option to the CH_2Cl_2 washing of the glassware is to combust the glassware in a muffle oven at 400° C for 4 hours.

7.0 Calibration and Standardization

7.1 General

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The water bath and Kontes tube heater should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

7.2 Sephadex LH-20 Column Calibration

Information on preparing the Sephadex LH-20 column is presented in Attachment A.

1. Add enough azulene (approximately 10 mg/mL) and perylene (approximately 1 mg/mL) to approximately 50 mL of 6:4:3 solvent to produce a deeply colored solution.

   NOTE: Make sure that the azulene and perylene are <u>completely</u> dissolved.

2. Place a 100 mL cylinder beneath the column and using a transfer pipet, cautiously remove any excess 6:4:3 solvent from the top of the packing.
3. Using a transfer pipet, cautiously apply 2 mL of the azulene/perylene calibration solution onto the column. Use a circular motion to dispense the solution just above the packing, and drip the solution slowly down the column wall so as not to disturb the packing.
4. Open the stopcock, drain to the packing top, and close the stopcock.
5. Add approximately 0.5 mL of solvent to the top of the column. Drain to the packing top, and close the stopcock.
6. Repeat step 5 once.

7. Add 100 mL of solvent, and open the stopcock.
8. Elute the solvent until all of the perylene has emerged, using the UV light to monitor the perylene. Record the volumes at which the azulene and perylene start and finish eluting.
9. If the azulene emerges in the 50-65 mL range, and the perylene emerges in the 60-80 mL range without distinct tailing on the packing, proceed to step 10. Otherwise, recycle the packing (Attachment A).
10. Discard the eluate. Add 50 mL of solvent to the column, and flush the packing by eluting 50 mL into the cylinder. Again, discard the eluate.
11. The column is now ready for the next sample.

NOTE: If the column is to be stored, maintain 30-50 mL of solvent in the column reservoir, and cover the top with aluminum foil. Remove the solvent if it separates into 2 phases, add 80 mL of fresh 6:4:3 solvent, and elute 50 mL.

7.3 GC Calibration

Calibration standards at a minimum of five concentration levels should be prepared. One of the calibration standards should be at a concentration near, but above, the method detection limit; the others should correspond to the range of concentrations found in real samples but should not exceed the working range of the GC/MS system. Each standard should contain each analyte for detection by this method (e.g., some or all of the compounds listed in Tables 2 and 3 may be included). All initial calibration standards should be stored at -10° C to -20° C and should be freshly prepared once a year, or sooner if check standards indicate a problem. The daily calibration standard (ongoing calibration should be prepared weekly and stored at 4° C.

Using the PCB/pesticide primary dilution standard solution, prepare the following suggested standards in 10 mL volumetric flasks:

Primary Dilution Standard Solution (µL)	Concentration (µg/L)
50	5
200	20
400	40
800	80
2000	200

Each standard should be brought to volume with hexane.

Linearity of the GC is determined by calculation of the individual response factors (RF) for each standard concentration using the following formula:

RF = total peak area/mass of injected analyte.

The calibration curve will be considered linear if the %RSD is $\leq$30% for each compound.

8.0 Procedure

8.1 Sediment Extraction

1. Decant the excess water from the sediment.

   NOTE: Discard all extraneous materials. However, wood chip layers are common in Great Lakes sediments and may be an important part of the sample. If so, depending upon the project needs, the wood chips may be part of the sample.

2. Using a spatula and powder funnel, weigh a 10 ± .5 g aliquot of the homogenized, field-moist sediment to the nearest 0.01 g into a tared bottle.
3. Centrifuge each sample bottle at <1500 rpm for 5 min. Decant and discard the H_2O.
4. To each sediment sample, add 100 mL of CH_2Cl_2.
5. Add all surrogate spike solutions (see section 9.8).

   NOTE: Make certain that the solutions are placed into the CH_2Cl_2.

6. Add 50 g of Na_2SO_4.
7. Clean bottle lip and threads to remove all sediment particles. Cap the bottle.

   NOTE: Do not over tighten so as deform the cap and cause leakage.

8. Put Teflon™ tape around outside of cap and bottle.
9. Manually shake each bottle until the contents are loose.

10. Roll for approximately 16 hr (i.e., overnight) on the tumbler at 100-250 rpm.
11. Remove the tape from each bottle and decant the extract into a labeled flask.

    NOTE: If the sample does not immediately settle, centrifuge at $\leq$1500 rpm for 5 min.

12. Add 100 mL of CH_2Cl_2 to each sample, and repeat steps 6-9, except roll each bottle for 6 hr (i.e., during the day).
13. Decant the 2nd extract into the flask from step 10.
14. Repeat step 12, except roll each bottle for 16 hr (i.e., overnight).
15. Add the 3rd extract from step 13 to the flask from step 10.

8.2 Extract Concentration

1. Add 3-4 Teflon boiling chips to the flask containing the CH_2Cl_2 extract from step 8.1 step 14, and attach a Snyder column.
2. Concentrate the extract in a 60° C water bath to 10-15 mL, and transfer concentrated extract to a labeled concentrator tube.
3. Wash down the flask with 3-4 mL of CH_2Cl_2, and add the washings to the tube.
4. Repeat step 3 once.
5. Add one boiling chip to the tube, and using the tube heater, concentrate the extract to between 0.9 and 1.0 mL.
6. Add 3 mL of hexane to the tube, and concentrate the extract to 2 mL using the tube heater.

8.3 Silica Gel/Alumina Chromatography

NOTE: The laboratory temperature must be <80° F (27° C). On warm days proceed more slowly to avoid vapor bubbles.

NOTE: Columns should be prepared just prior to use.

1. Add 100 mL of CH_2Cl_2 and between 5 and 15 mm glass wool plug to a 19 mm ID column with a stopcock. Tamp the plug well to remove any bubbles.

2. Add the 10 g alumina to a beaker and slowly add 20 mL of CH_2Cl_2. Gently swirl the beaker for 30 sec, and let it stand for 5 min (to remove all air bubbles).
3. Add the 20 g silica gel to a 2nd beaker. Slowly add 40 mL of CH_2Cl_2 to the beaker. Gently swirl the beaker for 30 sec, and let it stand for 5 min (to remove all air bubbles).
4. Place a curved-stem funnel into the column reservoir so that the funnel tip hangs well off-center. Swirl the beaker to resuspend the alumina from step 2, and pour the slurry into the column.
5. Wash the beaker with approximately 5 mL of CH_2Cl_2, and add the washings to the column. Repeat the wash twice.
6. After the particles settle, open the stopcock for 30 sec to allow the alumina to pack more tightly, then close the stopcock.

   NOTE: Gentle tapping of the column while the stopcock is open will assist in the settling of the alumina and silica gel.

7. Add the silica gel from step 3 to the column, as in steps 4 and 5.
8. After the particles settle, open the stopcock. While the solvent still drains, add 1 mL of sand through the powder funnel.
9. Drain CH_2Cl_2 to the packing top, then close the stopcock.
10. Add 30 mL of 1:1 CH_2Cl_2:pentane to the column. Drain to the packing top, then close the stopcock. Discard the eluates.
11. With a transfer pipet, cautiously transfer the sediment extract to the top of the packing. Drain to the packing top, then close the stopcock.
12. Wash down the sediment extract tube with 0.5 mL of 1:1 CH_2Cl_2:pentane, and add the washings to the top of the packing. Drain to the packing top, then close the stopcock.
13. Repeat step 12 three times.
14. Add 200 mL of 1:1 CH_2Cl_2:pentane, and continue eluting at approximately 3 mL/min.
15. Collect 20 mL of eluate, then close the stopcock, and discard the contents of the cylinder.
16. Replace the cylinder with a labeled flask and collect eluate until the column runs dry.

8.4 Concentration of Extract

1. Add 3-4 boiling chips and a few grains of activated copper to the flask from step 15, section 8.3 until no further discoloring of the copper occurs.

   NOTE: Activated copper is added to the flask to remove elemental sulfur, a potential interferant for GC/ECD analyses.

2. Attach a Snyder column and concentrate the fraction in a 60° C water bath to 10-15 mL, and transfer it to a concentrator tube.

   NOTE: It is necessary to wet the Snyder column by adding CH_2Cl_2 to the top of the column prior to sample boiling.

3. Wash down the flask with 3-4 mL of CH_2Cl_2, and add the washings to the tube.
4. Repeat step 3 once.
5. Add one boiling chip to the tube and concentrate the fraction to between 0.9 and 1.0 mL.
6. Add 2 mL of hexane to the tube and concentrate to between 0.9 and 1.0 mL.
7. Add approximately 0.7 mL of CH_3OH and 0.5 mL of CH_2Cl_2.

   NOTE: This step results in a final solution ratio of 6:4:3 hexane:CH_3OH:CH_2Cl_2 (v:v:v).

   NOTE: The extract must be dissolved in the solvent (no layers), with the total volume $\leq$ 2.3 mL.

8.5 Sephadex LH-20 Chromatography

NOTE: It is important to check column calibration on a monthly basis.
NOTE: During column storage, maintain 30-50 mL of the solvent in the column reservoir and cover the top with aluminum foil to minimize evaporation. If the solvent in the reservoir separates into 2 phases, remove it and replace it with >80 mL of fresh 6:4:3 solvent, then elute 50 mL.

1. Remove the excess solvent from the top of the column using a transfer pipet.

2. Add 10 mL of the 6:4:3 solvent to the column. Drain to the packing top, and close the stopcock. Discard the eluate.
3. Wash the column top with 2 mL of CH_2Cl_2, and place the 50-mL cylinder under the column.
4. Using a transfer pipet, carefully apply the extract from step 7, section 8.4 to the column.
5. Use a circular motion to dispense the sample immediately above the packing, dripping it slowly down the column wall so as not to disturb the packing.
6. Drain to the packing top, and close the stopcock.
7. Wash down the tube with 0.5 mL of 6:4:3 solvent, and apply the washings to the column. Drain to the packing top, and close the stopcock.
8. Repeat step 7 once.
9. Wash down the column wall with approximately 3 mL of 6:4:3 solvent, applied above the base of the reservoir. Drain to the packing top, and close the stopcock.
10. Repeat step 9 once.
11. Cautiously add approximately 150 mL of 6:4:3 solvent to the column without disturbing the packing.
12. Collect 25 mL of eluate in the 50 mL cylinder. Close the stopcock, and discard this eluate.
13. Replace the cylinder with a concentrator tube. Open the stopcock, collect approximately 15 to 20 mL of eluate (the amount calibrated in Section 7.2 steps 8 and 9 from just before where azulene first emerges from the column), then close the stopcock.
14. Archive this fraction.

    NOTE: This fraction is archived in case early eluting compounds are not identified in the next fraction. If early eluting compounds are not identified in the next fraction, analyze the archived fraction for these compounds. If the compounds are identified in the archived fraction, a re-calibration of the Sephadex LH-20 column is necessary.

15. Place a 100 mL cylinder under the column. Open the stopcock, and collect approximately 50 to 55 mL of eluate (the amount calibrated in Section 7.2, steps 8 and 9 from 5 mL after the last perylene has eluted). Close the stopcock, and transfer the eluate to a flask.

16. Wash down the cylinder with 3 to 4 mL of CH_2Cl_2, and add the washings to the flask.
17. Repeat step 16 once.
18. Replace the 100 mL cylinder with a waste cylinder, and elute to the top of the packing. Discard this eluate. Add 50 mL of solvent and cap. The column is now ready for the next sample.

8.6 Concentration of Sephadex LH-20 Fraction

1. Add 3-4 boiling chips to the flask from step 17 section 8.5, and attach a Snyder column.

   NOTE: It is necessary to wet the Snyder column by adding CH_2Cl_2 to the top of the column prior to sample boiling.

2. Concentrate the fraction in a 75° C water bath to 10-15 mL, and transfer it to a concentrator tube.
3. Wash down the flask with 3-4 mL of CH_2Cl_2, and add the washings to the tube.
4. Repeat step 3 once.
5. Add one boiling chip to the tube and concentrate the fraction to between 0.9 and 1.0 mL.
6. Add 7 mL of hexane to the tube and concentrate to between 0.9 and 1.0 mL.

8.7 GC/ECD Analysis

The analyst should follow the instructions provided by the instrument's manufacturer for GC operation and maintenance. The following machine operating conditions are required for the proper determination and separation of the PCB congeners and pesticides:

Machine Operating Conditions

Helium carrier	1.2 mL/min
Initial oven temperature	100° C
Initial hold time	1 min
First ramp rate	5°/min
First ramp final temperature	140°
Second hold time	1 min

Second ramp rate	1.5°/min
Second ramp final temperature	250° C
Third hold time	1 min
Third ramp rate	10°/min
Final temperature	300° C
Final hold time	5 min
ECD temperature	325° C
Injector port temperature	275° C

The primary quantification column should be a DB-5 0.25 mm ID column with a 30 m length. The secondary confirmation column should be a DB-17HT 0.25 mm ID column with a 30 m length.

When a PCB congener or pesticide is identified on the quantification column, the chromatogram of the confirmation column should also be checked to verify the identification of the analyte. If, however, the area of the confirmation column is lower than that of the quantification column, the area of the analyte in the confirmation column should be used to calculate the concentration of the analyte (along with the areas of the surrogates from the confirmation column).

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit.

The method detection limit for PCBs in sediments is 1 µg/kg per congener.

The method detection limits for pesticides in sediments are 10 µg/kg per compound.

NOTE: Method detection limits can be lowered by extracting larger amounts of sediments or by further concentrating the final extract volume (<1 mL).

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 25 percent or less between the measured total PCB or pesticide concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 30% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a routine sediment sample collected and homogenized in bulk that has undergone multiple analyses by the analytical laboratory. Alternately, a LCS can be created by spiking a known quantity of the contaminant(s) of concern into a clean sediment, homogenizing the bulk sample, followed by multiple analyses at the analytical laboratory. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the

ongoing calibration check sample should be ± 30% of the mean RF from the initial calibration curve.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - PCBs or pesticides, to the 10 g aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 30% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 25%.

9.8 Surrogate Spikes

A surrogate spike is defined as the addition of an organic compound which is similar to analytes of interest in chemical composition, extraction, and chromatography, but which are not normally found in the environmental sample (USEPA, 1986). These compounds are spiked into all blanks, standards, samples, and spiked samples prior to extraction. Surrogate spikes should be spiked at between 50 and 100 times the method detection limit. Surrogate spike recoveries should be ± 30% of the known spiking concentration.

The following surrogate spike compounds are recommended:

4,4'-dibromooctafluorobiphenyl (DBOFB)
decachlorobiphenyl (DCB)
PCB congener 103
PCB congener 198

9.9 Internal Standards

An internal standard (also known as GC standard) is added immediately prior to analysis by GC. The compound(s) added are sensitive to the detector and are a measure of analyte recovery without (or with highly reduced) matrix effects. These compounds are spiked into all blanks, standards, samples, and spiked samples. Internal standards should be spiked at between 50 and 100

times the method detection limit. Internal standard recoveries should be ± 30% of the known concentration. The recommended internal standard for this method is tetrachloro-m-xylene (TCMX).

Control charts for the internal standards, with ± 2 and 3 σ values as warning and action limits, respectively, will be required to be created and updated after each day of analysis to control any systematic bias that may be adding to the overall measurement uncertainty for a given parameter. A value outside the control limits is considered unacceptable, hence, the instrument should be recalibrated and the samples in that batch should be reanalyzed. If bias for a given analysis is indicated, i.e., at least seven successive points occurring on one side of the cumulative means, sample analysis should cease until an explanation is found and the system is brought under control.

10.0 Method Performance

Precision and accuracy information are not available at this time.

11.0 Calculations and Reporting

Identify the analyte peaks in the chromatograms of the extract fractions by comparing them with the analyte retention times obtained from the chromatogram of the ongoing calibration standard.

> **NOTE**: When a PCB congener or pesticide is identified on the quantification column, the chromatogram of the confirmation column should also be checked to verify the identification of the analyte. If, however, the area of the confirmation column is lower than that of the quantification column, the area of the analyte in the confirmation column should be used to calculate the concentration of the analyte (along with the areas of the surrogates from the confirmation column).

The concentration of an analyte in the sediment sample, dry weight basis:

$$\text{PCB/pesticide, ηg/g (dry weight)} = \frac{R_1 \times R_2 \times ss \times 100}{R_3 \times g \times dw}$$

where:

R_1 = $\frac{\text{analyte peak area from the sample}}{\text{surrogate spike peak area from the sample}}$

R_2 = analyte concentration in the ongoing calibration standard (ηg/µL)
surrogate spike concentration in the ongoing calibration standard (ηg/µL)

R_3 = analyte peak area from the ongoing calibration standard
surrogate spike peak area from the ongoing calibration standard

ss = surrogate spike concentration added to sample (ηg)
g = wet weight of sediment sample, g
dw = % dry weight of sample determined from total solids analysis

The concentration of the total PCBs in the sediment sample is calculated by summation of the 20 congeners (Table 3) as follows:

Total PCBs, ηg/g (dry weight) = Σ congener concentrations

If the congener concentration is < method detection limit, then a "0" value should be used during summation (i.e., do not add the method detection limit for non-identified congeners).

> **NOTE**: The USEPA/USACE Task Group that developed the Great Lakes Dredged Material Testing & Evaluation Manual never intended that regulatory decisions should be made by the comparison of concentrations of individual congeners. The summation of congeners should be the only value reported, unless the values of individual congeners summed is explicitly requested.

12.0 References

Brown, D.W., A.J. Friedman, D.G. Burrows, G.R. Snyder, B.G. Patten, W.E. Ames, L.S. Ramos, P.G. Prohaska, D.D. Gennero, D.D. Dungan, M.Y. Uyeda, and W.D. MacLeod, Jr. 1979. Investigation of petroleum in the marine environments of the Strait of Juan de Fuca and Northern Puget Sound. EPA-600/7-79/164. U.S. Environmental Protection Agency, Washington D.C.

Federal Register. 1979. Guidelines establishing test procedures for the analysis of pollutants; proposed regulations. Federal Register 44(233):69464-69575.

Horwitz, W., L.P. Kamps, and K.W. Boyer. 1980. Quality assurance in the analysis of foods for trace constituents. J. Assoc. Off. Anal. Chem. 63:1344-1354.

MacLeod, W.D., Jr., D.W. Brown, R.G. Jenkins, L.S. Ramos, and Y.D. Henry. 1977. A pilot study on the design of a petroleum hydrocarbon baseline investigation for Northern Puget Sound and the Strait of Juan de Fuca. EPA-600/7-77-098. U.S. Environmental Protection Agency, Washington, D.C.

MacLeod, W.D., Jr., L.S. Ramos, A.J. Friedman, D.G. Burrows, P.G. Prohaska, D.L. Fisher, and D.W. Brown. 1981. Analysis of residual chlorinated hydrocarbons, aromatic hydrocarbons and related compounds in selected sources, sinks, and biota of the New York Bight. U.S. Dep. Commerce. NOAA Tech. Memo. OMPA-6. 128 p.

MacLeod, W.D., Jr., P.G. Prohaska, D.D. Gennero, and D.W. Brown. 1982. Interlaboratory comparisons of selected trace hydrocarbons from marine sediments. Anal. Chem. 54:386-392.

Malins, D.C., B.B. McCain, D.W. Brown, A.K. Sparks, and H.O. Hodgins. 1980. Chemical contaminants in Central and Southern Puget Sound. U.S. Dep. Commerce. NOAA Tech. Memo. OMPA-2. 295 p.

Malins, D.C., B.B. McCain, D.W. Brown, A.K. Sparks, H.O. Hodgins, and S.L. Chan. 1982. Chemical contaminants and abnormalities in fish and Invertebrates from Puget Sound. U.S. Dep. Commerce. NOAA Tech. Memo. OMPA-19.

Malins, D.C., B.B. McCain, D.W. Brown, S.L. Chan, M.S. Myers, J.T. Landahl, P.G. Prohaska, A.J. Friedman, L.D. Rhodes, D.G. Burrows, W.D. Gronlund, and H.O. Hodgins. 1984. Chemical pollutants in sediments and diseases of bottom-dwelling fish in Puget Sound, Washington. Environ. Sci. Technol. 18:705-713.

National Oceanic and Atmospheric Administration. 1985. Standard Analytical Procedures of the NOAA National Analytical Facility 1985-1985: Extractable toxic organic compounds. 2nd ed. NOAA Tech. Memo. NMFS F/NWC-92.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA/U.S. Army Corps of Engineers. 1998. Evaluation of material proposed for discharge to waters of the United States: Testing Manual (Inland Testing Manual), EPA-823-B-98-004, Office of Water, Washington D.C.

Attachment A - Sephadex LH-20 Column Packing and Recycling

A.1 Column Packing

1. Fit a 19 mm ID column with a stopcock, add 10 mL of 6:4:3 solvent and between 5 and 10 mm glass wool plug. Tamp the plug to remove any air bubbles.
2. Add approximately 1 mL of sand to the column, and tap the column gently so that the sand forms a smooth layer on top of the glass wool.
3. Pour the swelled Sephadex gel through the funnel into the column until the gel fills the column and about 1/4 of the reservoir.
4. Allow 10 min for the Sephadex to settle. Open the stopcock, and elute 80 mL of solvent to ensure firm packing. Add more solvent as needed. Leave 30 mL of solvent in the column reservoir. Cover the top with aluminum foil, and allow the packing to settle overnight.
5. Open the stopcock, and elute 10 mL of solvent, then close the stopcock. Remove the excess Sephadex packing from the top with a transfer pipet until the height of the Sephadex is 26.5 cm.
6. Gently add approximately 1 mL of sand onto the packing so that it forms an even layer on the top. (The column may be tapped or tilted slightly to get an even layer of sand.)
7. Examine the packing for air bubbles. If bubbles are evident, elute approximately 250 mL of warm (about 35° C) solvent through the column. If the bubbles persist, recycle the packing (see section A.2).

A.2 Recycling Sephadex LH-20 Column Packing

NOTE: When the column no longer maintains its calibration with azulene/perylene, recycle the packing.

1. Decant any solvent in the column reservoir.
2. Empty the column packing into a beaker 4 times the volume of the packing.
3. Wash with CH_2Cl_2.
4. Add enough CH_2Cl_2 to float Sephadex particles in the upper half of the beaker.
5. Remove <u>all</u> glass wool with forceps (mandatory).
6. Cover the beaker and let stand for 1 to 2 hours.
7. Decant the floating particles leaving the sand in the beaker.
8. Aspirate the CH_2Cl_2 from the Sephadex particles and set them aside.
9. Swell these particles overnight in 6:4:3 solvent before reusing.

POLYNUCLEAR AROMATIC HYDROCARBONS IN SEDIMENTS (GC/MS, CAPILLARY COLUMN)

1.0 Scope and Application

This method is appropriate for the determination of polynuclear aromatic hydrocarbons (PAHs) in sediment samples. Individual polynuclear aromatic compounds that are soluble in methylene chloride (CH_2Cl_2) and capable of being eluted without derivitization as sharp peaks from a gas chromatographic fused-silica capillary column coated with a slightly polar silicone are listed in Table 1.

This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatograph/mass spectrometers and skilled in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method.

Extraction and quantification techniques are based on SW-846 Method 8270 (USEPA, 1986). The extract clean-up procedures are based on a National Oceanic and Atmospheric Administration (NOAA) method for the determination of Extractable Toxic Organic Compounds in marine sediments (NOAA, 1985).

The extracts produced from this method (sections 8.1 through 8.6) can be used in the determination of polychlorinated biphenyls (PCBs), pesticides, and PAHs.

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" sediments. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

The sample is extracted with methylene chloride (CH_2Cl_2) and sodium sulfate (Na_2SO_4). The resultant extract is cleaned-up with silica gel and alumina. Additional clean-up steps to remove biological macromolecules are performed using Sephadex LH-20. The final sample extract is injected into a gas chromatograph/mass spectrometer system using a capillary column for

separation, identification, and quantification of the individual PAHs present in the sample.

The same extract used to analyze for PAHs can be used to analyze for PCBs and pesticides using gas chromatography with electron capture detection (GC/ECD). The method for PCB and pesticide determination is provided in this methods manual.

3.0 Interferences

Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences during the analysis of samples. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing reagent blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

Contamination by carryover can occur whenever high-level and low-level samples are sequentially analyzed. To reduce carryover, the sample syringe must be rinsed out between samples with solvent. Whenever an unusually concentrated sample is encountered, it should be followed by the analysis of solvent to check for cross contamination.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.001 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Centrifuge, capable of holding 250 mL centrifuge tubes and maintaining speeds of 1500 rpm.
4. Desiccator and desiccant. Desiccants generally used include: anhydrous calcium sulfate, silica gel, or phosphorus pentoxide. Indicating desiccants are preferable since they show when the desiccant needs to be changed or regenerated.
5. Gas chromatograph/mass spectrometer system with:
   a. gas chromatograph system complete with a temperature-programmable gas chromatograph suitable for splitless injection and all required accessories, including syringes,

analytical columns, and gases. The capillary column should be directly coupled to the source.

b. mass spectrometer capable of scanning from 35 to 500 amu every 1 sec or less, using 70 volts (nominal) electron energy in the electron impact ionization mode.
c. GC/MS interface that gives acceptable calibration points at 50 ηg per injection for each compound of interest and achieves acceptable tuning performance criteria may be used.
d. data system: A computer system must be interfaced to the mass spectrometer. The system must allow the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the ration of the chromatographic program. The computer must have software that can search any GC/MS data file for ions of a specific mass and that can plot such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software must also be available that allows integrating the abundances in any EICP between specified time or scan-number limits. The most recent version of the EPA/NIH Mass Spectral Library should also be available.

6. Modified Kontes tube heater (block contains: Al inserts fitted to the 0.7 mL line of the tube tip and an Al-foil shroud.
7. Molecular sieve traps (for gas cylinder)

   NOTE: One suggested source for the molecular sieve traps is Hydro-Purge model ASC-l, Coast Engineering Laboratory, Gardena, California.

8. Oxygen traps.
9. UV light source.
10. Water bath, capable of maintaining a temperature of 80 ± 2° C.

    NOTE: The bath should be used in a hood.

4.2 Materials

1. Beakers, 250 mL, or equivalent.
2. Centrifuge tubes, 250 mL, amber, with Teflon™ caps.

3. Chromatography column with reservoir 250 mL, 19 mm ID, 30 cm.
4. Erlenmeyer flask, 500 mL, with stopper.
5. Erlenmeyer flask, 1 L, with stopper.
6. Funnel, curved-stem (curve must be glassblown).
7. Funnel, 200 mm OD, long-stem.
8. Funnel, powder.
9. GC column, silicon-coated fused-silica capillary column, DB-5, 30 m x 0.25 mm I.D. (or 0.32 mm I.D.).
10. Graduated cylinder, 500 mL.
11. Graduated cylinder, 100 mL.
12. Graduated cylinder, 50 mL.
13. Kontes concentrator tube, 25 mL, with stopper.
14. Kuderna-Danish concentrator tube, 10 mL, graduated.
15. Kuderna-Danish evaporative flask, 500 mL.
16. pH paper, wide range, capable of determining pH from 4 to 10.
17. Separatory funnel, 2 L, with Teflon™ stopcock.
18. Snyder column, 3-ball macro.
19. Syringe, 2000 µL.
20. Syringe, 800 µL.
21. Syringe, 400 µL.
22. Syringe, 200 µL.
23. Syringe, 100 µL.
24. Syringe, 50 µL.
25. Syringe, 10 µL.
26. Teflon wash-bottle, 500 mL (to be filled with CH_2Cl_2).
27. Transfer pipets (Pasteur style) with rubber bulbs.
28. GC vials, 2 mL.
29. GC vials, 100 µL, conical.
30. Volumetric flask, class A, 100 mL
31. Volumetric flask, class A, 50 mL
32. Volumetric flask, class A, 10 mL
33. Volumetric pipet, 50 mL.

5.0 Reagents

1. Alumina, 80-200 mesh. Alumina should be activated at 120° C for 2 hr and then cooled to room temperature in a desiccator just before weighing and use.

2. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
3. Azulene, reagent grade ($C_{15}H_{18}$).
4. Copper, reagent grade, fine granular. Copper should be activated < 1 hr before use. To activate copper, cover with concentrated. HCl and stir with a glass rod. Allow to stand for 5 min followed by washing twice with CH_3OH and then 3 times with CH_2Cl_2. Leave copper covered with CH_2Cl_2 to avoid contact with air.
5. Helium, grade 4.5 (purified, $\geq$99.995 %).
6. Hexane, high purity (C_6H_{14}). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
7. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
8. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
9. Methanol, high purity (CH_3OH). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
10. Methylene chloride (dichloromethane), high purity (CH_2Cl_2). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
11. Pentane, high purity (C_5H_{12}). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
12. Perylene, reagent grade ($C_{20}H_{12}$).
13. Sand, Ottawa, MCB, kiln-dried, 30-40 mesh. Sand should be acid-washed (steeped in *aqua regia* (ACS grades $HN0_3$:HCl, 1:3, v:v) overnight, then washed three times each with Type II H_2O, CH_3OH and CH_2Cl_2, dried, and stored at 120° C.

14. Sephadex LH-20, size-exclusion gel. Sephadex LH-20 should be swelled overnight in 6:4:3 solvent.
15. Silica gel, Davison Type 923 or Amicon No. 84080. Silica should be activated at 700° C for 18 hr, stored at 170° C, and cooled to room temperature in a desiccator just before weighing and use.
16. Sodium hydroxide, 10 N (NaOH). Add 20 g of NaOH to 400 mL Type II water. Dilute to 500 mL with Type II water.
17. Sodium sulfate, reagent grade, anhydrous granular (Na_2S0_4). Sodium sulfate should be CH_2Cl_2 washed, dried, stored at 120° C, and cooled to room temperature in a desiccator before weighing and use.
18. PAH standard stock solution (1.00 µg/µL). PAH stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

    NOTE: Prepare stock standard solutions by accurately weighing 0.0100 g of pure material. Dissolve the material in pesticide quality acetone or other suitable solvent and dilute 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 may be used without correction to calculate the concentration of the stock standard.

    Commercially prepared stock standards may 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 4° C and protect from light. Stock standard solutions 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 1 yr, or sooner, if comparison with quality control check samples indicates a problem.

19. GC/MS tuning standard. A methylene chloride solution containing 50 ηg/µL of decafluorotriphenylphosphine (DFTPP) should be prepared. Store at 4° C or less when not being used.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Sample containers should be filled with care so as to prevent contamination due to any portion of the collected sample coming in contact with the sampler's gloves.

Samples should not be collected or stored in the presence of exhaust fumes.

Since field-moist samples are used in the determination, preservation of the sample is not practical. Therefore, analysis should begin as soon as possible after sample collection. A holding time of 7 days until extraction and 40 days from extraction to analysis is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C) in the dark.

NOTE: Samples can be frozen to extend the holding time for up to 1 year.

All sample containers must be prewashed with detergents, acids, and Type II water. Glass containers should be used for the storage of samples to be analyzed for PAHs in sediments. All glassware and materials contacting the solvents should be washed with CH_2Cl_2 three times prior to use.

An option to the CH_2Cl_2 washing of the glassware is to combust the glassware in a muffle oven at 400° C for 4 hours.

7.0 Calibration and Standardization

7.1 General

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The water bath and Kontes tube heater should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

7.2 Sephadex LH-20 Column Calibration

Information on preparing the Sephadex LH-20 column is presented in Attachment A.

1. Add enough azulene (approximately 10 mg/mL) and perylene (approximately 1 mg/mL) to approximately 50 mL of 6:4:3 solvent to produce a deeply colored solution.

   NOTE: Make sure that the azulene and perylene are <u>completely</u> dissolved.

2. Place a 100 mL cylinder beneath the column and using a transfer pipet, cautiously remove any excess 6:4:3 solvent from the top of the packing.
3. Using a transfer pipet, cautiously apply 2 mL of the azulene/perylene calibration solution onto the column. Use a circular motion to dispense the solution just above the packing, and drip the solution slowly down the column wall so as not to disturb the packing.
4. Open the stopcock, drain to the packing top, and close the stopcock.
5. Add approximately 0.5 mL of solvent to the top of the column. Drain to the packing top, and close the stopcock.
6. Repeat step 5 once.

7. Add 100 mL of solvent, and open the stopcock.
8. Elute the solvent until all of the perylene has emerged, using the UV light to monitor the perylene. Record the volumes at which the azulene and perylene start and finish eluting.
9. If the azulene emerges in the 50-65 mL range, and the perylene emerges in the 60-80 mL range without distinct tailing on the packing, proceed to step 10. Otherwise, recycle the packing (Attachment A).
10. Discard the eluate. Add 50 mL of solvent to the column, and flush the packing by eluting 50 mL into the cylinder. Again, discard the eluate.
11. The column is now ready for the next sample.

    NOTE: If the column is to be stored, maintain 30-50 mL of solvent in the column reservoir, and cover the top with aluminum foil. Remove the solvent if it separates into 2 phases, add 80 mL of fresh 6:4:3 solvent, and elute 50 mL.

7.3 GC Calibration

Calibration standards at a minimum of five concentration levels should be prepared. One of the calibration standards should be at a concentration near, but above, the method detection limit; the others should correspond to the range of concentrations found in real samples but should not exceed the working range of the GC/MS system. Each standard should contain each analyte for detection by this method. All initial calibration standards should be stored at -10° to -20° C and should be freshly prepared once a year, or sooner if check standards indicate a problem. The daily calibration standard (ongoing calibration standard) should be prepared weekly and stored at 4° C.

Each GC/MS system must be hardware-tuned to meet the criteria in Table 2 for the GC/MS tuning standard. Analyses should not begin until all these criteria are met. Background subtraction should be straightforward and designed only to eliminate column bleed or instrument background ions. The GC/MS tuning standard should also be used to assess GC column performance and injection port inertness. If chromatogram peak degradation is excessive and/or poor chromatography is noted, the injection port may require cleaning. It may also be necessary to break off the first 6 to 12 in. of the capillary column.

Analyze each calibration standard (1 µL containing internal standards) and tabulate the area of the primary characteristic ion against concentration for each compound (Table 1). Calculate response factors (RFs) for each compound as follows:

$$RF = (A_x C_{is})/(A_{is} C_x)$$

where:
A_x = area of the characteristic ion for the compound being measured.
A_{is} = area of the characteristic ion for the specific internal standard.
C_x = concentration of the compound being measured (ηg/µL).
C_{is} = concentration of the specific internal standard (ηg/µL).

The average RF should be calculated for each compound. The percent relative standard deviation (%RSD) should also be calculated for each compound. The %RSD should be less than 30% for each compound. The relative retention times of each compound in each calibration run should agree within 0.06 relative retention time units.

NOTE: Late eluting compounds usually have much better agreement.

8.0 Procedure

8.1 Sediment Extraction

1. Decant the excess water from the sediment.

   NOTE: Discard all extraneous materials. However, wood chip layers are common in Great Lakes sediments and may be an important part of the sample. If so, depending upon the project needs, the wood chips may be part of the sample.

2. Using a spatula and powder funnel, weigh a 10 ± .5 g aliquot of the homogenized, field-moist sediment to the nearest 0.01 g into a tared bottle.
3. Centrifuge each sample bottle at <1500 rpm for 5 min. Decant and discard the H_2O.
4. To each sediment sample, add 100 mL of CH_2Cl_2.

NOTE: Be sure to add all surrogate spike solutions at this point. Make certain that the solutions are placed into the CH_2Cl_2.

5. Add 50 g of Na_2SO_4.
6. Clean bottle lip and threads to remove all sediment particles. Cap the bottle.

   NOTE: Do not over tighten so as deform the cap and cause leakage.

7. Put Teflon™ tape around outside of cap and bottle.
8. Manually shake each bottle until the contents are loose.
9. Roll for approximately 16 hr (i.e., overnight) on the tumbler at 100-250 rpm.
10. Remove the tape from each bottle and decant the extract into a labeled flask.

    NOTE: If the sample does not immediately settle, centrifuge at $\leq$1500 rpm for 5 min.

11. Add 100 mL of CH_2Cl_2 to each sample, and repeat steps 6-9, except roll each bottle for 6 hr (i.e., during the day).
12. Decant the 2nd extract into the flask from step 10.
13. Repeat step 12, except roll each bottle for 16 hr (i.e., overnight).
14. Add the 3rd extract from step 13 to the flask from step 10.

8.2 Extract Concentration

1. Add 3-4 Teflon boiling chips to the flask containing the CH_2Cl_2 extract from step 8.1 step 14, and attach a Snyder column.
2. Concentrate the extract in a 60° C water bath to 10-15 mL, and transfer concentrated extract to a labeled concentrator tube.
3. Wash down the flask with 3-4 mL of CH_2Cl_2, and add the washings to the tube.
4. Repeat step 3 once.
5. Add one boiling chip to the tube, and using the tube heater, concentrate the extract to between 0.9 and 1.0 mL.
6. Add 3 mL of hexane to the tube, and concentrate the extract to 2 mL using the tube heater.

8.3 Silica Gel/Alumina Chromatography

NOTE: The laboratory temperature must be <80° F (27° C). On warm days proceed more slowly to avoid vapor bubbles.

NOTE: Columns should be prepared just prior to use.

1. Add 100 mL of CH_2Cl_2 and between 5 and 15 mm glass wool plug to a 19 mm ID column with a stopcock. Tamp the plug well to remove any bubbles.
2. Add the 10 g alumina to a beaker and slowly add 20 mL of CH_2Cl_2. Gently swirl the beaker for 30 sec, and let it stand for 5 min (to remove all air bubbles).
3. Add the 20 g silica gel to a 2nd beaker. Slowly add 40 mL of CH_2Cl_2 to the beaker. Gently swirl the beaker for 30 sec, and let it stand for 5 min (to remove all air bubbles).
4. Place a curved-stem funnel into the column reservoir so that the funnel tip hangs well off-center. Swirl the beaker to resuspend the alumina from step 2, and pour the slurry into the column.
5. Wash the beaker with approximately 5 mL of CH_2Cl_2, and add the washings to the column. Repeat the wash twice.
6. After the particles settle, open the stopcock for 30 sec to allow the alumina to pack more tightly, then close the stopcock.

   NOTE: Gentle tapping of the column while the stopcock is open will assist in the settling of the alumina and silica gel.

7. Add the silica gel from step 3 to the column, as in steps 4 and 5.
8. After the particles settle, open the stopcock. While the solvent still drains, add 1 mL of sand through the powder funnel.
9. Drain CH_2Cl_2 to the packing top, then close the stopcock.
10. Add 30 mL of 1:1 CH_2Cl_2:pentane to the column. Drain to the packing top, then close the stopcock. Discard the eluates.
11. With a transfer pipet, cautiously transfer the extract to the top of the packing. Drain to the packing top, then close the stopcock.
12. Wash down the extract tube with 0.5 mL of 1:1 CH_2Cl_2:pentane, and add the washings to the top of the packing. Drain to the packing top, then close the stopcock.
13. Repeat step 12 three times.
14. Add 200 mL of 1:1 CH_2Cl_2:pentane, and continue eluting at approximately 3 mL/min.

15. Collect 20 mL of eluate, then close the stopcock, and discard the contents of the cylinder.
16. Replace the cylinder with a labeled flask and collect eluate until the column runs dry.

8.4 Concentration of Extract

1. Add 3-4 boiling chips and a few grains of activated copper to the flask from step 15, section 8.3 until no further discoloring of the copper occurs.

   NOTE: Activated copper is added to the flask to remove elemental sulfur, a potential interferant for GC/ECD analyses.

2. Attach a Snyder column and concentrate the fraction in a 60° C water bath to 10-15 mL, and transfer it to a concentrator tube.

   NOTE: It is necessary to wet the Snyder column by adding CH_2Cl_2 to the top of the column prior to sample boiling.

3. Wash down the flask with 3-4 mL of CH_2Cl_2, and add the washings to the tube.
4. Repeat step 3 once.
5. Add one boiling chip to the tube and concentrate the fraction to between 0.9 and 1.0 mL.
6. Add 2 mL of hexane to the tube and concentrate to between 0.9 and 1.0 mL.
7. Add approximately 0.7 mL of CH_3OH and 0.5 mL of CH_2Cl_2.

   NOTE: This step results in a final solution ratio of 6:4:3 hexane:CH_3OH:CH_2Cl_2 (v:v:v).

   NOTE: The extract must be dissolved in the solvent (no layers), with the total volume $\leq$ 2.3 mL.

8.5 Sephadex LH-20 Chromatography

NOTE: It is important to check column calibration on a monthly basis.

NOTE: During column storage, maintain 30-50 mL of the solvent in the column reservoir and cover the top with aluminum foil to minimize evaporation. If the solvent in the reservoir separates into 2 phases, remove it and replace it with >80 mL of fresh 6:4:3 solvent, then elute 50 mL.

1. Remove the excess solvent from the top of the column using a transfer pipet.
2. Add 10 mL of the 6:4:3 solvent to the column. Drain to the packing top, and close the stopcock. Discard the eluate.
3. Wash the column top with 2 mL of CH_2Cl_2, and place the 50-mL cylinder under the column.
4. Using a transfer pipet, carefully apply the extract from step 7, section 8.4, to the column.
5. Use a circular motion to dispense the sample immediately above the packing, dripping it slowly down the column wall so as not to disturb the packing.
6. Drain to the packing top, and close the stopcock.
7. Wash down the tube with 0.5 mL of 6:4:3 solvent, and apply the washings to the column. Drain to the packing top, and close the stopcock.
8. Repeat step 7 once.
9. Wash down the column wall with approximately 3 mL of 6:4:3 solvent, applied above the base of the reservoir. Drain to the packing top, and close the stopcock.
10. Repeat step 9 once.
11. Cautiously add approximately 150 mL of 6:4:3 solvent to the column without disturbing the packing.
12. Collect 25 mL of eluate in the 50 mL cylinder. Close the stopcock, and discard this eluate.
13. Replace the cylinder with a concentrator tube. Open the stopcock, collect approximately 15 to 20 mL of eluate (the amount calibrated in Section 7.2 steps 8 and 9 from just before where azulene first emerges from the column), then close the stopcock.
14. Archive this fraction.

    NOTE: This fraction is archived in case early eluting compounds are not identified in the next fraction. If early eluting compounds are not identified in the next fraction, analyze the archived fraction for these compounds. If the compounds are

identified in the archived fraction, a re-calibration of the Sephadex LH-20 column is necessary.

15. Place a 100 mL cylinder under the column. Open the stopcock, and collect approximately 50 to 55 mL of eluate (the amount calibrated in Section 7.2, steps 8 and 9 from 5 mL after the last perylene has eluted). Close the stopcock, and transfer the eluate to a flask.
16. Wash down the cylinder with 3 to 4 mL of CH_2Cl_2, and add the washings to the flask.
17. Repeat step 16 once.
18. Replace the 100 mL cylinder with a waste cylinder, and elute to the top of the packing. Discard this eluate. Add 50 mL of solvent and cap. The column is now ready for the next sample.

8.6 Concentration of Sephadex LH-20 Fraction

1. Add 3-4 boiling chips to the flask from step 17 section 8.5, and attach a Snyder column.

   NOTE: It is necessary to wet the Snyder column by adding CH_2Cl_2 to the top of the column prior to sample boiling.

2. Concentrate the fraction in a 75° C water bath to 10-15 mL, and transfer it to a concentrator tube.
3. Wash down the flask with 3-4 mL of CH_2Cl_2, and add the washings to the tube.
4. Repeat step 3 once.
5. Add one boiling chip to the tube and concentrate the fraction to between 0.9 and 1.0 mL.
6. Add 7 mL of hexane to the tube and concentrate to between 0.9 and 1.0 mL.

8.7 GC/MS Analysis

The analyst should follow the instructions provided by the instrument's manufacturer for GC operation and maintenance. The recommended GC/MS operating conditions for PAH quantification are:

Mass Range	35-500 amu
Scan time	1 sec/scan
Initial column temperature	40° C

Initial hold time:	4 min
Column temperature program	40-270° C at 10°C/min
Final column temperature hold	270° C (until benzo[g,h,i]perylene has eluted)
Injector temperature	250-300° C
Transfer line temperature	250-300° C
Source temperature	According to manufacturer's specifications
Injector	Grob-type, splitless
Sample volume	1-2 µL
Carrier gas	Helium at 30 cm/sec.

The primary quantification column should be a DB-5 0.25 mm I.D. column with a 30 m length.

The volume to be injected should ideally contain 100 ηg of the PAHs (for a 1 µL injection).

NOTE: It is highly recommended that the extract be screened on a GC with flame ionization detection (FID) or GC with photoionization detection (PID) using the same type of capillary column (DB-5 0.25 mm I.D. with a 30 m length). This will minimize contamination of the GC/MS system from unexpectedly high concentrations of organic compounds.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit.

The method detection limit for PAHs in sediments is 50 µg/kg.

NOTE: Method detection limits can be lowered by extracting larger amounts of sediments or by further concentrating the final extract volume (<1 mL).

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 25 percent or less between the measured PAH concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 30% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

One reagent blank should also be analyzed prior to any routine sample analyses to ensure interferences and contamination are under control.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 30% of the mean RF from the initial calibration curve.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - PAHs, to the 1 L aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at

the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 30% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 30%.

9.7 Surrogate Spikes

A surrogate spike is defined as the addition of an organic compound which is similar to analytes of interest in chemical composition, extraction, and chromatography, but which are not normally found in the environmental sample (USEPA, 1986). These compounds are spiked into all blanks, standards, samples, and spiked samples prior to extraction. Surrogate spikes should be spiked at between 50 and 100 times the method detection limit. Surrogate spike recoveries should be ± 30% of the known spiking concentration.

The following surrogate spike compounds are recommended:

naphthalene-d_8
acenaphthene-d_{10}
perylene-d_{12}

Other surrogate spike compounds that are also commonly used are phenanthrene-d_{10} and chrysene-d_{12}.

Control charts for the surrogate spikes, with ± 2 and 3 σ values as warning and action limits, respectively, will be required to be created and updated after each day of analysis to control any systematic bias that may be adding to the overall measurement uncertainty for a given parameter. A value outside the control limits is considered unacceptable, hence, the instrument should be recalibrated and the samples in that batch should be reanalyzed. If bias for a given analysis is indicated, i.e., at least seven successive points occurring on one side of the cumulative means, sample analysis should cease until an explanation is found and the system is brought under control.

9.8 Internal Standards

An internal standard (also known as GC standard) is added immediately prior to analysis by GC. The compound(s) added are sensitive to the detector

and are a measure of analyte recovery without (or with highly reduced) matrix effects. These compounds are spiked into all blanks, standards, samples, and spiked samples. Internal standards should be spiked at between 50 and 100 times the method detection limit. Internal standard recoveries should be ± 30% of the known concentration. The recommended internal standard for this method is tetrachloro-m-xylene (TCMX). An alternate internal standard often used is hexamethylbenzene (HMB).

Control charts for the internal standards, with ± 2 and 3 σ values as warning and action limits, respectively, will be required to be created and updated after each day of analysis to control any systematic bias that may be adding to the overall measurement uncertainty for a given parameter. A value outside the control limits is considered unacceptable, hence, the instrument should be recalibrated and the samples in that batch should be reanalyzed. If bias for a given analysis is indicated, i.e., at least seven successive points occurring on one side of the cumulative means, sample analysis should cease until an explanation is found and the system is brought under control.

9.9 Ongoing GC/MS Tuning Standard

A 50 ηg injection of the GC/MS tuning standard (DFTPP) must be made during each 12 hour shift. Acceptance criteria in the mass spectrum for DFTPP must meet the criteria given in Table 2.

10.0 Method Performance

Precision and accuracy information are not available at this time.

11.0 Calculations and Reporting

11.1 Qualitative Analysis

An analyte is identified by comparison of the sample mass spectrum with the mass spectrum of a standard of the suspected compound (standard reference spectrum). Mass spectra for the standard reference should be obtained on the GC/MS within the same 12 hours as the sample analysis. These standard reference spectra may be obtained through analysis of the calibration standards. Two criteria must be satisfied to verify identification: (1) elution of sample component at the same GC relative retention time (RRT) as

the standard component; and (2) correspondence of the sample component and the standard component mass spectrum.

The sample component RRT must compare within ± 0.06 RRT units of the RRT of the standard component. If coelution of interfering components prohibits accurate assignment of the sample component RRT from the total ion chromatogram, the RRT should be assigned by using extracted ion current profiles for ions unique to the component of interest.

All ions present in the standard mass spectra at a relative intensity greater than 10% (most abundant ion in the spectrum equals 100%) must be present in the sample spectrum. The relative intensities of ions must agree within plus or minus 20% between the standard and sample spectra (i.e., an ion with an abundance of 50% in the standard spectra must have the corresponding sample abundance between 30 and 70 percent).

For samples containing components not associated with the calibration standards, a library search may be made for the purpose of tentative identification. The necessity to perform this type of identification will be determined by the type of analyses being conducted. Computer-generated library search routines should not use normalization routines that would misrepresent the library or unknown spectra when compared to each other. Only after visual comparison of sample spectra with the nearest library searches will the mass spectral interpretation specialist assign a tentative identification. Guidelines for making tentative identification are:

1. Relative intensities of major ions in the reference spectrum (ion >10% of the most abundant ion) should be present in the sample spectrum.
2. The relative intensities of the major ions should agree within ±20%. (i.e., an ion with an abundance of 50% in the standard spectrum must have the corresponding sample ion abundance between 30 and 70%).
3. Molecular ions present in the reference spectrum should be present in sample the spectrum.
4. Ions present in the sample spectrum but not in the reference spectrum should be reviewed for possible background contamination or presence of coeluting compounds.
5. Ions present in the reference spectrum but not in the sample spectrum should be reviewed for possible subtraction from the sample spectrum because of background contamination or coeluting

peaks. Data system library reduction programs can sometimes create these discrepancies.

11.2 Quantitative Analysis

When a compound has been identified, the quantitation of that compound will be based on the integrated abundance from the EICP of the primary characteristic ion. Quantitation will take place using the internal standard technique. The internal standard used shall be the one nearest the retention time of that of a given analyte.

Calculate the concentration of each identified analyte in the sample as follows:

$$\text{PAH, µg/kg (dry weight)} = \frac{A_x \times I_s \times V_t}{A_{is} \times RF \times V_i \times g \times dw}$$

where:
A_x = area of characteristic ion for compound being measured.
I_s = amount of internal standard injected (ηg).
V_t = volume of total extract (µL).
A_{is} = area of characteristic ion for the internal standard.
R_F = response factor for compound being measured.
V_i = volume of extract injected (µL).
g = wet weight of sediment sample, g.
dw = % dry weight of sample determined from total solids analysis.

Where applicable, an estimate of concentration for noncalibrated components in the sample should be made. The formulas given above should be used with the following modifications: the areas A_x and A_{is} should be from the total ion chromatograms and the RF for the compound should be assumed to equal 1. The concentration obtained using this method should be reported indicating (1) that the value is an estimate and (2) which internal standard was used to determine concentration. Use the nearest internal standard free of interferences.

Report results without correction for recovery data in µg/kg of each PAH.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

J.W. Eichelberger, L.E. Harris, and W.L. Budde. 1975. Reference Compound to Calibrate Ion Abundance Measurement in Gas Chromatography-Mass Spectrometry. Anal. Chem. 47:995.

National Oceanic and Atmospheric Administration. 1985. Standard Analytical Procedures of the NOAA National Analytical Facility 1985-1985: Extractable toxic organic compounds. 2nd ed. NOAA Tech. Memo. NMFS F/NWC-92.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Table 1. Characteristic Ions for PAHs.

Compound	Retention Time (min)	Primary Ion	Secondary Ion(s)
Acenaphthene	15.13	154	153, 152
Acenaphthene-d_{10} (SS)	15.05	164	162, 160
Acenaphthylene	14.57	152	151, 153
Anthracene	19.77	178	176, 179
Benzo(a)anthracene	27.83	228	229, 226
Benzo(b)fluoranthene	31.45	252	253, 125
Benzo(k)fluoranthene	31.55	252	253, 125
Benzo(g,h,i)perylene	41.43	276	138, 277
Benzo(a)pyrene	32.80	252	253, 125
Chrysene	27.97	228	226, 229
Dibenz(a,h)anthracene	39.82	278	139, 279
Fluoranthene	23.33	202	101, 203
Fluorene	16.70	166	165, 167
Indeno(1,2,3-cd)pyrene	39.52	276	138, 227
2-Methylnaphthalene	11.87	142	141
Naphthalene-d_8 (SS)	9.75	136	68
Perylene-d_{12} (SS)	33.05	264	260, 265
Phenanthrene	19.62	178	179, 176
Pyrene	24.02	202	200, 203
Tetrachloro-m-xylene (IS)			

IS = internal standard
SS = surrogate spike
[a]estimated retention times.

Table 2. DFTPP Key Ions and Ion Abundance Criteria[a]

Mass	Ion Abundance Criteria
51	30-60% of mass 198
68	<2% of mass 69
70	<2% of mass 69
127	40-60% of mass 198
197	<1% of mass 198
198	Base peak, 100% relative abundance
199	5-9% of mass 198
275	10-30 of mass 198
365	>1% of mass 198
441	Present but less than mass 443
442	>40% of mass 198
443	17-23% of mass 442

a = from Eichelberger et al., 1975.

Attachment A - Sephadex LH-20 Column Packing and Recycling

A.1 Column Packing

1. Fit a 19 mm ID column with a stopcock, add 10 mL of 6:4:3 solvent and between 5 and 10 mm glass wool plug. Tamp the plug to remove any air bubbles.
2. Add approximately 1 mL of sand to the column, and tap the column gently so that the sand forms a smooth layer on top of the glass wool.
3. Pour the swelled Sephadex gel through the funnel into the column until the gel fills the column and about 1/4 of the reservoir.
4. Allow 10 min for the Sephadex to settle. Open the stopcock, and elute 80 mL of solvent to ensure firm packing. Add more solvent as needed. Leave 30 mL of solvent in the column reservoir. Cover the top with aluminum foil, and allow the packing to settle overnight.
5. Open the stopcock, and elute 10 mL of solvent, then close the stopcock. Remove the excess Sephadex packing from the top with a transfer pipet until the height of the Sephadex is 26.5 cm.
6. Gently add approximately 1 mL of sand onto the packing so that it forms an even layer on the top. (The column may be tapped or tilted slightly to get an even layer of sand.)
7. Examine the packing for air bubbles. If bubbles are evident, elute approximately 250 mL of warm (about 35° C) solvent through the column. If the bubbles persist, recycle the packing (see section A.2).

A.2 Recycling Sephadex LH-20 Column Packing

NOTE: When the column no longer maintains its calibration with azulene/perylene, recycle the packing.

1. Decant any solvent in the column reservoir.
2. Empty the column packing into a beaker 4 times the volume of the packing.
3. Wash with CH_2Cl_2.
4. Add enough CH_2Cl_2 to float Sephadex particles in the upper half of the beaker.
5. Remove <u>all</u> glass wool with forceps (mandatory).
6. Cover the beaker and let stand for 1 to 2 hours.
7. Decant the floating particles leaving the sand in the beaker.
8. Aspirate the CH_2Cl_2 from the Sephadex particles and set them aside.
9. Swell these particles overnight in 6:4:3 solvent before reusing.

TOTAL SUSPENDED SOLIDS IN WATERS AND ELUTRIATES

1.0 Scope and Application

This method determines the weight of material associated with an aqueous (surface water or elutriate) sample that is suspended and capable of being removed from the sample by filtration.

The filtrate from this procedure may be used in the determination of total dissolved solids.

The end product or residue created from this procedure can be used in the determination of total volatile solids (TVS) in aqueous samples.

Since this method is based on the difference between two weighings, the range and sensitivity of the method is dependent upon the balance used.

The practical range of the determination is 4 mg/L to 20,000 mg/L.

This method is based on EPA Method 160.2 (USEPA, 1983).

2.0 Summary of Method

A well-mixed sample is filtered through a glass fiber filter, and the residue retained on the filter is dried to constant weight at 103-105° C.

3.0 Interferences

Large floating particles or submerged agglomerates of nonhomogeneous materials should be excluded from the sample if it is determined that their inclusion is not desired in the final result.

For samples high in dissolved solids, thoroughly wash the filter to ensure removal of the dissolved material.

Prolonged filtration times resulting from filter clogging may produce high results owing to excessive solids capture on the clogged filter.

Since the procedure is operationally defined and based on the difference between two weighings, the test is subject to errors due to loss of water of

crystallization, loss of volatile organic matter prior to combustion, incomplete oxidation of certain complex organics, and decomposition of mineral salts during combustion.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.001 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Convection oven.
4. Thermometer, 0 to 200° C range, graduated to 1° C.
5. Desiccator and desiccant. Desiccants generally used include: anhydrous calcium sulfate, silica gel, or phosphorus pentoxide. Indicating desiccants are preferable since they show when the desiccant needs to be changed or regenerated.
6. Filter support. Filtering apparatus with reservoir and a coarse (40-60 µm) fritted disc as a filter support.

   NOTE: Many funnel designs are available in glass or porcelain. Some of the most common are Hirsch or Buchner funnels, membrane filter holders and Gooch crucibles. All are available with coarse fritted disc.

7. Planchet, aluminum or stainless steel, or equivalent.

4.2 Materials

1. Glass fiber filter discs, without organic binder, such as Millipore AP-40, Reeves Angel 934-AH, Gelman type A/E, or equivalent.

   NOTE: Because of the physical nature of glass fiber filters, the absolute pore size cannot be controlled or measured. Terms such as "pore size," collection efficiencies and effective retention are used to define this property in glass fiber filters. Values for these parameters vary for the filters listed above.

2. Suction flask. Should be of sufficient capacity for sample size selected.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Preservation of the sample is not practical; analysis should begin as soon as possible. A holding time of 7 days is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C) to minimize microbiological decomposition of solids between sample collection and sample analysis.

All sample containers must be prewashed with detergents. If samples are to be analyzed for other parameters, acids and Type II water washings may also be required. Either glass or plastic containers can be used for the storage of samples to be analyzed for total suspended solids.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The oven should be monitored to ensure that temperature fluctuation does not exceed ± 2° C.

8.0 Procedure

8.1 Preparation of Glass-Fiber Filter Disk

1. Place the glass fiber filter on the membrane filter apparatus or insert into bottom of a suitable Gooch crucible with wrinkled surface up.
2. While vacuum is applied, wash the disc with three successive 20 mL volumes of Type II water.
3. Remove all traces of water by continuing to apply vacuum after water has passed through.
4. Remove filter from membrane filter apparatus or both crucible and filter if Gooch crucible is used.
5. Dry in an oven at 103-105° C for one hour.

   NOTE: If total volatile solids are to be measured, ignite at 550 ± 10° C for 15 minutes in a muffle furnace.

   NOTE: Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg).

6. Remove to desiccator and store until needed.
7. Weigh immediately before use.

   NOTE: After weighing, handle the filter or crucible/filter with forceps or tongs only.

8.2 Selection of Filter and Sample Sizes

1. For a 4.7 cm diameter filter, filter 100 mL of sample.

   NOTE: If weight of captured residue is less than 1.0 mg, the sample volume must be increased to provide at least 1.0 mg of residue.

   NOTE: If other filter diameters are used, start with a sample volume equal to 7 mL/cm^2 of filter area and collect at least a weight of residue proportional to the 1.0 mg stated above.

NOTE: If during filtration of this initial volume the filtration rate drops rapidly, or if filtration time exceeds 5 to 10 minutes, the following scheme is recommended:

a. Use an unweighed glass fiber filter of choice affixed in the filter assembly.
b. Add a known volume of sample to the filter funnel and record the time elapsed after selected volumes have passed through the filter. Twenty-five mL increments for timing are suggested.
c. Continue to record the time and volume increments until filtration rate drops rapidly.
d. Add additional sample if the filter funnel volume is inadequate to reach a reduced rate.
e. Plot the observed time versus volume filtered.
f. Select the proper filtration volume as that just short of the time a significant change in filtration rate occurred.

8.3 Sample Analysis

1. Assemble the filtering apparatus and begin suction.
2. Wet the filter with a small volume of Type II water to seat it against the fritted support.
3. Shake the sample vigorously and quantitatively transfer the sample volume selected in 8.2 to the filter using a graduated cylinder.
4. Remove all traces of water by continuing to apply vacuum after sample has passed through.
5. With suction on, wash the graduated cylinder, filter, suspended solids, and filter funnel wall with three portions of Type II water allowing complete drainage between washing.

   NOTE : Total volume of wash water used should equal approximately 2 mL per cm^2. For a 4.7 cm filter. the total volume is 30 mL.

6. Remove all traces of water by continuing to apply vacuum after water has passed through.
7. Carefully remove the filter from the filter support. Alteratively, remove crucible and filter from crucible adapter if a Gooch crucible is used.

8. Dry at least one hour at 103-105° C.
9. Cool in a desiccator and weigh.
10. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg or less than 4% of the previous weight, whichever is less).

9.0 Quality Control

9.1 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 10 percent or less.

9.2 Blanks

A minimum of one blank per sample batch should be analyzed. A blank for total suspended solids consists of a filter disk. The weight change of the blank should not be greater than ± 0.5 mg.

10.0 Method Performance

The standard deviation was 5.2 mg/L (coefficient of variation = 33%) at 15 mg/L, 24 mg/L at 242 mg/L, and 13 mg/L 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.

11.0 Calculations and Reporting

Use the results from the individual weighings to calculate the total suspended solid content of the sample as follows:

$$\text{Total Suspended Solids, mg/L} = \frac{(A - B) \times 1000}{V}$$

where:

A = weight of filter plus dried residue, mg.
B = weight of filter, mg.
V = sample volume, mL.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition, APHA, New York, New York. p. 2-71 - 2-79.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. Environmental Protection Agency. 1983. "Methods for the Chemical Analysis of Water and Wastes". EPA-600/4-79-020. U.S. Environmental Protection Agency Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. March.

TOTAL DISSOLVED SOLIDS IN WATERS AND ELUTRIATES

1.0 Scope and Application

This method determines the weight of material associated with an aqueous (surface water or elutriate) sample that is dissolved and is carried through a glass fiber filter into the filtrate.

The filtrate from the total suspended solids procedure may be used in the determination of total dissolved solids in aqueous samples.

The end product or residue created from this procedure can be used in the determination of total volatile solids (TVS) in aqueous samples.

Since this method is based on the difference between two weighings, the range and sensitivity of the method is dependent upon the balance used.

The practical range of the determination is 10 mg/L to 20,000 mg/L.

This method is based on EPA Method 160.1 (USEPA, 1983).

2.0 Summary of Method

A well-mixed sample is filtered through a glass fiber filter. The filtrate is evaporated to dryness in a weighed dish and dried to a constant weight at 180° C. The increase in dish weight represents the total dissolved solids.

3.0 Interferences

Highly mineralized waters with considerable calcium, magnesium, chloride, and/or sulfate content may be hygroscopic and require prolonged drying, proper desiccation, and rapid weighing.

Samples high in bicarbonate require careful and possibly prolonged drying to 180° C to insure complete conversion of bicarbonate to carbonate.

Excessive residue in the dish may form a water-trapping crust, therefore, sample size should be limited to no more than 200 mg of residue.

Since the procedure is operationally defined and based on the difference between two weighings, the test is subject to errors due to loss of water of crystallization, loss of volatile organic matter prior to combustion, incomplete oxidation of certain complex organics, and decomposition of mineral salts during combustion.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.001 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Convection oven, capable of maintaining 180 ± 2° C.
4. Thermometer, 0 to 200° C range, graduated to 1° C.
5. Desiccator and desiccant. Desiccants generally used include: anhydrous calcium sulfate, silica gel, or phosphorus pentoxide. Indicating desiccants are preferable since they show when the desiccant needs to be changed or regenerated.
6. Filter support. Filtering apparatus with reservoir and a coarse (40-60 µm) fritted disc as a filter support.

   NOTE: Many funnel designs are available in glass or porcelain. Some of the most common are Hirsch or Buchner funnels, membrane filter holders and Gooch crucibles. All are available with coarse fritted disc.

7. Steam bath.

4.2 Materials

1. Evaporating dishes, porcelain, 90 mm, 100 mL capacity. (aluminum, Vycor, or platinum weighing dishes may be substituted and smaller size dishes may be used, if required.)
2. Glass fiber filter discs, without organic binder, such as Millipore AP-40, Reeves Angel 934-AH, Gelman type A/E, or equivalent.

   NOTE: Because of the physical nature of glass fiber filters, the absolute pore size cannot be controlled or measured. Terms such as "pore size," collection efficiencies and effective retention

are used to define this property in glass fiber filters. Values for these parameters vary for the filters listed above.

3. Suction flask. Should be of sufficient capacity for sample size selected.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Preservation of the sample is not practical; analysis should begin as soon as possible. A holding time of 7 days is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C) to minimize microbiological decomposition of solids between sample collection and sample analysis.

All sample containers must be prewashed with detergents. If samples are to be analyzed for other parameters, acids and Type II water washings may also be required. Either glass or plastic containers can be used for the storage of samples to be analyzed for total dissolved solids.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The oven should be monitored to ensure that temperature fluctuation does not exceed ± 2° C.

8.0 Procedure

8.1 Preparation of Glass-Fiber Filter Disk

1. Place the glass fiber filter on the membrane filter apparatus or insert into bottom of a suitable Gooch crucible with wrinkled surface up.
2. While vacuum is applied, wash the disc with three successive 20 mL volumes of Type II water.
3. Remove all traces of water by continuing to apply vacuum after water has passed through.
4. Discard washings.

8.2 Preparation of Evaporating Dish

1. If total volatile solids are to be determined on the sample, the evaporating dish should be heated at 550 ± 10° C for one hour in a muffle furnace prior to use. Cool and store in desiccator until ready for use.
2. If only total dissolved solids are to be measured, heat the clean evaporating dish to 180 ± 2° C for one hour. Cool and store in desiccator until ready for use.
3. Weigh evaporating dish immediately before use.

8.3 Selection of Filter and Sample Sizes

1. Choose sample volume to yield between 2.5 and 200 mg of dried residue.
2. If more than 10 minutes are required to complete filtration, increase filter size or decrease sample volume but do <u>not</u> produce less than 2.5 mg of residue.

8.4 Sample Analysis

1. Assemble the filtering apparatus and begin suction.
2. Wet the filter with a small volume of Type II water to seat it against the fritted support.
3. Shake the sample vigorously and quantitatively transfer the well-mixed sample volume to the filter.

4. Wash with three 10 mL portions of Type II water allowing complete drainage between washing.
5. Continue suction for about 3 minutes after filtration is complete.
6. Transfer filtrate to a weighed evaporating dish and evaporate to dryness on a steam bath.

   NOTE: If filtrate volume exceeds dish capacity, add successive portions to the same dish after evaporation.

7. Dry at least one hour at 180 ± 2° C.
8. Cool in a desiccator to balance temperature and weigh.
9. Repeat the drying cycle (steps 7 and 8) until a constant weight is obtained (weight loss is less than 0.5 mg or less than 4% of the previous weight, whichever is less).

9.0 Quality Control

9.1 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 10 percent or less.

9.2 Blanks

A minimum of one blank per sample batch should be analyzed. A blank for total dissolved solids consists of a clean evaporating dish. The weight change of the blank should not be greater than ± 0.5 mg.

10.0 Method Performance

Single laboratory analyses of 77 samples of a known of 293 mg/L were made with a standard deviation of differences of 21.20 mg/L.

11.0 Calculations and Reporting

Use the results from the individual weighings to calculate the total dissolved solid content of the sample as follows:

$$\text{Total Dissolved Solids, mg/L} = \frac{(A - B) \times 1000}{V}$$

where:

A = weight of dish plus dried residue, mg.
B = weight of dish, mg.
V = sample volume, mL.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition, APHA, New York, New York. p. 2-71 - 2-79.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. Environmental Protection Agency. 1983. "Methods for the Chemical Analysis of Water and Wastes". EPA-600/4-79-020. U.S. Environmental Protection Agency Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. March.

TOTAL VOLATILE SOLIDS IN WATERS AND ELUTRIATES

1.0 Scope and Application

This method determines the weight of material associated with an aqueous (surface water or elutriate) sample that is volatile or combustible at 550° C. The test is useful in obtaining a rough approximation of the amount of organic matter present in the solid fraction of waters or elutriates.

The solid materials used for the determination of total volatile solids may be obtained from the residue obtained in the determination of total dissolved or total suspended solids or from the original sample.

> **NOTE**: If the aliquot is obtained from the original sample, then the procedure for total suspended or total dissolved solids must be performed on the aliquot prior to ashing in the muffle furnace.

Since this method is based on the difference between two weighings, the range and sensitivity of the method is dependent upon the balance used.

This method is based on EPA Method 160.4 (USEPA, 1983).

2.0 Summary of Method

The residue obtained from the determination of total suspended or total dissolved solids is ignited at 550° C in a muffle furnace. The loss of weight on ignition is reported as mg/L volatile residue.

3.0 Interferences

Since the procedure is operationally defined and based on the difference between two weighings, the test is subject to errors due to loss of water of crystallization, loss of volatile organic matter prior to combustion, incomplete oxidation of certain complex organics, and decomposition of mineral salts during combustion.

The principal source of error in the determination is failure to obtain a representative sample.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.001 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Muffle furnace.
4. Thermocouple, 0 to 600° C range, graduated to 1° C.
5. Desiccator and desiccant. Desiccants generally used include: anhydrous calcium sulfate, silica gel, or phosphorus pentoxide. Indicating desiccants are preferable since they show when the desiccant needs to be changed or regenerated.

4.2 Materials

1. Evaporating dishes or crucibles, porcelain, 90 mm, 100 mL capacity. (Aluminum, Vycor, or platinum weighing dishes or crucibles may be substituted and smaller size dishes may be used, if required.)

5.0 Reagents and Standards

No reagents are required for this procedure.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Preservation of the sample is not practical; analysis should begin as soon as possible. A holding time of 7 days is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C) to minimize microbiological decomposition of solids between sample collection and sample analysis.

All sample containers must be prewashed with detergents. If samples are to be analyzed for other parameters, acids and Type II water washings may also be required. Either glass or plastic containers can be used for the storage of samples to be analyzed for total volatile solids.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The oven should be monitored to ensure that temperature fluctuation does not exceed ± 2° C.

8.0 Procedure

1. Heat muffle furnace to 550 ± 10° C.
2. Ignite residue produced from the determination of either total dissolved or total suspended solids at 550 ± 10° C for one hour in a muffle furnace.
3. Remove the sample dish from the furnace and allow to partially cool until most of the heat has been dissipated (about 15 minutes).
4. Transfer the sample to a desiccator for final cooling.
5. Weigh sample dish to the nearest mg and record final weight of the sample + dish.

9.0 Quality Control

9.1 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 10 percent or less.

9.2 Blanks

A minimum of one blank per sample batch should be analyzed. A blank for total volatile solids consists of an empty dish. The weight change of the blank should not be greater than ± 0.5 mg.

10.0 Method Performance

A collaborative study involving three laboratories examining four samples by means of ten replicates produced a standard deviation of ± 11 mg/L at a volatile residue concentration of 170 mg/L (APHA, 1989; USEPA, 1983).

11.0 Calculations and Reporting

Use the results from the individual weighings to calculate the total volatile solid content of the sample as follows:

$$\text{Total Volatile Solids, mg/L} = \frac{(A - B) \times 100}{V}$$

where:

A = weight of residue plus dish before ignition, mg.
B = weight of the ashed sample plus dish, mg.
V = sample volume, mL.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition, APHA, New York, New York. p. 2-71 - 2-79.

U.S. Environmental Protection Agency. 1983. "Methods for the Chemical Analysis of Water and Wastes". EPA-600/4-79-020. U.S. Environmental Protection Agency Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. March.

ELUTRIATE PREPARATION FROM SEDIMENTS

1.0 Scope and Application

This method is applicable to the preparation of elutriates from sediment and dredging site water samples. The elutriate test is a simplified simulation of the dredging and disposal process wherein predetermined amounts of dredging site water and sediment are mixed together to approximate a dredged material slurry.

This method is based on the elutriate preparation procedures presented by Plumb (1981).

2.0 Summary of Method

Water and sediment samples collected at the dredging site are collected and mixed in a ratio of 1:4 sediment:water. The mixture is shaken vigorously for 30 minutes and allowed to settle undisturbed for 1 hour. The liquid phase is then centrifuged and filtered through a 45 µm membrane filter to remove all suspended particulate matter. The filtrate is the elutriate to be used for testing purposes.

3.0 Interferences

Non-representative particulates such as leaves, sticks, fish and lumps of fecal matter should be excluded from the sediment sample if it is determined that their inclusion is not desired in the final result.

Excessive quantities of suspended fine particulates may clog the membrane filter. Additional high speed centrifugation can be used to alleviate this interference.

4.0 Apparatus and Materials

4.1 Apparatus

1. Centrifuge, capable of handling six 1 or 0.5 L centrifuge bottles at 3000 to 5000 rpm.

2. Laboratory shaker capable of shaking 2 L flasks at approximately 100 excursions/minute. Box type or wrist action shakers are acceptable.
3. Vacuum or pressure filtration equipment including:
   a. vacuum pump or compressed air source
   b. appropriate filter holder capable of handling 47, 105, or 155 mm diameter filters

4.2 Materials

1. Erlenmeyer flasks, graduated, 1 L, or equivalent.
2. Graduated cylinders, 1 L.
3. Large powder funnels, 15 cm.
4. Membrane filters, 0.45 µm pore size.
5. Volumetric flasks, class A, 1 L.

5.0 Reagents and Standards

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
3. Hydrochloric acid, 5 M. Add 417 mL concentrated HCl to 500 mL Type II water. Dilute to 1 liter with Type II water.

6.0 Sample Collection, Handling, and Preservation

Sample collection procedures for the waters and sediments should be described in the approved sampling manual. Further information on bulk water and sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

For this procedure, approximately 3 liters of sediment should be collected to provide sufficient elutriates for all parameters and to cover most reanalyses that may be needed/requested.

For this procedure, approximately 12 liters of water should be collected from the dredging site.

NOTE: If fill material disposal activity is to be assessed, 12 liters of water should be collected from the disposal site.

No preservation techniques (other that refrigeration - see below) should be applied to the water or sediments to be used in the preparation of elutriates. Analysis should begin as soon as possible after sample collection. A holding time of 7 days is generally cited for the site waters used in the elutriate preparation procedure.

For the elutriate, required preservation techniques for the various parameters are presented in Section 6.0 of each individual method.

Elutriate, sediment, and water samples should be stored under refrigerated conditions (4° C).

NOTE: Water and sediment samples should not be frozen or dried prior to use.

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of samples used in the elutriate preparation procedure.

NOTE: If trace organic analyses are to be performed, glass containers with Teflon-lined lids are required.

7.0 Calibration and Standardization

No calibration or standardization procedures are required during elutriate preparation.

8.0 Procedure

1. Place 100 mL of unfiltered dredging site water into a 1 L Erlenmeyer flask.
2. Add homogenized, field-moist sediment via a powder funnel to obtain a total volume of 300 mL.
3. Fill the flask to the 1 L mark with unfiltered dredging site water.

NOTE: This procedure should provide 700 - 800 mL of elutriate for analysis.

4. Cap the flask tightly with a noncontaminating stopper and shake vigorously on an automatic shaker at about 100 excursions/minute.

   NOTE: A polyfilm-covered rubber stopper is generally acceptable for minimum contamination.

   NOTE: During the mixing step, the oxygen demand of the dredged material may cause the dissolved oxygen concentration to drop to zero. This change can alter the release of chemical contaminants from dredged material to the disposal site water and reduce the reproducibility of the elutriate test. If it is known that anoxic conditions will not occur at the disposal site or if reproducibility of the elutriate test is a potential problem, the mixing may be accompanied by compressed air-mixing instead of by mechanical mixing using the following procedure:

   a. After preparation of the slurry mixture, an air-diffuser tube is inserted almost to the bottom of the flask.
   b. Compressed air is passed through a Type II water trap and then through the diffuser tube and slurry.
   c. The air flow rate should be adjusted to agitate the mixture vigorously for 30 minutes.
   d. Flasks should be manually stirred at 10 minute intervals to ensure complete mixing.

5. After 30 minutes of shaking or mixing with air, allow the suspension to settle undisturbed for 1 hour.
6. Carefully decant the supernatant into appropriate centrifuge bottles.
7. Select a time and centrifuge speed that will substantially reduce the suspended solids concentration.

   NOTE: The time and speed will be vary depending upon the particle-size distribution of the sediment. More clayey sediment may require longer times at higher speeds than sandy sediments.

8. Filter approximately 100 mL of sample through a 45 µm membrane filter and discard the filtrate.

NOTE: The filters should be soaked in 5 M HCl for at least 2 hours prior to use.

9. Filter the remainder of the sample to give a clear elutriate.

NOTE: Store the elutriate at 4° C.

NOTE: Analyze the elutriate as soon as possible after extraction using the methods specified in this appendix. If necessary, addition of the preservatives specified for the parameters in their respective analytical methods may be added to a subsample of the elutriate sample.

9.0 Quality Control

No quality control procedures are required for elutriate preparation. If precision within a given bulk sediment sample (i.e., a very heterogenous sediment) is a concern, multiple elutriate samples can be prepared and analyzed as separate routine samples.

NOTE: Analytical replicates are required within each of the analytical procedures within this appendix.

10.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Plumb, R.H., Jr. 1981. Procedure for Handling and Chemical Analysis of Sediment and Water Samples. Tech. Rep. EPA/CE-81-1. U.S. Army Engineer Waterways Expt. Station, Vicksburg, MS.

AMMONIA NITROGEN IN WATERS AND ELUTRIATES (COLORIMETRIC, AUTOMATED)

1.0 Scope and Application

This method covers the determination of ammonia in surface water and sediment elutriates in the range of 0.01 to 2.0 mg/L NH_3 as N. This range is for photometric measurements made at 630-660 ηm in a 15 mm or 50 mm tubular flow cell. Higher concentrations can be determined by sample dilution. Approximately 20 to 60 samples per hour can be analyzed.

This procedure is based on Method 350.1 (APHA, 1989).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside. The intensity of the color is automatically determined by measuring sample absorbance at 630 ηm.

3.0 Interferences

Calcium and magnesium ions may be present in concentration sufficient to cause precipitation problems that may interfere with the colorimetric analysis. A 5% EDTA solution is used to prevent the precipitation of calcium and magnesium ions when fresh water samples are analyzed.

Sample turbidity and color may interfere with this method. Turbidity must be removed by filtration prior to analysis. Sample color that absorbs in the photometric range used will also interfere. It may be necessary to distill ammonia from high-color content samples prior to analysis.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Technicon AutoAnalyzer Unit (AAI or AAII) consisting of:
   a. sampler,
   b. manifold (AAI) or analytical cartridge (AAII),
   c. proportioning pump,
   d. heating bath with double delay coil (AAI),
   e. colorimeter equipped with 15 mm tubular flow cell and 630-660 ηm filters,
   f. recorder, and
   g. digital printer for AAII (optional).

4.2 Materials

1. Volumetric flask, class A, 100 mL.
2. Volumetric flask, class A, 1 L.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.

   NOTE: Type II water: Special precaution must be taken to insure that this Type II water is free of ammonia. Such water is prepared by passage of Type II water 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 instruction of the manufacturer. All solutions must be made using ammonia-free Type II water.

2. Disodium ethylenediamine-tetraacetate (EDTA), 5%. Dissolve 50 g of EDTA (disodium salt) and approximately six pellets of NaOH in 1 liter of Type II water.

3. Sodium hypochlorite (NaOCl) solution. Dilute 250 mL of a bleach solution containing 5.25% NaOCl (such as "Clorox") to 500 mL with Type II water. Available chlorine level should approximately 2 to 3%.

   NOTE: Since "Clorox" is a proprietary product, its formulation is subject to change. The analyst must remain alert to detecting any variation in this product significant to its use in this procedure. Due to the instability of this product, storage over an extended period should be avoided.

4. Sodium nitroprusside ($Na_2Fe(CN)_5NO \cdot H_2O$), 0.05%. Dissolve 0.5 g of sodium nitroprusside in 1 liter of Type II water.
5. Sodium phenolate. Using a 1 liter Erlenmeyer flask, dissolve 83 g phenol (C_6H_5OH) in 500 mL of Type II water. In small increments, cautiously add with agitation, 32 g of NaOH. Periodically cool flask under water faucet. When cool, dilute to 1 liter with Type II water.
6. Concentrated sulfuric acid (H_2SO_4), reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
7. Sulfuric acid (H_2SO_4), 5 N, for use as the air scrubber solution. Carefully add 139 mL of concentrated sulfuric acid to approximately 500 mL of ammonia-free Type II water. Cool to room temperature and dilute to 1 liter with ammonia-free Type II water.
8. Ammonia stock solution. Dissolve 3.819 g of anhydrous ammonium chloride (NH_4Cl), dried at 105° C, in Type II water. Dilute to 1 liter with Type II water. (1.0 mg/mL NH_3-N).
9. Standard solution A. Dilute 10.0 mL of ammonia stock solution to 1 liter with Type II water. (0.01 mg/mL NH_3-N).
10. Standard solution B. Dilute 10.0 mL of standard solution A to 1 liter with Type II water. (0.001 mg/mL NH_3-N).

6.0 Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Aqueous samples should be preserved by adding 2 mL concentrated H_2SO_4 per liter. The final pH of acid-preserved samples should be between 1.5 and 2.0. When samples are preserved in this manner, a pH adjustment of the samples and/or the analytical standards may be required before analysis is completed.

A holding time of 28 days after sample collection is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of aqueous samples to be analyzed for ammonia.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Using standard solutions A and B, prepare the following standards in 100 mL volumetric flasks (prepare fresh daily):

NH_3-N, mg/L	mL Standard Solution/100 mL
	Solution B
0.01	1.0
0.02	2.0
0.05	5.0
0.10	10.0
	Solution A
0.20	2.0
0.50	5.0
0.80	8.0
1.00	10.0
1.50	15.0
2.00	20.0

8.0 Procedure

The intensity of the color developed between ammonia and the colorimetric reagents is pH dependent. In order to compensate for this effect, the pH of the samples, the standard ammonia solutions, and the wash water should be similar. This can be accomplished by either (1) adding 2 mL concentrated H_2SO_4 per liter to the standards and wash water or (2) neutralizing the pH of the samples with NaOH or KOH.

1. Select the appropriate manifold for the automated analyses to be run. For a working range of 0.01 to 2.00 mg NH_3-N/L use the AAI set up. For a working range of.01 to 1.0 mg NH_3-N/L, use the AAII set up. Higher concentrations may be accommodated by sample dilution.

   NOTE: Manifold flow rates for the AAI set up are as follows:

Wash Water	2.0 mL/min.
Sample	0.42 mL/min.
EDTA	0.8 mL/min.
Air*	0.23 mL/min.
Na-phenolate	0.42 mL/min.
Na-hypochlorite	0.32 mL/min.
Na-nitroprusside	0.42 mL/min.

   NOTE: Manifold flow rates for the AAII set up are as follows:

Wash Water	2.9 mL/min.
Sample	2.0 mL/min.
EDTA	0.8 mL/min.
Air*	2.0 mL/min.
Na-phenolate	0.6 mL/min.
Na-hypochlorite	0.6 mL/min.
Na-nitroprusside	0.6 mL/min.

   * = air should be scrubbed through 5 N H_2SO_4

2. Allow both colorimeter and recorder to warm up for 30 minutes.
3. Obtain a stable baseline with all reagents, feeding Type II water through sample line.

4. For the AAI system, sample at a rate of 20/hr. 1:1. For the AAII, use a 60/hr 6:1 cam with a common wash.
5. Arrange ammonia standards in the sampler in order of decreasing ammonia concentration.
6. Complete loading of the sampler tray with routine and quality assurance/quality control samples.
7. Switch sample line from distilled water to sampler and begin analysis.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for ammonia in aqueous solutions is 30 µg/L.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured ammonia concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

10.0 Method Performance

In a single laboratory (EMSL-CIN), using surface water samples at concentrations of 1.41, 0.77, 0.59 and 0.43 mg NH_3-N/L, the standard deviation was ± 0.005. In a second single laboratory study (Alberta Pollution Control Laboratory), the calculated coefficient of variation for surface water samples with ammonia concentrations of 0.029, 0.060, and 0.093 mg/L NH_3-N were 4.7, 2.0, and 1.1%, respectively.

In a single laboratory (EMSL-CIN), using surface water samples at concentrations of 0.16 and 1.44 mg NH_3-N/L, recoveries were 107 and 99%, respectively. In a second single laboratory study (Alberta Pollution Control Laboratory), using surface water samples with NH_3-N concentrations of 0.008, 0.015, and 0.039 mg/L, the recoveries were 104, 97, and 105%, respectively.

11.0 Calculations and Reporting

The resultant ammonia concentrations can obtained by comparison of sample peak heights to the standard curve of peak heights of the standards plotted against their corresponding known concentration values.

12.0 References

Alberta Environmental Centre. 1981. Methods Manual for Chemical Analysis of Water and Wastes. Environment Canada, Vegreville, Alberta, Canada.

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. Method 4500-NH_3 H. 17th Edition, APHA, New York, New York. p. 4-126.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Booth, R. L., and L.B. Lobring. 1973. Evaluation of the AutoAnalyzer II: A Progress Report". In Advances in Automated Analysis 8:7-10. 1972 Technicon International Congress. Mediad Incorporated, Tarrytown, N.Y.

Fiore, J., and J.E. O'Brien. 1962. Ammonia Determination by Automatic Analysis. Wastes Engineering 33:352.

Hiller, A., and D. Van Slyke. 1933. Determination of Ammonia in Blood. J. Biol. Chem. 102:499.

O'Connor, B., R. Dobbs, B. Villiers, and R. Dean. 1967. Laboratory Distillation of Municipal Waste Effluents. JWPCF 39:25.

AMMONIA NITROGEN IN WATERS AND ELUTRIATES (COLORIMETRIC, MANUAL)

1.0 Scope and Application

This method covers the determination of ammonia in surface water and sediment elutriates in the range of 0.05 to 1.0 mg NH_3-N/L for the colorimetric procedure. The measurements are made colorimetrically at 425 ηm. Higher concentrations can be determined by sample dilution.

This procedure is based on Method 350.2 (APHA, 1989).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

The sample is buffered at a pH of 9.5 with a borate buffer in order to decrease hydrolysis of cyanates and organic nitrogen compounds, and is then distilled into a solution of boric acid. The ammonia in the distillate is determined colorimetrically by nesslerization.

3.0 Interferences

A number of aromatic and aliphatic amines, as well as other compounds, both organic and inorganic, will cause turbidity upon the addition of Nessler reagent, so direct nesslerization (i.e., without distillation), has been discarded as an official method.

Cyanate, which may be encountered in certain industrial effluents, will hydrolyze to some extent even at the pH of 9.5 at which distillation is carried out. Volatile alkaline compounds, such as certain ketones, aldehydes, and alcohols, may cause an off-color upon nesslerization in the distillation method. Some of these, such as formaldehyde, may be eliminated by boiling off at a low pH (approximately 2 to 3) prior to distillation and nesslerization.

Residual chlorine must also be removed by pretreatment of the sample with sodium thiosulfate or sodium arsenite before distillation.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Kjeldahl distillation apparatus.
4. Spectrophotometer or filter photometer for use at 425 ηm and providing a light path of 1 cm or more.

4.2 Materials

1. Erlenmeyer flask, 500 mL. These flasks should be marked at the 350 and the 500 mL volumes. With such marking, it is not necessary to transfer the distillate to volumetric flasks.
2. Kjeldahl flask, 800 mL.
3. Nessler tubes. Matched Nessler tubes (APHA Standard) about 300 mm long, 17 mm inside diameter, and marked at 225 mm ± 1.5 mm inside measurement from bottom.
4. Volumetric flask, class A, 100 mL.
5. Volumetric flask, class A, 1 L.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.

   NOTE: Special precaution must be taken to insure that this Type II water is free of ammonia. Such water is prepared by passage of Type II water 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 instruction of the manufacturer. All solutions must be made using ammonia-free Type II water.

2. Borate buffer. Add 88 mL of 0.1 N NaOH solution to 500 mL of 0.025 M sodium tetraborate solution (5.0 g anhydrous $Na_2B_4O_7$ or 9.5 g $Na_2B_4O_7 \cdot 10H_2O$ per liter). Dilute to 1 liter with Type II water.

3. Boric acid solution, 2%. Dissolve 20 g H_3BO_3 in ammonia-free Type II water. Dilute to 1 liter with Type II water.
4. Dechlorinating reagents. A number of dechlorinating reagents may be used to remove residual chlorine prior to distillation. These include:

    a. Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$), 0.014 N. Dissolve 3.5 g $Na_2S_2O_3 \cdot 5H_2O$ in ammonia-free Type II water. Dilute to 1 liter with Type II water.

    NOTE: One mL of this solution will remove 1 mg/L of residual chlorine in 500 mL of sample.

    b. Sodium arsenite ($NaAsO_2$), 0.014 N. Dissolve 1.0 g $NaAsO_2$ in ammonia-free Type II water. Dilute to 1 liter with Type II water.

6. Nessler reagent. Dissolve 100 g of mercuric iodide (HgI) and 70 g of potassium iodide (KI) in a small amount of water. Add this mixture slowly, with stirring, to a cooled solution of 160 g of NaOH in 500 mL of Type II water. Dilute to 1 liter with Type II water.

    NOTE: If this reagent is stored in a Pyrex bottle out of direct sunlight, it will remain stable for a period of up to 1 year.

    NOTE: This reagent should give the characteristic color with ammonia within 10 minutes after addition and should not produce a precipitate with small amounts of ammonia ($\leq$0.04 mg/50 mL).

7. Sodium hydroxide (NaOH), 1 N: Dissolve 40 g NaOH in ammonia-free Type II water. Dilute to 1 liter with Type II water.
8. Concentrated sulfuric acid (H_2SO_4), reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
9. Sulfuric acid stock solution, 0.1 N. Add 3 mL of concentrated H_2SO_4 to 1 liter of CO_2-free Type II water.
10. Sulfuric acid standard solution, 0.02 N (0.28 mg/mL NH_3-N). Dilute 200 mL of the sulfuric acid stock solution to 1 liter with CO_2-free Type II water.
11. Ammonia stock solution. Dissolve 3.819 g of anhydrous ammonium chloride (NH_4Cl), dried at 105° C, in Type II water. Dilute to 1 liter with Type II water. (1.0 mg/mL NH_3-N).

12. Ammonia standard solution. Dilute 10.0 mL of ammonia stock solution to 1 liter with Type II water. (0.01 mg/mL NH_3-N).

6.0 Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Aqueous samples should be preserved by adding 2 mL concentrated H_2SO_4 per liter. The final pH of acid-preserved samples should be between 1.5 and 2.0. When samples are preserved in this manner, a pH adjustment of the samples and/or the analytical standards may be required before analysis is completed.

A holding time of 28 days after sample collection is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of aqueous samples to be analyzed for ammonia.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Prepare a series of Nessler tube standards as follows:

mL of Standard (0.01 mg/mL NH_3-N)	mg NH_3-N/50.0 mL
0.0	0.0
0.5	0.005
1.0	0.01
2.0	0.02
3.0	0.03
4.0	0.04
5.0	0.05
8.0	0.08
10.0	0.10

Dilute each tube to 50 mL with Type II water. Add 2.0 mL of Nessler reagent. Mix. After 20 minutes read the absorbance at 425 ηm against the blank. From the values obtained, plot absorbance vs. mg NH_3-N for the standard curve.

The sulfuric acid standard solution must be standardized following one of the two following methods:

a. Standardize the approximately 0.02 N acid against 0.0200 N Na_2CO_3 solution. This sodium carbonate solution is prepared by dissolving 1.060 g anhydrous Na_2CO_3, oven-dried at 140° C, and diluting to 1 liter with CO_2-free Type II water.
b. Standardize the approximately 0.1 N H_2SO_4, solution against a 0.100 N Na_2CO_3 solution. By proper dilution, the 0.02 N acid can then be prepared.

The later method (b.) is preferable.

8.0 Procedure

8.1 Preparation of Equipment

1. Add 500 mL of Type II water to an 800 mL Kjeldahl flask.

NOTE: The addition of boiling chips which have been previously treated with dilute NaOH will prevent bumping.

2. Steam out the distillation apparatus until the distillate shows no trace of ammonia with Nessler reagent.

8.2 Sample Preparation

1. Remove the residual chlorine in the sample by adding dechlorinating agent equivalent to the chlorine residual.
2. Add 1 N NaOH to 400 mL of sample, until the pH is 9.5, checking the pH during addition with a pH meter or by use of a short range pH paper.

8.3 Sample Distillation

1. Transfer the sample, the pH of which has been adjusted to 9.5, to a 800 mL Kjeldahl flask.
2. Add 25 ml of the borate buffer.
3. Distill 300 mL at the rate of 610 mL/min. into 50 mL of 2% boric acid contained in a 500 mL Erlenmeyer flask.

   NOTE: The condenser tip or an extension of the condenser tip must extend below the level of the boric acid solution.

4. Dilute the distillate to 500 mL with Type II water.

8.4 Colorimetric Analysis

1. Allow the spectrophotometer to warm up for 30 minutes.
2. Add 2.0 mL of Nessler reagent to 50 mL of the distillate in a Nessler tube. Mix.
3. After 20 minutes, read the absorbance at 425 ηm.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for ammonia in aqueous solutions is 30 µg/L.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured ammonia concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

10.0 Method Performance

Twenty-four analysts in sixteen laboratories analyzed natural water samples containing exact increments of an ammonium salt with the following results:

Increment as Nitrogen, Ammonia		Precision as Standard Deviation	Accuracy as Bias
mg N/L	mg N/L	%	mg N/L
0.21	0.122	-5.54	-0.01
0.26	0.070	-18.12	-0.05
1.71	0.244	+0.46	+0.01
1.92	0.279	-2.01	-0.04

11.0 Calculations and Reporting

The initial ammonia concentrations can obtained by comparison of sample peak heights to the standard curve of peak heights of the standards plotted against their corresponding known concentration values. These values are then adjusted for dilution effects using the following formula:

$$NH_3\text{-N, mg/L} = \frac{A \times B \times 1{,}000}{D \times C}$$

where:

A = NH_3-N read from standard curve, mg.
B = total distillate collected including boric acid and any dilution, mL.
C = distillate taken for nesslerization, mL.
D = volume of original sample taken, mL.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. Method 4500-NH_3 H. 17th Edition, APHA, New York, New York. p. 4-126.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

CYANIDE IN WATERS AND ELUTRIATES (COLORIMETRIC, AUTOMATED UV)

1.0 Scope and Application

This method is used to determine the concentration of inorganic cyanide in aqueous samples, such as surface water and elutriate samples. The method detects inorganic cyanides that may be present as either simple soluble salts or complex radicals.

The colorimetric method is sensitive to approximately 0.02 mg/L of cyanide and is recommended for concentrations below 1 mg/L. The range of the procedure can be adjusted by modifying the sample preparation technique or the cell path length. However, the amount of sodium hydroxide in the standards and the sample to be analyzed must be the same.

This procedure is based on SW-846 Method 9012 (EPA, 1986).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" to waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

A sample aliquot is treated with strong acid to convert any cyanides that may be present to hydrocyanic acid (HCN). This material is isolated from the original sample matrix by a process of distillation and trapped in a sodium hydroxide solution. The resultant cyanide concentration is determined by automated UV colorimetry.

Cyanide in the distillate is reacted with chloramine-T at a pH less than 8 to produce cyanogen chloride (CNCl). After this reaction is complete, the addition of pyridine-barbituric acid reagent produces a red-blue color that is proportional to the cyanide concentration. The intensity of the color is automatically determined by measuring sample absorbance at 570 ηm. The concentration of NaOH must be the same in the standards, the sample distillate, and any dilutions of the original sample distillate to obtain colors of comparable intensity.

3.0 Interferences

Oxidizing agents such as chlorine will decompose most cyanides. Chlorine interferences can be eliminated by adding an excess of ascorbic acid to the sample prior to preservation and storage (Section 6). This will reduce the chlorine to chloride which does not interfere with the colorimetric procedure.

Sulfides adversely affect the development of color in the analytical procedure. This interference can be reduced or eliminated by adding bismuth nitrate to the samples to precipitate the sulfide prior to distillation (Section 8.1.4). Samples that contain hydrogen sulfide, metal sulfides, or other compounds that may produce sulfide during the distillation procedure should also be treated with bismuth nitrate prior to distillation.

Nitrate and/or nitrite in samples can act as a positive interference when present at concentrations above 10 mg/L and in the presence of certain organic compounds. These nitrogen compounds can form nitrous acid during the distillation process which will react with some organic compounds to form oxides. These oxides will decompose under conditions developed in the colorimetric procedure to generate HCN. This interference is eliminated by treating the samples with sulfamic acid prior to distillation (Section 8.1.5).

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Reflux distillation apparatus. The boiling flask should be of 1-liter size with inlet tube and provision for condenser. The gas absorber is a Fisher-Milligan scrubber (Fisher Catalog #07-513), or equivalent.
4. Automated continuous-flow analytical instrument with:
   a. sampler,
   b. manifold with UV digestor,
   c. proportioning pump,
   d. heating bath with distillation coil,
   e. distillation head,

f. colorimeter equipped with a 15 mm flow cell and 570 ηm filter, and
g. recorder.

4.2 Materials

1. Potassium iodide-starch test paper.
2. Volumetric flasks, class A, 250 mL.
3. Volumetric flasks, class A, 100 mL.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Ascorbic acid ($C_6H_8O_6$), analytical reagent grade crystals.
3. Bismuth nitrate solution ($Bi(NO_3)_3$). Dissolve 30.0 grams of $Bi(NO_3)_3$ in 100 mL of Type II water. While stirring, add 250 mL of glacial acetic acid. Stir until dissolved. Dilute to 1 liter with Type II water.
4. Chloramine-T solution. Dissolve 1.0 g of white, water-soluble, chloramine-T in 100 mL of Type II water. Refrigerate until ready to use.
5. Concentrated acetic acid ($C_4H_6O_3$), glacial, reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
6. Concentrated sulfuric acid (H_2SO_4), reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
7. Sulfuric acid (1:1). Slowly add 500 mL of concentrated H_2SO_4 to 500 mL of Type II water.

   CAUTION: This is an exothermic reaction.

8. Magnesium chloride solution ($MgCl_2 \cdot 6H_2O$). Dissolve 510 g of $MgCl_2 \cdot 6H_2O$ into a 1 liter flask. Dilute to 1 liter with Type II water.
9. Pyridine-barbituric acid reagent. Place 15 g of barbituric acid ($C_4H_4O_3N_2$) in a 250 mL volumetric flask. Add just enough Type II water to wash the sides of the flask and wet the barbituric acid. Add 75 mL of pyridine (C_5H_5N) and mix. Add 15 mL of concentrated HCl. Allow to

cool to room temperature. Dilute to 250 mL with Type II water. This reagent is stable for approximately six months, if stored in a cool, dark place.

10. Sodium dihydrogenphosphate ($NaH_2PO_4.H_2O$), 1 M. Dissolve 138 g of $NaH_2PO_4.H_2O$ in 1 liter of Type II water.
11. Sodium hydroxide solution (NaOH), 1.25 N. Dissolve 50 g of NaOH in Type II water. Dilute to 1 liter with Type II water.
12. Sodium hydroxide solution (NaOH), 1 N. Dissolve 40 g of NaOH in Type II water. Dilute to 1 liter with Type II water.
13. Sodium hydroxide dilution water and receptacle wash water (NaOH), 0.25 N. Dissolve 10.0 g NaOH in 500 mL of Type II water. Dilute to 1 liter with Type II water.
14. Sulfamic acid solution (NH_2SO_3H). Dissolve 40 g of sulfamic acid in Type II water. Dilute to 1 liter with Type II water.
15. Cyanide stock solution. Dissolve 2.51 g of KCN and 2 g KOH in 900 mL of Type II water. Standardize with 0.0192 N $AgNO_3$. Dilute to appropriate concentration of 1 mg/mL.
16. Intermediate standard cyanide solution. Dilute 100.0 mL of stock cyanide solution to 1 liter with Type II water (100 µg/mL CN).
17. Working standard cyanide solution. Prepare fresh daily by diluting 100.0 mL of intermediate cyanide solution to 1 liter with Type II water (10.0 µg/mL CN). Store in a glass-stoppered bottle.

    NOTE: All working standards should contain 2 mL of 1 N NaOH per 100 mL.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Aqueous samples should be immediately preserved in the field by the addition of 10 N sodium hydroxide until the sample pH is $\geq$ 12. A holding time of 14 days after sample collection is generally cited for this parameter.

Oxidizing agents, such as chlorine, decompose most cyanides. To determine whether oxidizing agents are present, test a drop of the sample with

acidified potassium iodide (KI)-starch test paper as soon as the sample is collected. A blue color indicates the need for treatment. Add ascorbic acid a few crystals at a time until a drop of sample produces no color on the indicator. Then add an additional 0.6 g of ascorbic acid for each liter of water.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of aqueous samples to be analyzed for cyanide.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Two methods are given for preparing a standard cyanide curve. Section 7.1 should be followed if the samples do not contain sulfide and Section 7.2 should be followed if the samples to be analyzed contain sulfide. The difference between these two methods is that all the cyanide standards must be carried through the sample distillation process when sulfide is present.

7.1 Standard Curve for Samples without Sulfide.

1. Prepare a series of standards by pipetting suitable volumes of the working standard cyanide solution into 250 mL volumetric flasks. To each standard add 50 mL of 1.25 N sodium hydroxide and dilute to 250 mL with Type II water. Prepare as follows:

mL of Working Standard Solution (1 mL = 10 µg CN)	Concentration (µg CN/250 mL)
0.0	BLANK
1.0	10
2.0	20
5.0	50
10.0	100
15.0	150
20.0	200

It is not imperative that all standards be distilled in the same manner as the samples. It is recommended that at least two standards (a high and a low) be distilled and compared with similar values on the curve to ensure that the distillation technique is reliable. If distilled standards do not agree within ± 10% of the undistilled standards, the analyst should find the cause of the apparent error before proceeding.

2. Prepare a standard curve by plotting absorbances of standards vs. cyanide concentrations.
3. To check the efficiency of the sample distillation, add an increment of cyanide from either the intermediate standard cyanide solution or the working standard cyanide solution to 500 mL of sample to ensure a level of 20 µg/L. Proceed with the analysis as in Section 8.1 - Sample Distillation.

7.2 Standard Curve for Samples with Sulfide

1. All standards must be distilled in the same manner as the samples. A minimum of three standards shall be distilled.
2. Prepare a standard curve by plotting absorbance of standards vs. cyanide concentration.

8.0 Procedure

8.1 Sample Distillation

1. Place 500 mL of sample, or an aliquot diluted to 500 mL, in a 1 liter boiling flask.
2. Pipet 50 mL of 1.25 N sodium hydroxide solution into the absorbing tube of the reflux distillation apparatus. Connect the boiling flask, condenser, absorber, and trap in the train.
3. Adjust the vacuum source to allow a slow stream of air to enter the boiling flask. Approximately two bubbles of air per second should enter the boiling flask.
4. Use lead acetate paper to check the sample for the presence of sulfide. A positive test is indicated by a black color on the paper.

   NOTE: If test is positive, treat the sample by adding 50 mL of bismuth nitrate solution through the air inlet tube after the air rate is set. Mix for 3 minutes prior to addition of H_2SO_4 in step 6.

5. If samples are suspected to contain NO_3 and/or NO_2, add 50 mL of sulfamic acid solution after the air rate is set through the air inlet tube. Mix for 3 min prior to addition of H_2SO_4.
6. Slowly add 50 mL 1:1 H_2SO_4 through the air inlet tube.
7. Rinse the tube with Type II water and allow the airflow to mix the flask contents for 3 min.
8. Pour 20 mL of magnesium chloride into the air inlet and wash down with stream of water.
9. Heat the solution to boiling. Reflux for 1 hr. Turn off heat and continue the airflow for at least 15 min. After cooling the boiling flask, disconnect absorber and close off the vacuum source.
10. Drain the solution from the absorber into a 250 mL volumetric flask. Wash the absorber with Type II water and add the washings to the flask. Dilute to the 250 mL with Type II water. The sample is now ready for analysis.

8.2 Automated Colorimetric Determination

1. Set up the manifold in a hood or a well-ventilated area.
2. Allow colorimeter and recorder to warm up for 30 min.
3. Run a baseline with all reagents feeding Type II water through the sample line.
4. Place appropriate standards in the sampler in order of decreasing concentration.
5. Complete loading of the sampler tray with unknown and quality assurance/quality control samples.
6. When the baseline becomes steady, begin the analysis.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for cyanide in aqueous solutions is 5000 µg/L.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured cyanide concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the instrument detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - cyanide, to the 100 mL aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The matrix spike should be prepared by adding cyanide from the working standard or intermediate cyanide standard to ensure a final concentration of approximately 40 µg/L. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration.

Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

10.0 Method Performance

In a single laboratory (EMSL-CIN) test, using mixed industrial and domestic waste samples at concentrations of 0.06, 0.13, 0.28, and 0.62 mg/L CN, the precision standard deviations were ± 0.005, ± 0.007, ± 0.031, and ± 0.094, respectively.

In the same single laboratory (EMSL-CIN) test, using mixed industrial and domestic waste samples at 0.28 and 0.62 mg/L CN, the analyte recoveries were 85% and 102%, respectively.

11.0 Calculations and Reporting

The method of standard additions shall be used for the analysis of all samples that suffer from matrix interferences, such as those that contain sulfide (see section 11.1). The results of all other colorimetric analyses can obtained by comparison of sample peak heights to the standard curve of peak heights of the standards plotted against their corresponding known concentration values.

11.1 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. Method 4500-CN C and 4500-CN E. 17th Edition, APHA, New York, New York. p. 4-29 - 4-31.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Goulden, P.D., B.K. Afghan, and P. Brooksbank. 1972. Determination of Nanogram Quantities of Simple and Complex Cyanides in Water. Anal. Chem. 44:1845-49.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

CYANIDE IN WATERS AND ELUTRIATES (COLORIMETRIC, MANUAL)

1.0 Scope and Application

This method is used to determine the concentration of inorganic cyanide in aqueous samples, such as surface water and elutriate samples. The method detects inorganic cyanides that may be present as either simple soluble salts or complex radicals.

The colorimetric method is sensitive to approximately 0.02 mg/L of cyanide and is recommended for concentrations below 1 mg/L. The range of the procedure can be adjusted by modifying the sample preparation technique or the cell path length. However, the amount of sodium hydroxide in the standards and the sample to be analyzed must be the same.

This procedure is based on SW-846 Method 9010A (EPA, 1986).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

A sample aliquot is treated with strong acid to convert any cyanides that may be present to hydrocyanic acid (HCN). This material is isolated from the original sample matrix by a process of distillation and trapped in a sodium hydroxide solution. The resultant cyanide concentration is determined colorimetrically.

Cyanide in the distillate is reacted with chloramine-T at a pH less than 8 to produce cyanogen chloride (CNCl). After this reaction is complete, the addition of pyridine-barbituric acid reagent produces a red-blue color that is proportional to the cyanide concentration. The intensity of the color is measured by measuring sample absorbance at 578 ηm. The concentration of NaOH must be the same in the standards, the sample distillate, and any dilutions of the original sample distillate to obtain colors of comparable intensity.

3.0 Interferences

Oxidizing agents such as chlorine will decompose most cyanides. Chlorine interferences can be eliminated by adding an excess of ascorbic acid to the sample prior to preservation and storage (Section 6). This will reduce the chlorine to chloride which does not interfere with the colorimetric procedure.

Sulfides adversely affect the development of color in the analytical procedure. This interference can be reduced or eliminated by adding bismuth nitrate to the samples to precipitate the sulfide prior to distillation (Section 8.1.4). Samples that contain hydrogen sulfide, metal sulfides, or other compounds that may produce sulfide during the distillation procedure should also be treated with bismuth nitrate prior to distillation.

Nitrate and/or nitrite in samples can act as a positive interference when present at concentrations above 10 mg/L and in the presence of certain organic compounds. These nitrogen compounds can form nitrous acid during the distillation process which will react with some organic compounds to form oxides. These oxides will decompose under conditions developed in the colorimetric procedure to generate HCN. This interference is eliminated by treating the samples with sulfamic acid prior to distillation (Section 8.1.5).

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Reflux distillation apparatus. The boiling flask should be of 1-liter size with inlet tube and provision for condenser. The gas absorber is a Fisher-Milligan scrubber (Fisher Catalog #07-513), or equivalent.
4. Spectrophotometer. Suitable for measurements at 578 ηm with a 1.0-cm cell or larger.

4.2 Materials

1. Potassium iodide-starch test paper.
2. Volumetric flasks, class A, 250 mL.

3. Volumetric flasks, class A, 100 mL.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Ascorbic acid ($C_6H_8O_6$), analytical reagent grade crystals.
3. Bismuth nitrate solution ($Bi(NO_3)_3$). Dissolve 30.0 grams of $Bi(NO_3)_3$ in 100 mL of Type II water. While stirring, add 250 mL of glacial acetic acid. Stir until dissolved. Dilute to 1 liter with Type II water.
4. Chloramine-T solution. Dissolve 1.0 g of white, water-soluble, chloramine-T in 100 mL of Type II water. Refrigerate until ready to use.
5. Concentrated acetic acid ($C_4H_6O_3$), glacial, reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
6. Concentrated sulfuric acid (H_2SO_4), reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
7. Sulfuric acid (1:1). Slowly add 500 mL of concentrated H_2SO_4 to 500 mL of Type II water.

   CAUTION: This is an exothermic reaction.

8. Magnesium chloride solution ($MgCl_2 \cdot 6H_2O$). Dissolve 510 g of $MgCl_2 \cdot 6H_2O$ into a 1 liter flask. Dilute to 1 liter with Type II water.
9. Pyridine-barbituric acid reagent. Place 15 g of barbituric acid ($C_4H_4O_3N_2$) in a 250 mL volumetric flask. Add just enough Type II water to wash the sides of the flask and wet the barbituric acid. Add 75 mL of pyridine (C_5H_5N) and mix. Add 15 mL of concentrated HCl. Allow to cool to room temperature. Dilute to 250 mL with Type II water. This reagent is stable for approximately six months, if stored in a cool, dark place.
10. Sodium dihydrogenphosphate ($NaH_2PO_4.H_2O$), 1 M. Dissolve 138 g of $NaH_2PO_4.H_2O$ in 1 liter of Type II water.
11. Sodium hydroxide solution (NaOH), 1.25 N. Dissolve 50 g of NaOH in Type II water. Dilute to 1 liter with Type II water.

12. Sulfamic acid solution (NH_2SO_3H). Dissolve 40 g of sulfamic acid in Type II water. Dilute to 1 liter with Type II water.
13. Cyanide stock solution. Dissolve 2.51 g of KCN and 2 g KOH in 900 mL of Type II water. Standardize with 0.0192 N $AgNO_3$. Dilute to appropriate concentration of 1 mg/mL.
14. Intermediate standard cyanide solution. Dilute 100.0 mL of stock cyanide solution to 1 liter with Type II water (100 µg/mL CN).
15. Working standard cyanide solution. Prepare fresh daily by diluting 100.0 mL of intermediate cyanide solution to 1 liter with Type II water (10.0 µg/mL CN). Store in a glass-stoppered bottle.

    NOTE: All working standards should contain 2 mL of 1 N NaOH per 100 mL.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Aqueous samples should be immediately preserved in the field by the addition of 10 N sodium hydroxide until the sample pH is $\geq$ 12. A holding time of 14 days after sample collection is generally cited for this parameter.

Oxidizing agents, such as chlorine, decompose most cyanides. To determine whether oxidizing agents are present, test a drop of the sample with acidified potassium iodide (KI)-starch test paper as soon as the sample is collected. A blue color indicates the need for treatment. Add ascorbic acid a few crystals at a time until a drop of sample produces no color on the indicator. Then add an additional 0.6 g of ascorbic acid for each liter of water.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of aqueous samples to be analyzed for cyanide.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Two methods are given for preparing a standard cyanide curve. Section 7.1 should be followed if the samples do not contain sulfide and Section 7.2 should be followed if the samples to be analyzed contain sulfide. The difference between these two methods is that all the cyanide standards must be carried through the sample distillation process when sulfide is present.

7.1 Standard Curve for Samples without Sulfide.

1. Prepare a series of standards by pipetting suitable volumes of the working standard cyanide solution into 250 mL volumetric flasks. To each standard add 50 mL of 1.25 N sodium hydroxide and dilute to 250 mL with Type II water. Prepare as follows:

mL of Working Standard Solution (1 mL = 10 µg CN)	Concentration (µg CN/250 mL)
0.0	BLANK
1.0	10
2.0	20
5.0	50
10.0	100
15.0	150
20.0	200

It is not imperative that all standards be distilled in the same manner as the samples. It is recommended that at least two standards (a high and a low) be distilled and compared with similar values on the curve to ensure that the distillation technique is reliable. If distilled standards do not agree within ± 10% of the undistilled standards, the analyst should find the cause of the apparent error before proceeding.

2. Prepare a standard curve by plotting absorbances of standards vs. cyanide concentrations.

3. To check the efficiency of the sample distillation, add an increment of cyanide from either the intermediate standard cyanide solution or the working standard cyanide solution to 500 mL of sample to ensure a level of 20 µg/L. Proceed with the analysis as in Section 8.1 - Sample Distillation.

7.2 Standard Curve for Samples with Sulfide

1. All standards must be distilled in the same manner as the samples. A minimum of three standards shall be distilled.
2. Prepare a standard curve by plotting absorbance of standards vs. cyanide concentration.

8.0 Procedure

8.1 Sample Distillation

1. Place 500 mL of sample, or an aliquot diluted to 500 mL, in a 1 liter boiling flask.
2. Pipet 50 mL of 1.25 N sodium hydroxide solution into the absorbing tube of the reflux distillation apparatus. Connect the boiling flask, condenser, absorber, and trap in the train.
3. Adjust the vacuum source to allow a slow stream of air to enter the boiling flask. Approximately two bubbles of air per second should enter the boiling flask.
4. Use lead acetate paper to check the sample for the presence of sulfide. A positive test is indicated by a black color on the paper.

   NOTE: If test is positive, treat the sample by adding 50 mL of bismuth nitrate solution through the air inlet tube after the air rate is set. Mix for 3 minutes prior to addition of H_2SO_4 in step 6.

5. If samples are suspected to contain NO_3 and/or NO_2, add 50 mL of sulfamic acid solution after the air rate is set through the air inlet tube. Mix for 3 min prior to addition of H_2SO_4.
6. Slowly add 50 mL 1:1 H_2SO_4 through the air inlet tube.
7. Rinse the tube with Type II water and allow the airflow to mix the flask contents for 3 min.
8. Pour 20 mL of magnesium chloride into the air inlet and wash down with stream of water.

9. Heat the solution to boiling. Reflux for 1 hr. Turn off heat and continue the airflow for at least 15 min. After cooling the boiling flask, disconnect absorber and close off the vacuum source.
10. Drain the solution from the absorber into a 250 mL volumetric flask. Wash the absorber with Type II water and add the washings to the flask. Dilute to the 250 mL with Type II water. The sample is now ready for analysis.

8.2 Manual Spectrophotometric Analysis

1. Withdraw 50 mL, or a smaller aliquot diluted to 50 mL with 1.25 N sodium hydroxide solution, of the final sample distillate and transfer to a 100 mL volumetric flask.
2. Add 15.0 mL of sodium dihydrogenphosphate solution. Mix.
3. Add 2 mL of Chloramine-T. Mix.

   NOTE: Some distillates may contain compounds that have a chlorine demand. One minute after the addition of chloramine-T, test for residual chlorine with KI-starch paper. If the test is negative, add an additional 0.5 mL chloramine-T. Recheck after 1 min.

   NOTE: Temperature of reagents may affect the response factor of the colorimetric determination. The reagents stored under refrigerated conditions should be warmed to ambient temperature before use. Also, samples should not be left in a warm instrument to develop color but, instead, should be aliquoted to a cuvette immediately prior to reading the sample absorbance.

4. After 1 to 2 min, add 5 mL of pyridine-barbituric acid solution. Mix.
5. Dilute to 100 mL with Type II water. Mix.
6. Allow 8 min for color development and then read absorbance at 578 ηm in a 1-cm cell within 15 min.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for cyanide in aqueous solutions is 5000 µg/L.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured cyanide concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the instrument detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - cyanide, to the 100 mL aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The matrix spike should be prepared by adding cyanide from the working standard or intermediate cyanide standard to ensure a final concentration of approximately 40 µg/L. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

10.0 Method Performance

In a single laboratory (EMSL-CIN) test, using mixed industrial and domestic waste samples at concentrations of 0.06, 0.13, 0.28, and 0.62 mg/L CN, the precision standard deviations were ± 0.005, ± 0.007, ± 0.031, and ± 0.094, respectively.

In the same single laboratory (EMSL-CIN) test, using mixed industrial and domestic waste samples at 0.28 and 0.62 mg/L CN, the analyte recoveries were 85% and 102%, respectively.

11.0 Calculations and Reporting

The method of standard additions shall be used for the analysis of all samples that suffer from matrix interferences, such as those that contain sulfide (see section 11.1). The results of all other colorimetric analyses can be used to calculate the cyanide concentration in the original sample as follows:

$$\text{CN, µg/L} = \frac{(A \times 1{,}000) \times 50}{(B \times C)}$$

where:

A = CN read from standard curve, µg.
B = volume of original sample for distillation, mL.
C = volume taken for colorimetric analysis, mL.

11.1 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. Method 4500-CN C and 4500-CN E. 17th Edition, APHA, New York, New York. p. 4-29 - 4-31.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Bark, L.S., and H.G. Higson. 1964. Investigation of Reagents for the Colorimetric Determination of Small Amounts of Cyanide. Talanta 2: 471-479.

Elly, C.T. 1968. Recovery of Cyanides by Modified Serfass Distillation. J. Water Poll. Control Fed. 40:848-856.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

ARSENIC IN WATERS AND ELUTRIATES (ICP)

1.0 Scope and Application

Inductively coupled plasma atomic emission spectroscopy (ICP) determines arsenic and numerous other elements that are present in aqueous samples, such as surface water and elutriate samples. The aqueous samples must first be digested with nitric acid prior to analysis.

Detection limits, sensitivity, and optimum concentration ranges for arsenic, and other metals stable in a mixed standard solution with arsenic, will vary with the sample aliquot size, the spectrophotometer being used, and possible matrix interferences. The data shown in Table 1 provide concentration ranges for clean aqueous samples.

The method of standard addition (MSA) shall be used for the analysis of all sample digests unless either serial dilution or matrix spike addition demonstrates that it is not required (see section 9).

This method should only be used by spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

This procedure is based on SW-846 Methods 3010 (for digestion) and 6010 for arsenic quantitation (EPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Prior to analysis, the samples to be analyzed are refluxed with nitric acid in a covered beaker. This step is repeated with additional HNO_3 until the final digestate is light in color and diluted to volume.

This instrumental method measures light emitted by arsenic in the final digest by optical spectrometry. The digested samples are nebulized and the resulting aerosol is transported to a plasma torch. Arsenic-specific atomic-line

emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

The possibility of additional interferences named in section 3.0 should also be recognized and appropriate corrections made when necessary. The procedures that can be used for this purpose are described in section 9.

3.0 Interferences

There are three main forms of interferences that can occur during the analysis of water and elutriate samples by ICP. These three types of interferences include: (1) spectral, (2) physical, and (3) chemical interferences. Each is discussed separately in the following sections.

3.1 Spectral Interferences

Spectral interferences are caused by: (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of simultaneous multi-element instruments must verify the absence of spectral interference from an element in a sample for which there is no instrument detection channel. Potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended

as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferants can be assumed.

The magnitude of the interference effects summarized in Table 2 are expressed as analyte concentration equivalents (i.e., false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that As is to be determined (at 193.696 ηm) in a sample containing approximately 10 mg/L of V. According to Table 2, 100 mg/L of V would yield a false signal for As equivalent to approximately 1.1 mg/L. Therefore, the presence of 10 mg/L of V would result in a false signal for As equivalent to approximately 0.11 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, and argon flow rate.

The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.2 Physical Interferences

Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, or by using the standard additions method. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Further, it has been reported that better control of the argon flow rate improves instrument performance. Improved argon flow rate control can be accomplished with the use of mass flow controllers.

3.3 Chemical Interferences

Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant

with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, etc.), buffering of the sample, matrix matching, and standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Centrifuge and centrifuge tubes.
4. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
5. Inductively coupled argon plasma emission spectrometer.
6. Computer-controlled emission spectrometer with background correction.
7. Radio frequency generator.
8. Argon gas supply, welding grade or better.

4.2 Materials

1. Griffin beakers, 150 mL, or equivalent.
2. Watch glasses, ribbed and plain.
3. Whatman No. 41 filter paper, or equivalent.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
3. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.

4. Nitric acid (1:1). Add 500 mL concentrated HNO_3 to 400 mL Type II water. Dilute to 1 liter with Type II water.
5. Hydrochloric acid (1:1). Add 500 mL concentrated HCl to 400 mL Type II water. Dilute to 1 liter with Type II water.
6. Standard stock solutions. May be purchased or prepared from ultrahigh purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hr at 105° C, unless otherwise specified.

   CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.
7. Arsenic standard stock solution, (100 µg/mL). Dissolve 0.13 g of As_2O_3 weighed accurately to at least four significant figures, in 100 mL of Type II water containing 0.4 g NaOH. Acidify the solution with 2 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
8. Molybdenum standard stock solution, (100 µg/mL). Dissolve 0.20 g $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, weighed accurately to at least four significant figures, in Type II water. Dilute to 1 liter with Type II water.
9. Silica standard stock solution, (100 µg/mL). Do not dry. Dissolve 0.47 g $Na_2SiO_3{\cdot}9H_2O$, weighed accurately to at least four significant figures, in Type II water. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
10. Mixed calibration standard solutions. Care should be taken when preparing mixed standards for ICP analysis to ensure that the elements in the final mixed standard are compatible and stable together. One set of mixed calibration standards that has been found to be useful is listed in Table 3. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interferences or the presence of impurities.

    To prepare the mixed calibration standard for arsenic, combine appropriate volumes of the individual stock solutions indicated in Table 3 in volumetric flask. Add 2 mL 1:1 HNO_3 and 10 mL of 1:1 HCl and dilute to 100 mL with Type II water. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see section 9.8) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should be scanned using a sequential spectrometer to verify

the absence of interelement spectral interference in the recommended mixed standard solutions.

TABLE 3. MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo, and Si
IV	Al, Ca, Cr, K, Na, and Ni

NOTE: Premixed standard solutions (traceable to NIST) containing the combined elements as listed in solutions I through IV are available from a number of commercial vendors.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Aqueous samples should be acidified to a pH of <2 with HNO_3. A holding time of 6 months after sample collection is generally cited for this parameter.

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of aqueous samples to be analyzed for arsenic.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Prepare a calibration blank (see section 9.4.1) and at least three mixed calibration standards in the appropriate concentration range to correlate arsenic concentrations with the ICP's linear response range. Prepare standards for

instrument calibration as instructed in section 5.13. Match the sample matrix and that of the standards as closely as possible.

Calibrate the instrument according to the instrument manufacturer's recommended procedures using typical mixed calibration standard solutions. Flush the system with the calibration blank between each standard. Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.

8.0 Procedure

1. Transfer a 100 mL representative aliquot of the well-mixed aqueous sample to a 150 mL Griffin beaker.
2. Add 3 mL of concentrated HNO_3. Cover the beaker with a ribbed watch glass.
3. Place the beaker on a hot plate and cautiously evaporate to a low volume (5 mL) making certain that the sample does not boil and that no portion of the beaker is allowed to go dry.

   NOTE: If a sample is allowed to go to dryness, low recoveries may result. Should this occur, repeat the steps 1 through 3 with a fresh sample aliquot.

4. Cool the beaker and add 3 mL of concentrated HNO_3.
5. Cover the beaker with a nonribbed watch glass and return to the hot plate. Increase the temperature of the hot plate so that a gentle reflux action occurs.
6. Continue heating, adding additional acid as necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change in appearance with continued refluxing).
7. Uncover the beaker, or use a ribbed watch glass, and evaporate to a low volume (3 mL), not allowing any portion of the bottom of the beaker to go dry. Cool the beaker.
8. Add a small quantity of 1:1 HCl (10 mL/100 of final solution) and warm the beaker for an additional 15 min to dissolve any precipitate or residue resulting from evaporation.
9. Wash down the beaker walls and watch glass with Type II water and, when necessary, filter or centrifuge the sample to remove silicates and other insoluble material that could clog the nebulizer.

NOTE: Filtration should be done only if there is concern that insoluble materials may clog the nebulizer. This additional step can cause sample contamination unless the filter and the filtering apparatus are thoroughly cleaned and prerinsed with dilute HNO_3.

10. Adjust to the final volume of 100 mL with Type II water. The sample is now ready for analysis.
11. The analyst should follow the instructions provided by the instrument's manufacturer. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 min of operation prior to calibration). For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within instrument linear range where coordination factors are valid. The analyst must: (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.
12. Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5% (or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this conditions.
13. Flush the system with the calibration blank solution for at least 1 min before the analysis of each sample. Analyze samples.

NOTE: Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for arsenic in aqueous solutions is 75 µg/L.

The method detection limit specified in this manual is sufficient to meet the Great Lakes Water Quality Criteria for arsenic (360 µg/l). For projects requiring more sensitive analyses of arsenic in waters and elutriates, a graphite furnace atomic absorption procedure has been provided in this methods appendix.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured arsenic concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance of the ICP and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

9.4.1 Calibration Blank

The calibration blank is prepared by diluting 2 mL of 1:1 HNO_3 and of 1:1 HCl to 100 mL with Type II water. Prepare a sufficient quantity to flush the system between standards and samples.

The calibration blank should be analyzed prior to routine sample analysis, after every 10 samples, and at the end of the analytical run. The measured concentration in the calibration blank should be less than or equal to the method detection limit.

9.4.2 Reagent Blank

The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - arsenic, to the 100 mL aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and

analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of ≤ 20%.

9.7 Interference Check Sample

The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the method detection limit for each element. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

The interference check sample should be analyzed at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Results should be within ± 20% of the known concentration.

9.8 Calibration Control Sample

A calibration control sample should be prepared in the same acid matrix as the calibration standards at 10 times the method detection limit. This sample should be prepared in accordance with the instructions provided by the supplier. The calibration control sample is used to verify the integrity of the calibration standards on a weekly basis.

9.9 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

9.9.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the method detection limit after dilution), an analysis of a 1:4 dilution should

agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.9.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The

abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 µg/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported in µg/L with up to three significant figures.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Winge, R.K., V.J. Peterson, and V.A. Fassel. 1979. Inductively coupled plasma-atomic emission spectroscopy: Prominent lines, final report, March 1977 - February 1978, Ames laboratory, Ames, IA. EPA-600/4-79-017. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Table 1. Recommended Wavelengths and Estimated Instrumental Detection Limits.

Element	Wavelength[a] (ηm)	Estimated Detection Limit[b] (µg/L)
Arsenic	193.696	53
Molybdenum	202.030	8
Silicon	288.158	58

a - The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see section 3.1).

b - The estimated instrumental detection limits are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

Table 2. Analyte Concentration Equivalents arising from Interference at the 100 mg/L Level.

		Interferant[a,b]									
Analyte	Wavelength (ηm)	Al	Ca	Cr	Cu	Fe	Mg	Mn	Mo	Tl	V
Arsenic	193.696	1.3	-	0.44	-	-	-	-	-	-	1.1
Molybdenum	202.030	0.05	-	-	-	0.03	-	-	-	-	-
Silicon	288.158	-	-	0.07	-	-	-	-	-	-	0.01

a - Dashes indicate that no interference was observed even when interferants were introduced at the following levels:

Al - 1000 mg/L,
Ca - 1000 mg/L,
Cr - 200 mg/L,
Cu - 200 mg/L,
Fe - 1000 mg/L.
Mg - 1000 mg/L,
Mn - 200 mg/L,
Tl - 200 mg/L,
V - 200 mg/L, and

b - The figures recorded as analyte concentrations are not the actual observed concentrations; to obtain those figures, add the listed concentration to the interferant figure.

Table 4. ICP Precision and Accuracy Data[a]

Element	Sample No. 1			Sample No. 2			Sample No. 3		
	True Value (µg/L)	Mean Reported Value (µg/L)	Mean SD[b] (%)	True Value (µg/L)	Mean Reported Value (µg/L)	Mean SD[b] (%)	True Value (µg/L)	Mean Reported Value (µg/L)	Mean SD[b] (%)
As	200	208	7.5	22	19	23	60	63	17

a - Not all elements were analyzed by all laboratories.
b - SD = standard deviation.

ARSENIC IN WATERS AND ELUTRIATES (GFAA)

1.0 Scope and Application

This method is an atomic absorption procedure suitable for the determination of arsenic in natural water samples and mobility extracts such as sediment elutriates. All samples must be subjected to an acid digestion/oxidation step prior to analysis.

This procedure is based on EPA SW-846 Method 7060 (USEPA, 1986).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Prior to analysis, samples are treated with a mixture of hydrogen peroxide and nitric acid in order to convert organic forms of arsenic to inorganic forms and minimize organic interferences.

Following sample pretreatment, a representative aliquot is spiked with a nickel nitrate solution and placed into a graphite tube furnace. The sample aliquot is then slowly evaporated to dryness, charred (ashed), and atomized. The absorption of hollow cathode or EDL radiation during sample atomization is proportional to the arsenic concentration.

3.0 Interferences

Elemental arsenic and many of its compounds are volatile; therefore, samples may be subject to potential loss of arsenic during sample preparation. Spiked samples and relevant standard reference materials should be processed to determine if the analytical procedure is performing adequately.

Caution should also be employed when selecting the temperature and duration of the sample drying and charring (ashing) cycles. A nickel nitrate solution must be added to all prepared samples prior to analysis to minimize volatilization losses during drying and ashing.

In addition to the normal interferences experienced during graphite furnace analysis, arsenic analysis can suffer from severe nonspecific absorption and light scattering caused by matrix components during atomization. Arsenic analysis is particularly susceptible to these problems because of its low analytical wavelength (193.7 ηm). Simultaneous background correction must be employed to avoid erroneously high results. Aluminum is a severe positive interferant in the analysis of arsenic, especially using D_2 arc background correction. Zeeman background correction is very useful in this situation.

If the analyte is not completely volatilized and removed from the furnace during atomization, memory effects will occur. If this situation is detected by means of blank burns, the tube should be cleaned by operating the furnace at full power at regular intervals in the analytical scheme.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, 3-5 weights covering expected weight range.
3. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
4. Thermometer, 0 to 100° C range.
5. Atomic absorption spectrophotometer, single or dual channel, single- or double-beam instrument having a grating monochromator, photo-multiplier detector, adjustable slits, a wavelength range of 190 to 800 ηm, and provisions for simultaneous background correction and interfacing with a strip-chart recorder.
6. Arsenic hollow cathode lamp, or electrodeless discharge lamp (EDL). EDLs are recommended since they provide better sensitivity for arsenic analyses.
7. Graphite furnace. Any graphite furnace device with the appropriate temperature and timing controls.
8. Strip-chart recorder. A recorder is strongly recommended for furnace work so that there will be a permanent record and so that any problems with the analysis such as drift, incomplete atomization, losses during charring, and changes in sensitivity can easily be recognized.

4.2 Materials

1. Griffin beakers, 250 mL, or equivalent.
2. Volumetric flasks, class A, 10 mL.
3. Volumetric flasks, class A, 50 mL.
4. Pipets. Microliter with disposable tips. Sizes can range from 5 to 1,000 µL, as required.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid is <MDL, then the acid can be used.
3. Hydrogen peroxide, 30% (H_2O_2). Oxidant should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the H_2O_2 is <MDL, then the acid can be used.
4. Arsenic standard stock solution (1,000 mg/L). Either procure a certified aqueous standard from a supplier and verify by comparison with a second standard, or dissolve 1.320 g of arsenic trioxide (As_2O_3, analytical reagent grade), or equivalent, in 100 mL of Type II water containing 4 g NaOH. Acidify the solution with 20 mL concentrated HNO_3 and dilute to 1 liter (1 mg/mL As).
5. Nickel nitrate solution, 5%. Dissolve 24.780 g of ACS reagent grade $Ni(N0_3)_2 \cdot 6H_2O$, or equivalent, in Type II water and dilute to 100 mL.
6. Nickel nitrate solution, 1%. Dilute 20 mL of the 5% nickel nitrate to 100 mL with Type II water.
7. Arsenic working standards. Prepare dilutions of the stock solution to be used as calibration standards at the time of the analysis. Withdraw appropriate aliquot of the stock solution, add 1 mL of concentrated HNO_3, 2 mL of 30% H_2O_2, and 2 mL of the 5% nickel nitrate solution. Dilute to 100 mL with Type II water.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Water and elutriate samples should be acidified to a pH <2 with HNO_3. A holding time of 6 months is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of samples to be analyzed for arsenic in sediments.

NOTE: Special containers (e.g., containers used for volatile organic analysis) may have to be used if the samples are to be analyzed for very volatile arsenic compounds.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The hot plate should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

Prepare a method blank and at least three standards in the appropriate concentration range to correlate arsenic concentrations with the atomic absorption spectrophotometer's linear response range. Prepare standards for instrument calibration by appropriate dilution of the stock arsenic solution. These standards should be prepared fresh on the day of use. Match the sample matrix and that of the standards as closely as possible.

Inject a suitable portion of each standard into the graphite furnace in order of increasing concentration. It is recommended that each standard solution be

analyzed in triplicate in order to assess method precision. Instrument calibration curves should be composed of a minimum of a blank and three standards. A calibration curve should be prepared every day of continuous sample analysis and prior to the initiation of the project's routine sample analysis.

Construct an analytical curve by plotting the average peak absorbance or peak area for the standard solutions as a function of sample concentration on a linear graph. Prepare this graph daily when new initial calibration information is obtained. Alternatively, electronic instrument calibration can be used if the instrument is appropriately equipped.

8.0 Procedure

1. Transfer 100 mL of well-mixed sample to a 250 mL Griffin beaker.
2. Add 2 mL of 30% H_2O_2 and sufficient concentrated HNO_3 to result in an acid concentration of 1% (v/v).
3. Heat for 1 hr at 95° C or until the volume is slightly less than 50 mL.
4. Cool the digested sample. Quantitatively transfer the sample to a 50 mL volumetric flask and dilute to a 50 mL volume with Type II water.
5. Pipet 5 mL of the digested solution into a 10-mL volumetric flask.
6. Add 1 mL of the 1% nickel nitrate solution and dilute to 10 mL with Type II water. The sample is now ready for injection into the furnace.
7. The 193.7 ηm wavelength line and a background correction system are required. Follow the manufacturer's suggestions for all other spectrophotometer parameters.
8. Furnace parameters suggested by the manufacturer should be employed as guidelines. Because temperature-sensing mechanisms and temperature controllers can vary between instruments or with time, the validity of the furnace parameters must be periodically confirmed by systematically altering the furnace parameters while analyzing a standard. In this manner, losses of analyte due to overly high temperature settings or losses in sensitivity due to less than optimum settings can be minimized. Similar verification of furnace parameters may be required for complex sample matrices.
9. Inject a measured microliter (µL) aliquot of sample digest into the furnace and atomize. If the digest concentration is greater than the highest standard, or if the instrument response falls on the plateau of the calibration curve, the sample should be diluted in the same acid matrix and reanalyzed. The use of multiple injections can improve accuracy and help detect furnace pipetting errors.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for arsenic in waters and elutriates is 75 µg/L.

The method detection limit specified in this manual is sufficient to meet the Great Lakes Water Quality Criteria for arsenic (360 µg/l).

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured arsenic concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standard 1643c - Water, should be run to monitor the life and performance of the graphite tube and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a water sample, prepared by spiking ASTM Type II water at the laboratory, and that has undergone multiple analyses by the analytical laboratory. The lot numbers of the As stock solution used in the creation of the LCS should be different from those used to prepare the calibration (both initial and ongoing) standards. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the instrument detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 15 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - arsenic, to the 100 mL sample aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 3 times the estimated or actual method detection limit. The added matrix spike should have a negligible volume when compared to the routine sample volume to eliminate/control sample dilution effects. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

10.0 Method Performance

The optimal concentration range for this method is 5-100 µg/L.

Data summarized in Table 1 provide an estimate of the precision that can be obtained with the method (APHA, 1989).

11.0 Calculations and Reporting

Calculate metal concentrations by (1) the method of standard additions, (2) from a calibration curve, or (3) directly from the instrument's concentration readout. All dilution or concentration factors must be taken into account.

The method of standard additions is the preferred method for the analysis of all sediment elutriates to compensate for any sample matrix effects.

Prepare a standard curve based on the absorbance and concentration of the arsenic standards. Determine the arsenic concentration in each of the sediment digests by comparing the digest absorbance with the standard calibration curve.

Arsenic concentrations in water or elutriate samples should be reported in µg/L.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition, APHA, New York, New York.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Table 1. Method Performance Data for Arsenic by Electrothermal Atomization (APHA, 1989).

Single-Analyst Precision, % RSD

Concentration µg/L	Lab Water	Drinking Water	Surface Water	Effluent 1	Effluent 2
9.78	40	25	15	74	23
227	10	6	8	11	15

Interlaboratory Precision, % RSD

Concentration µg/L	Lab Water	Drinking Water	Surface Water	Effluent 1	Effluent 2
9.78	43	26	37	72	50
227	18	12	13	20	15

Interlaboratory Relative Error, %

Concentration µg/L	Lab Water	Drinking Water	Surface Water	Effluent 1	Effluent 2
9.78	43	26	37	72	50
9.78	36	1	22	106	13
227	3	7	10	19	6

CADMIUM IN WATERS AND ELUTRIATES (ICP)

1.0 Scope and Application

Inductively coupled plasma atomic emission spectroscopy (ICP) determines cadmium and numerous other elements that are present in aqueous samples, such as surface water and elutriate samples. The aqueous samples must first be digested with nitric acid prior to analysis.

Detection limits, sensitivity, and optimum concentration ranges for cadmium, and other metals stable in a mixed standard solution with cadmium, will vary with the sample aliquot size, the spectrophotometer being used, and possible matrix interferences. The data shown in Table 1 provide concentration ranges for clean aqueous samples.

The method of standard addition (MSA) shall be used for the analysis of all sample digests unless either serial dilution or matrix spike addition demonstrates that it is not required (see section 9).

This method should only be used by spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

This procedure is based on SW-846 Methods 3010 (for digestion) and 6010 for quantitation (EPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" to waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Prior to analysis, the samples to be analyzed are refluxed with nitric acid in a covered beaker. This step is repeated with additional HNO_3 until the final digestate is light in color and diluted to volume.

This instrumental method measures light emitted by cadmium in the final digest by optical spectrometry. The digested samples are nebulized and the resulting aerosol is transported to a plasma torch. Cadmium-specific atomic-line

emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

The possibility of additional interferences named in section 3.0 should also be recognized and appropriate corrections made when necessary. The procedures that can be used for this purpose are described in section 9.

3.0 Interferences

There are three main forms of interferences that can occur during the analysis of water and elutriate samples by ICP. These three types of interferences include: (1) spectral, (2) physical, and (3) chemical interferences. Each is discussed separately in the following sections.

3.1 Spectral Interferences

Spectral interferences are caused by: (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of simultaneous multi-element instruments must verify the absence of spectral interference from an element in a sample for which there is no instrument detection channel. Potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended

as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferants can be assumed.

The magnitude of the interference effects summarized in Table 2 are expressed as analyte concentration equivalents (i.e., false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that Cd is to be determined (at 226.502 ηm) in a sample containing approximately 10 mg/L of Fe. According to Table 2, 100 mg/L of Fe would yield a false signal for Cd equivalent to approximately 0.03 mg/L. Therefore, the presence of 10 mg/L of Fe would result in a false signal for Cd equivalent to approximately 0.003 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, and argon flow rate.

The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.2 Physical Interferences

Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, or by using the standard additions method. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Further, it has been reported that better control of the argon flow rate improves instrument performance. Improved argon flow rate control can be accomplished with the use of mass flow controllers.

3.3 Chemical Interferences

Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant

with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, etc.), buffering of the sample, matrix matching, and standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Centrifuge and centrifuge tubes.
4. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
5. Inductively coupled argon plasma emission spectrometer.
6. Computer-controlled emission spectrometer with background correction.
7. Radio frequency generator.
8. Argon gas supply, welding grade or better.

4.2 Materials

1. Griffin beakers, 150 mL, or equivalent.
2. Watch glasses, ribbed and plain.
3. Whatman No. 41 filter paper, or equivalent.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
3. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.

4. Nitric acid (1:1). Add 500 mL concentrated HNO_3 to 400 mL Type II water. Dilute to 1 liter with Type II water.
5. Hydrochloric acid (1:1). Add 500 mL concentrated HCl to 400 mL Type II water. Dilute to 1 liter with Type II water.
6. Standard stock solutions. May be purchased or prepared from ultrahigh purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hr at 105° C, unless otherwise specified.

   CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

7. Beryllium standard stock solution (100 µg/mL). Dissolve 1.970 g $BeSO_4 \cdot 4H_2O$ (analytical reagent grade, undried), in 100 mL of Type II water, add 10.0 ml concentrated HNO_3. Dilute to 1 liter with Type II water.
8. Cadmium standard stock solution (100 µg/mL). Dissolve 0.1100 g CdO (analytical reagent grade) in a minimum amount of 1:1 HNO_3. Heat to increase rate of dissolution. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
9. Lead standard stock solution (100 µg/mL). Dissolve 0.1600 g $Pb(NO_3)_2$ (analytical reagent grade) in a minimum amount of 1:1 HNO_3. Add 10 mL 1:1 HNO_3. Add 10 mL 1:1 HNO_3. Dilute to 1 liter with Type II water.
10. Manganese standard stock solution (100 µg/mL). Dissolve 0.1000 g of manganese metal, in an acid mixture of 10 mL concentrated HCl and 1 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
11. Selenium standard stock solution (100 µg/mL). Dissolve 0.1700 g H_2SeO_3 (analytical reagent grade, undried) in 100 mL of Type II water. Dilute to 1 liter with Type II water.
12. Zinc standard stock solution (100 µg/mL). Dissolve 0.1200 g ZnO in a minimum amount of 1:1 HNO_3. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
13. Mixed calibration standard solutions. Care should be taken when preparing mixed standards for ICP analysis to ensure that the elements in the final mixed standard are compatible and stable together. One set of mixed calibration standards that has been found to be useful is listed in Table 3. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interferences or the presence of impurities.

    To prepare the mixed calibration standard for cadmium, combine appropriate volumes of the individual stock solutions indicated in Table

3 in volumetric flask. Add 2 mL 1:1 HNO_3 and 10 mL of 1:1 HCl and dilute to 100 mL with Type II water. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see section 9.8) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should be scanned using a sequential spectrometer to verify the absence of interelement spectral interference in the recommended mixed standard solutions.

TABLE 3. MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo, and Si
IV	Al, Ca, Cr, K, Na, and Ni

NOTE: Premixed standard solutions (traceable to NIST) containing the combined elements as listed in solutions I through IV are available from a number of commercial vendors.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Aqueous samples should be acidified to a pH of <2 with HNO_3. A holding time of 6 months after sample collection is generally cited for this parameter.

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of aqueous samples to be analyzed for cadmium.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Prepare a calibration blank (see section 9.4.1) and at least three mixed calibration standards in the appropriate concentration range to correlate cadmium concentrations with the ICP's linear response range. Prepare standards for instrument calibration as instructed in section 5.13. Match the sample matrix and that of the standards as closely as possible.

Calibrate the instrument according to the instrument manufacturer's recommended procedures using typical mixed calibration standard solutions. Flush the system with the calibration blank between each standard. Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.

8.0 Procedure

1. Transfer a 100 mL representative aliquot of the well-mixed aqueous sample to a 150 mL Griffin beaker.
2. Add 3 mL of concentrated HNO_3. Cover the beaker with a ribbed watch glass.
3. Place the beaker on a hot plate and cautiously evaporate to a low volume (5 mL) making certain that the sample does not boil and that no portion of the beaker is allowed to go dry.

   NOTE: If a sample is allowed to go to dryness, low recoveries may result. Should this occur, repeat the steps 1 through 3 with a fresh sample aliquot.

4. Cool the beaker and add 3 mL of concentrated HNO_3.
5. Cover the beaker with a nonribbed watch glass and return to the hot plate. Increase the temperature of the hot plate so that a gentle reflux action occurs.
6. Continue heating, adding additional acid as necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change in appearance with continued refluxing).

7. Uncover the beaker, or use a ribbed watch glass, and evaporate to a low volume (3 mL), not allowing any portion of the bottom of the beaker to go dry. Cool the beaker.
8. Add a small quantity of 1:1 HCl (10 mL/100 of final solution) and warm the beaker for an additional 15 min to dissolve any precipitate or residue resulting from evaporation.
9. Wash down the beaker walls and watch glass with Type II water and, when necessary, filter or centrifuge the sample to remove silicates and other insoluble material that could clog the nebulizer.

   NOTE: Filtration should be done only if there is concern that insoluble materials may clog the nebulizer. This additional step can cause sample contamination unless the filter and the filtering apparatus are thoroughly cleaned and prerinsed with dilute HNO_3.

10. Adjust to the final volume of 100 mL with Type II water. The sample is now ready for analysis.
11. The analyst should follow the instructions provided by the instrument's manufacturer. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 min of operation prior to calibration). For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within instrument linear range where coordination factors are valid. The analyst must: (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.
12. Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5% (or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this conditions.
13. Flush the system with the calibration blank solution for at least 1 min before the analysis of each sample. Analyze samples.

NOTE: Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for cadmium in aqueous solutions is 4 µg/L.

The method detection limit specified in this method is insufficient to meet the Great Lakes Water Quality Criteria for cadmium (2.1 µg/L). However, this method has been presented to allow for the use of the ICP as a screening tool for cadmium. If the measured concentrations are greater than 4 µg/L, the Great Lakes Water Quality Criteria has been violated and no further analyses are needed. However, if the measured concentration is below 4 µg/L, cadmium must be quantified using the graphite furnace atomic absorption procedure presented in this appendix.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured cadmium concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance of the ICP and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

9.4.1 Calibration Blank

The calibration blank is prepared by diluting 2 mL of 1:1 HNO_3 and of 1:1 HCl to 100 mL with Type II water. Prepare a sufficient quantity to flush the system between standards and samples.

The calibration blank should be analyzed prior to routine sample analysis, after every 10 samples, and at the end of the analytical run. The measured concentration in the calibration blank should be less than or equal to the method detection limit.

9.4.2 Reagent Blank

The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - cadmium, to the 100 mL aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

9.7 Interference Check Sample

The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the method detection limit for each element. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

The interference check sample should be analyzed at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Results should be within ± 20% of the known concentration.

9.8 Calibration Control Sample

A calibration control sample should be prepared in the same acid matrix as the calibration standards at 10 times the method detection limit. This sample should be prepared in accordance with the instructions provided by the supplier. The calibration control sample is used to verify the integrity of the calibration standards on a weekly basis.

9.9 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor

negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

9.9.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the method detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.9.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.

4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 µg/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported in µg/L with up to three significant figures.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Winge, R.K., V.J. Peterson, and V.A. Fassel. 1979. Inductively coupled plasma-atomic emission spectroscopy: Prominent lines, final report, March 1977 - February 1978, Ames laboratory, Ames, IA. EPA-600/4-79-017. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Table 1. Recommended Wavelengths and Estimated Instrumental Detection Limits.

Element	Wavelength[a] (ηm)	Estimated Detection Limit[b] (µg/L)
Beryllium	313.042	0.3
Cadmium	226.502	4
Lead	220.353	42
Manganese	257.610	2
Selenium	196.026	75
Zinc	213.856	2

a - The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see section 3.1).

b - The estimated instrumental detection limits are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

Table 2. Analyte Concentration Equivalents arising from Interference at the 100 mg/L Level.

Analyte	Wavelength (ηm)	Interferant[a,b] Al	Ca	Cr	Cu	Fe	Mg	Mn	Mi	Tl	V
Beryllium	313.042	-	-	-	-	-	-	-	-	0.04	0.05
Cadmium	226.502	-	-	-	-	0.03	-	-	0.02	-	-
Lead	220.353	0.17	-	-	-	-	-	-	-	-	-
Manganese	257.610	0.005	-	0.01	-	0.002	0.002	-	-	-	-
Selenium	196.026	0.23	-	-	-	0.09	-	-	-	-	-
Zinc	213.856	-	-	-	0.14	-	-	-	0.29	-	-

a - Dashes indicate that no interference was observed even when interferants were introduced at the following levels:

Al - 1000 mg/L,
Ca - 1000 mg/L,
Cr - 200 mg/L,
Cu - 200 mg/L,
Fe - 1000 mg/L.
Mg - 1000 mg/L,
Mn - 200 mg/L,
Tl - 200 mg/L,
V - 200 mg/L, and

b - The figures recorded as analyte concentrations are not the actual observed concentrations. To obtain those figures, add the listed concentration to the interferant figure.

Table 4. ICP Precision and Accuracy Data[a]

Element	Sample No. 1 True Value (µg/L)	Sample No. 1 Mean Reported Value (µg/L)	Sample No. 1 Mean SD[b] (%)	Sample No. 2 True Value (µg/L)	Sample No. 2 Mean Reported Value (µg/L)	Sample No. 2 Mean SD[b] (%)	Sample No. 3 True Value (µg/L)	Sample No. 3 Mean Reported Value (µg/L)	Sample No. 3 Mean SD[b] (%)
Be	750	733	6.2	20	20	9.8	180	176	5.2
Mn	350	345	2.7	15	15	6.7	100	99	3.3
Cd	50	48	12	2.5	2.9	16	14	13	16
Pb	250	236	16	24	30	32	80	80	14
Zn	200	201	5.6	16	19	45	80	82	9.4
Se[c]	40	32	21.9	6	8.5	42	1`0	8.5	8.3

a - Not all elements were analyzed by all laboratories.
b - SD = standard deviation.
c - Results for Se are from two laboratories.

CADMIUM IN WATERS AND ELUTRIATES (GFAA)

1.0 Scope and Application

This method is an graphite furnace atomic absorption (GFAA) procedure suitable for the determination of cadmium in natural water samples and mobility extracts such as sediment elutriates. All samples must be subjected to an acid digestion step prior to analysis.

This procedure is based on EPA SW-846 Methods 3020 for sample digestion and 7131 for cadmium quantitation (USEPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

A mixture of nitric acid and the sample to be analyzed is refluxed in a covered Griffin beaker. This step is repeated with additional portions of nitric acid until the digestate is light in color or until its color has stabilized. After the digestate has been brought to a low volume, it is cooled and brought up in dilute nitric acid such that the final dilution contains 3% (v/v) HNO_3. If the sample contains suspended solids, it must be centrifuged, filtered, or allowed to settle.

Following sample digestion, a representative aliquot is slowly evaporated to dryness, charred (ashed), and atomized in a graphite furnace. The absorption of hollow cathode or EDL radiation during sample atomization is proportional to the cadmium concentration.

3.0 Interferences

The composition of the sample matrix can have a major effect on the analysis. It is those effects which must be determined and taken into consideration in the analysis of each different matrix encountered. To help verify the absence of matrix or chemical interference, the serial dilution technique (see section 9.8.1) may be used. Those samples which indicate the presence of interference should be treated in one or more of the following ways:

1. Successively dilute and reanalyze the samples to eliminate interferences.
2. Modify the sample matrix either to remove interferences or to stabilize the analyte. Examples are the addition of ammonium nitrate to remove alkali chlorides and the addition of ammonium phosphate to retain cadmium. The mixing of hydrogen with the inert purge gas has also been used to suppress chemical interference. The hydrogen acts as a reducing agent and aids in molecular dissociation.
3. Analyze the sample by method of standard additions while noticing the precautions and limitations of its use (see section 9.8.2).

Gases generated in the furnace during atomization may have molecular absorption bands encompassing the analytical wavelength. When this occurs, use either background correction or choose an alternate wavelength. Background correction may also compensate for nonspecific broad-band absorption interference.

Continuum background correction cannot correct for all types of background interference. When the background interference cannot be compensated for, chemically remove the analyte or use an alternate form of background correction, e.g., Zeeman background correction.

Interference from a smoke-producing sample matrix can sometimes be reduced by extending the charring time at a higher temperature or utilizing an ashing cycle in the presence of air. Care must be taken, however, to prevent loss of the analyte.

Spectral interference can occur when an absorbing wavelength of an element present in the sample but not being determined falls within the width of the absorption line of the element of interest. The results of the determination will then be erroneously high, due to the contribution of the interfering element to the atomic absorption signal. Interference can also occur when resonant energy from another element in a multielement lamp, or from a metal impurity in the lamp cathode, falls within the bandpass of the slit setting when that other metal is present in the sample. This type of interference may sometimes be reduced by narrowing the slit width.

Cross-contamination and contamination of the sample can be major sources of error because of the extreme sensitivities achieved with the furnace. The sample preparation work area should be kept scrupulously clean. All glassware should be cleaned as directed in the Materials section (section 4.2). Pipet tips are

a frequent source of contamination. Many yellow plastic tips contain cadmium. Use "cadmium-free" tips. If other pipet tips are suspected of causing sample contamination, they should be acid soaked with 1:5 $HN0_3$ and rinsed thoroughly with tap and Type II water. The use of a better grade of pipet tip can greatly reduce this problem.

Pyrolytic graphite, because of the production process and handling, can become contaminated. As many as five to ten high-temperature burns may be required to clean the tube before use.

In addition to the normal interferences experienced during graphite furnace analysis, cadmium analysis can suffer from severe nonspecific absorption and light scattering caused by matrix components during atomization. Cadmium analysis is particularly susceptible to these problems because of its low analytical wavelength (228.8 ηm). Simultaneous background correction must be employed to avoid erroneously high results.

Excess chloride may cause premature volatilization of cadmium. Ammonium phosphate used as a matrix modifier minimizes this loss.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
4. Thermometer, 0 to 100° C range.
5. Atomic absorption spectrophotometer, single or dual channel, single- or double-beam instrument having a grating monochromator, photo-multiplier detector, adjustable slits, a wavelength range of 190 to 800 ηm, and provisions for simultaneous background correction and interfacing with a strip-chart recorder.
6. Cadmium hollow cathode lamp, or electrodeless discharge lamp (EDL).
7. Graphite furnace. Any graphite furnace device with the appropriate temperature and timing controls.

8. Strip-chart recorder. A recorder is strongly recommended for furnace work so that there will be a permanent record and so that any problems with the analysis such as drift, incomplete atomization, losses during charring, and changes in sensitivity can easily be recognized.

4.2 Materials

1. Commercial grade available argon and nitrogen are required for furnace work.
2. Griffin beakers, 150 mL, or equivalent.
3. Volumetric flasks, class A, 1 L.
4. Volumetric flasks, class A, 100 mL.
5. Pipets. Microliter with disposable tips. Sizes can range from 5 to 100 µL, as required.

   NOTE: All glassware, polypropylene, or Teflon containers, including sample bottles, should be washed in the following sequence: detergent, tap water, 1:1 HNO_3, tap water, 1:1 HCl, tap water, and Type II water.

   NOTE: Chromic acid (H_2CrO_4) should not be used as a cleaning agent for glassware if chromium is to be included in the analytical scheme for the sample digestate.

6. Watch glass, ribbed. Watch glass should be large enough to cover the mouth of the beaker.
7. Watch glass, non-ribbed. Watch glass should be large enough to cover the mouth of the beaker.

5.0 Reagents

1. Ammonium phosphate solution $(NH_4)_2HPO_4$), analytical reagent grade, 40%. Dissolve 40 g of $NH_4)_2HPO_4$ in 50 mL of Type II water. Dilute to 100 mL with Type II water.
2. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
3. Concentrated hydrochloric acid (HCl), spectrograde grade certified for AA use. Acid should be analyzed to verify that contaminants are not

present at levels that will interfere with method performance. If a method blank using the acid is <MDL, then the acid can be used.

4. Hydrochloric acid solution (HCl), 1:1. Add 500 mL of concentrated HCl to 400 mL of Type II water. Dilute to 1 liter with Type II water.
5. Concentrated nitric acid (HNO_3), spectrograde grade certified for AA use. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid is <MDL, then the acid can be used.
6. Nitric acid solution (HNO_3), 1:1. Add 500 mL of concentrated HNO_3 to 400 mL of Type II water. Dilute to 1 liter with Type II water.
7. Cadmium standard stock solution (1,000 mg/L). Either procure a certified aqueous standard from a supplier and verify by comparison with a second standard, or dissolve 1.000 g of cadmium metal, analytical reagent grade, in 20 mL of 1:1 HNO_3. Dilute to 1 liter with Type II water.
8. Cadmium working standards. Prepare dilutions of the stock solution to be used as calibration standards at the time of the analysis. To each 100 mL of standard, add 2 mL of ammonium phosphate solution. The calibration standard should be prepared to contain 0.5% (v/v) HNO_3.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Water and elutriate samples should be acidified to a pH <2 with HNO_3. A holding time of 6 months is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water (see note in section 4.2). Either glass or plastic containers can be used for the storage of samples to be analyzed for cadmium in aqueous samples.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The hot plate should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

Prepare a method blank and at least three standards in the appropriate concentration range to correlate cadmium concentrations with the atomic absorption spectrophotometer's linear response range. Prepare standards for instrument calibration by appropriate dilution of the cadmium standard stock solution. These standards should be prepared fresh on the day of use. Match the sample matrix and that of the standards as closely as possible.

Inject a suitable portion of each standard into the graphite furnace in order of increasing concentration. It is recommended that each standard solution be analyzed in triplicate in order to assess method precision. Instrument calibration curves should be composed of a minimum of a blank and three standards. A calibration curve should be prepared every day of continuous sample analysis and prior to the initiation of the project's routine sample analysis.

Construct an analytical curve by plotting the average peak absorbance or peak area for the standard solutions as a function of sample concentration on a linear graph. Prepare this graph daily when new initial calibration information is obtained. Alternatively, electronic instrument calibration can be used if the instrument is appropriately equipped.

8.0 Procedure

8.1 Sample Preparation

1. Transfer 100 mL of well mixed sample to a 150 mL Griffin beaker.
2. Add 3 mL of concentrated HNO_3.
3. Cover the beaker with a ribbed watch glass.

4. Place the beaker on a hot plate and cautiously evaporate to a low volume (approximately 5 mL) making certain that the sample does not boil and that no portion of the bottom of the beaker is allowed to go dry.
5. Cool the digested sample and add 3 mL of concentrated HNO_3.
6. Cover the beaker with a non-ribbed watch glass.
7. Place the beaker on a hot plate and increase the temperature so that a gentle reflux action occurs.
8. Continue heating, adding additional acid as necessary, until the digestion is complete.

   NOTE: Complete digestion is generally indicated by a light digestate color or if the digestate does not change in appearance with continued refluxing.

9. When the digestion is complete, cover with a ribbed watch glass and evaporate to a low volume (approximately 3 mL).

   NOTE: Do not allow any portion of the beaker bottom to go dry.

10. Add approximately 10 mL of Type II water and mix.
11. Continue warming the beaker for 10 to 15 minutes to allow additional solubilization of any residue to occur.
12. Remove beaker from hot plate and wash down the beaker walls and watch glass with Type II water.

    NOTE: It may be necessary to filter or centrifuge the sample to remove silicates and other insoluble material that may interfere with injecting the sample into the graphite furnace.

13. Adjust the final volume to 100 mL with Type II water.
14. Add 2 mL of ammonium phosphate solution to each sample. The sample is now ready for analysis.

8.2 Sample Analysis

1. The 228.8 ηm wavelength line and a background correction system are required. Follow the manufacturer's suggestions for all other spectrophotometer parameters.
2. Furnace parameters suggested by the manufacturer should be employed as guidelines. Because temperature-sensing

mechanisms and temperature controllers can vary between instruments or with time, the validity of the furnace parameters must be periodically confirmed by systematically altering the furnace parameters while analyzing a standard. In this manner, losses of analyte due to overly high temperature settings or losses in sensitivity due to less than optimum settings can be minimized. Similar verification of furnace parameters may be required for complex sample matrices.

3. Inject a measured microliter (µL) aliquot of sample digest into the furnace and atomize. If the digest concentration is greater than the highest standard, or if the instrument response falls on the plateau of the calibration curve, the sample should be diluted in the same acid matrix and reanalyzed. The use of multiple injections can improve accuracy and help detect furnace pipetting errors.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for cadmium in waters and elutriates is 1 µg/L.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured cadmium concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standard 1643c - Water, should be run to monitor the life and performance of the graphite tube and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a water sample, prepared by spiking ASTM Type II water at the laboratory, and that has undergone multiple analyses by the analytical laboratory. The lot numbers of the As stock solution used in the creation of the LCS should be different from those used to prepare the calibration (both initial and ongoing) standards. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 15 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - cadmium, to the 100 mL sample aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 3 times the estimated or actual method detection limit. The added matrix spike should have a negligible volume when compared to the routine sample volume to eliminate/control sample dilution effects. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

9.8 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values. Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition may be use (see Section 9.8.2).

9.8.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the method detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.8.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through

the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

The performance characteristics for an aqueous sample free of interferences are:

Optimum concentration range: 0.5-10 µg/L.
Detection limit: 0.1 µg/L.

Precision and accuracy data shown in Table 1 were obtained from records of state and contractor laboratories (USEPA, 1979).

11.0 Calculations and Reporting

Calculate metal concentrations by (1) the method of standard additions, (2) from a calibration curve, or (3) directly from the instrument's concentration readout. All dilution or concentration factors must be taken into account.

The method of standard additions is the preferred method for the analysis of all sediment elutriates to compensate for any sample matrix effects.

Prepare a standard curve based on the absorbance and concentration of the cadmium standards. Determine the cadmium concentration in each of the sample digests by comparing the digest absorbance with the standard calibration curve.

If dilution of sample was required, the following formula is provided to account for the dilution:

$$\text{Cd, } \mu g/L = \frac{A \times (B + C)}{C}$$

where:

A = metal in diluted aliquot from calibration curve, µg/L.
B = acid blank matrix used for dilution, mL.
C = sample aliquot, mL.

Cadmium concentrations in water or elutriate samples should be reported in µg/L.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition, APHA, New York, New York.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Table 1. Method Performance Data.

Number of Labs	True Value µg/L	Mean Value µg/L	Standard Deviation µg/L	Accuracy as % Bias
74	71	70	21	-2.2
73	78	74	18	-5.7
63	14	16.8	11.0	19.8
68	18	18.3	10.3	1.9
55	1.4	3.3	5.0	135
51	2.8	2.9	2.8	4.7

CHROMIUM IN WATERS AND ELUTRIATES (ICP)

1.0 Scope and Application

Inductively coupled plasma atomic emission spectroscopy (ICP) determines chromium and numerous other elements that are present in aqueous samples, such as surface water and elutriate samples. The aqueous samples must first be digested with nitric acid prior to analysis.

Detection limits, sensitivity, and optimum concentration ranges for chromium, and other metals stable in a mixed standard solution with chromium, will vary with the sample aliquot size, the spectrophotometer being used, and possible matrix interferences. The data shown in Table 1 provide concentration ranges for clean aqueous samples.

The method of standard addition (MSA) shall be used for the analysis of all sample digests unless either serial dilution or matrix spike addition demonstrates that it is not required (see section 9).

This method should only be used by spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

This procedure is based on SW-846 Methods 3010 (for digestion) and 6010 for quantitation (EPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Prior to analysis, the samples to be analyzed are refluxed with nitric acid in a covered beaker. This step is repeated with additional HNO_3 until the final digestate is light in color and diluted to volume.

This instrumental method measures light emitted by chromium in the final digest by optical spectrometry. The digested samples are nebulized and the resulting aerosol is transported to a plasma torch. Chromium-specific atomic-line

emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

The possibility of additional interferences named in section 3.0 should also be recognized and appropriate corrections made when necessary. The procedures that can be used for this purpose are described in section 9.

3.0 Interferences

There are three main forms of interferences that can occur during the analysis of water and elutriate samples by ICP. These three types of interferences include: (1) spectral, (2) physical, and (3) chemical interferences. Each is discussed separately in the following sections.

3.1 Spectral Interferences

Spectral interferences are caused by: (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of simultaneous multi-element instruments must verify the absence of spectral interference from an element in a sample for which there is no instrument detection channel. Potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended

as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferants can be assumed.

The magnitude of the interference effects summarized in Table 2 are expressed as analyte concentration equivalents (i.e., false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that Cd is to be determined (at 267.716 ηm) in a sample containing approximately 10 mg/L of Mn. According to Table 2, 100 mg/L of Mn would yield a false signal for Cr equivalent to approximately 0.04 mg/L. Therefore, the presence of 10 mg/L of Mn would result in a false signal for Cr equivalent to approximately 0.004 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, and argon flow rate.

The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.2 Physical Interferences

Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, or by using the standard additions method. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Further, it has been reported that better control of the argon flow rate improves instrument performance. Improved argon flow rate control can be accomplished with the use of mass flow controllers.

3.3 Chemical Interferences

Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant

with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, etc.), buffering of the sample, matrix matching, and standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Centrifuge and centrifuge tubes.
4. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
5. Inductively coupled argon plasma emission spectrometer.
6. Computer-controlled emission spectrometer with background correction.
7. Radio frequency generator.
8. Argon gas supply, welding grade or better.

4.2 Materials

1. Griffin beakers, 150 mL, or equivalent.
2. Watch glasses, ribbed and plain.
3. Whatman No. 41 filter paper, or equivalent.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
3. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.

4. Nitric acid (1:1). Add 500 mL concentrated HNO_3 to 400 mL Type II water. Dilute to 1 liter with Type II water.
5. Hydrochloric acid (1:1). Add 500 mL concentrated HCl to 400 mL Type II water. Dilute to 1 liter with Type II water.
6. Standard stock solutions. May be purchased or prepared from ultrahigh purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hr at 105° C, unless otherwise specified.

   CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.
7. Aluminum standard stock solution (100 µg/mL). Dissolve 0.1000 g of aluminum metal in an acid mixture of 4 mL of 1:1 HCl and 1 mL of concentrated HNO_3 in a beaker. Warm gently to effect solution. When solution is complete, transfer quantitatively to a liter flask, add an additional 10 mL of 1:1 HCl. Dilute to 1 liter with Type II water.
8. Calcium standard stock solution (100 µg/mL). Suspend 0.2500 g $CaCO_3$ dried at 180° C for 1 hr before weighing in Type II water and dissolve cautiously with a minimum amount of 1:1 HNO_3. Add 10.0 mL of concentrated HNO_3. Dilute to 1 liter with Type II water.
9. Chromium standard stock solution (100 µg/mL). Dissolve 0.1900 g CrO_3 in Type II water. When solution is complete, acidify with 10 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
10. Potassium standard stock solution (100 µg/mL). Dissolve 0.1900 g KCl dried at 110° C in Type II water. Dilute to 1 liter with Type II water.
11. Sodium standard stock solution (100 µg/mL). Dissolve 0.2500 g NaCl in Type II water. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
12. Nickel standard stock solution (100 µg/mL). Dissolve 0.1000 g of nickel metal in 10.0 mL hot concentrated HNO_3. Cool. Dilute to 1 liter with Type II water.
13. Mixed calibration standard solutions. Care should be taken when preparing mixed standards for ICP analysis to ensure that the elements in the final mixed standard are compatible and stable together. One set of mixed calibration standards that has been found to be useful is listed in Table 3. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interferences or the presence of impurities.

    To prepare the mixed calibration standard for chromium, combine appropriate volumes of the individual stock solutions indicated in Table 3 in volumetric flask. Add 2 mL 1:1 HNO_3 and 10 mL of 1:1 HCl and

dilute to 100 mL with Type II water. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see section 9.8) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should be scanned using a sequential spectrometer to verify the absence of interelement spectral interference in the recommended mixed standard solutions.

TABLE 3. MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo, and Si
IV	Al, Ca, Cr, K, Na, and Ni

NOTE: Premixed standard solutions (traceable to NIST) containing the combined elements as listed in solutions I through IV are available from a number of commercial vendors.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Aqueous samples should be acidified to a pH of <2 with HNO_3. A holding time of 6 months after sample collection is generally cited for this parameter.

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of aqueous samples to be analyzed for chromium.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Prepare a calibration blank (see section 9.4.1) and at least three mixed calibration standards in the appropriate concentration range to correlate chromium concentrations with the ICP's linear response range. Prepare standards for instrument calibration as instructed in section 5.14. Match the sample matrix and that of the standards as closely as possible.

Calibrate the instrument according to the instrument manufacturer's recommended procedures using typical mixed calibration standard solutions. Flush the system with the calibration blank between each standard. Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.

8.0 Procedure

1. Transfer a 100 mL representative aliquot of the well-mixed aqueous sample to a 150 mL Griffin beaker.
2. Add 3 mL of concentrated HNO_3. Cover the beaker with a ribbed watch glass.
3. Place the beaker on a hot plate and cautiously evaporate to a low volume (5 mL) making certain that the sample does not boil and that no portion of the beaker is allowed to go dry.

   NOTE: If a sample is allowed to go to dryness, low recoveries may result. Should this occur, repeat the steps 1 through 3 with a fresh sample aliquot.

4. Cool the beaker and add 3 mL of concentrated HNO_3.
5. Cover the beaker with a nonribbed watch glass and return to the hot plate. Increase the temperature of the hot plate so that a gentle reflux action occurs.
6. Continue heating, adding additional acid as necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change in appearance with continued refluxing).

7. Uncover the beaker, or use a ribbed watch glass, and evaporate to a low volume (3 mL), not allowing any portion of the bottom of the beaker to go dry. Cool the beaker.
8. Add a small quantity of 1:1 HCl (10 mL/100 of final solution) and warm the beaker for an additional 15 min to dissolve any precipitate or residue resulting from evaporation.
9. Wash down the beaker walls and watch glass with Type II water and, when necessary, filter or centrifuge the sample to remove silicates and other insoluble material that could clog the nebulizer.

   NOTE: Filtration should be done only if there is concern that insoluble materials may clog the nebulizer. This additional step can cause sample contamination unless the filter and the filtering apparatus are thoroughly cleaned and prerinsed with dilute HNO_3.

10. Adjust to the final volume of 100 mL with Type II water. The sample is now ready for analysis.
11. The analyst should follow the instructions provided by the instrument's manufacturer. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 min of operation prior to calibration). For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within instrument linear range where coordination factors are valid. The analyst must: (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.
12. Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5% (or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this conditions.
13. Flush the system with the calibration blank solution for at least 1 min before the analysis of each sample. Analyze samples.

NOTE: Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for chromium in aqueous solutions is 7 µg/L.

The method detection limit specified in this method is marginally sufficient to meet the Great Lakes Water Quality Criteria for chromium (16 µg/L). If concern is expressed for concentrations at or near the detection limit and Great Lakes Water Quality Criteria, a method for quantifying chromium using the graphite furnace atomic absorption procedure has been presented in this methods appendix.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured chromium concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance of the ICP and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to correct

for possible contamination resulting from varying amounts of the acids used in the sample processing.

9.4.1 Calibration Blank

The calibration blank is prepared by diluting 2 mL of 1:1 HNO_3 and of 1:1 HCl to 100 mL with Type II water. Prepare a sufficient quantity to flush the system between standards and samples.

The calibration blank should be analyzed prior to routine sample analysis, after every 10 samples, and at the end of the analytical run. The measured concentration in the calibration blank should be less than or equal to the method detection limit.

9.4.2 Reagent Blank

The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - chromium, to the 100 mL aliquot of a routine sample for which an independent estimate of the target analyte

concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of ≤ 20%.

9.7 Interference Check Sample

The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the method detection limit for each element. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

The interference check sample should be analyzed at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Results should be within ± 20% of the known concentration.

9.8 Calibration Control Sample

A calibration control sample should be prepared in the same acid matrix as the calibration standards at 10 times the method detection limit. This sample should be prepared in accordance with the instructions provided by the supplier. The calibration control sample is used to verify the integrity of the calibration standards on a weekly basis.

9.9 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

9.9.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the method detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.9.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 µg/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported in µg/L with up to three significant figures.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Winge, R.K., V.J. Peterson, and V.A. Fassel. 1979. Inductively coupled plasma-atomic emission spectroscopy: Prominent lines, final report, March 1977 - February 1978, Ames laboratory, Ames, IA. EPA-600/4-79-017. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Table 1. Recommended Wavelengths and Estimated Instrumental Detection Limits.

Element	Wavelength[a] (ηm)	Estimated Detection Limit[b] (µg/L)
Aluminum	308.215	45
Chromium	267.716	7
Copper	324.754	6
Nickel	231.604	15
Potassium	766.491	See footnote c
Sodium	588.995	29

a - The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see section 3.1).
b - The estimated instrumental detection limits are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.
c - Highly dependent on operating conditions and plasma position.

Table 2. Analyte Concentration Equivalents arising from Interference at the 100 mg/L Level.

Analyte	Wavelength (ηm)	Interferant[a,b] Al	Ca	Cr	Cu	Fe	Mg	Mn	Mo	Tl	V
Aluminum	308.215	-	-	-	-	-	-	0.21	-	-	1.4
Calcium	317.933	-	-	0.08	-	0.01	0.01	0.04	-	0.03	0.03
Chromium	267.716	-	-	-	-	0.003	-	0.04	-	-	0.04
Nickel	231.604	-	-	-	-	-	-	-	-	-	-
Sodium	588.995	0.30	-	-	-	-	-	-	-	-	-

a - Dashes indicate that no interference was observed even when interferants were introduced at the following levels:

Al - 1000 mg/L,
Ca - 1000 mg/L,
Cr - 200 mg/L,
Cu - 200 mg/L,
Fe - 1000 mg/L.
Mg - 1000 mg/L,
Mn - 200 mg/L,
Tl - 200 mg/L,
V - 200 mg/L, and

b - The figures recorded as analyte concentrations are not the actual observed concentrations; to obtain those figures, add the listed concentration to the interferant figure.

Table 4. ICP Precision and Accuracy Data[a]

Element	Sample No. 1 True Value (µg/L)	Sample No. 1 Mean Reported Value (µg/L)	Sample No. 1 Mean SD[b] (%)	Sample No. 2 True Value (µg/L)	Sample No. 2 Mean Reported Value (µg/L)	Sample No. 2 Mean SD[b] (%)	Sample No. 3 True Value (µg/L)	Sample No. 3 Mean Reported Value (µg/L)	Sample No. 3 Mean SD[b] (%)
Cr	150	149	3.8	10	10	18	50	50	3.3
Al	700	695	5.6	60	62	33	160	161	13
Ni	250	245	5.8	30	28	11	60	55	14

a - Not all elements were analyzed by all laboratories.
b - SD = standard deviation.

CHROMIUM IN WATERS AND ELUTRIATES (GFAA)

1.0 Scope and Application

This method is an graphite furnace atomic absorption (GFAA) procedure suitable for the determination of chromium in natural water samples and mobility extracts such as sediment elutriates. All samples must be subjected to an acid digestion step prior to analysis.

This procedure is based on EPA SW-846 Methods 3020 for sample digestion and 7191 for chromium quantitation (USEPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

A mixture of nitric acid and the sample to be analyzed is refluxed in a covered Griffin beaker. This step is repeated with additional portions of nitric acid until the digestate is light in color or until its color has stabilized. After the digestate has been brought to a low volume, it is cooled and brought up in dilute nitric acid such that the final dilution contains 3% (v/v) HNO_3. If the sample contains suspended solids, it must be centrifuged, filtered, or allowed to settle.

Following sample digestion, a representative aliquot is slowly evaporated to dryness, charred (ashed), and atomized in a graphite furnace. The absorption of hollow cathode or EDL radiation during sample atomization is proportional to the chromium concentration.

3.0 Interferences

The composition of the sample matrix can have a major effect on the analysis. It is those effects which must be determined and taken into consideration in the analysis of each different matrix encountered. To help verify the absence of matrix or chemical interference, the serial dilution technique (see section 9.8.1) may be used. Those samples which indicate the presence of interference should be treated in one or more of the following ways:

1. Successively dilute and reanalyze the samples to eliminate interferences.
2. Modify the sample matrix either to remove interferences or to stabilize the analyte. Examples are the addition of ammonium nitrate to remove alkali chlorides and the addition of ammonium phosphate to retain cadmium. The mixing of hydrogen with the inert purge gas has also been used to suppress chemical interference. The hydrogen acts as a reducing agent and aids in molecular dissociation.
3. Analyze the sample by method of standard additions while noticing the precautions and limitations of its use (see section 9.8.2).

Gases generated in the furnace during atomization may have molecular absorption bands encompassing the analytical wavelength. When this occurs, use either background correction or choose an alternate wavelength. Background correction may also compensate for nonspecific broad-band absorption interference.

Continuum background correction cannot correct for all types of background interference. When the background interference cannot be compensated for, chemically remove the analyte or use an alternate form of background correction, e.g., Zeeman background correction.

Interference from a smoke-producing sample matrix can sometimes be reduced by extending the charring time at a higher temperature or utilizing an ashing cycle in the presence of air. Care must be taken, however, to prevent loss of the analyte.

Spectral interference can occur when an absorbing wavelength of an element present in the sample but not being determined falls within the width of the absorption line of the element of interest. The results of the determination will then be erroneously high, due to the contribution of the interfering element to the atomic absorption signal. Interference can also occur when resonant energy from another element in a multielement lamp, or from a metal impurity in the lamp cathode, falls within the bandpass of the slit setting when that other metal is present in the sample. This type of interference may sometimes be reduced by narrowing the slit width.

Cross-contamination and contamination of the sample can be major sources of error because of the extreme sensitivities achieved with the furnace. The sample preparation work area should be kept scrupulously clean. All glassware should be cleaned as directed in the Materials section (section 4.2). Pipet tips are

a frequent source of contamination. If other pipet tips are suspected of causing sample contamination, they should be acid soaked with 1:5 $HN0_3$ and rinsed thoroughly with tap and Type II water. The use of a better grade of pipet tip can greatly reduce this problem.

Pyrolytic graphite, because of the production process and handling, can become contaminated. As many as five to ten high-temperature burns may be required to clean the tube before use.

Low concentrations of calcium and/or phosphate may cause interferences. At concentrations above 200 mg/L, calcium's effect is constant and eliminates the effect of phosphate. Calcium nitrate is therefore added to ensure a known constant effect.

Nitrogen should not be used as the purge gas because of a possible CN band interference.

Background correction may be required because nonspecific absorption and scattering can be significant at the analytical wavelength. Background correction with certain instruments may be difficult at this wavelength due to low-intensity output from hydrogen or deuterium lamps. Consult the specific instrument manufacturer's literature for details.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
4. Thermometer, 0 to 100° C range.
5. Atomic absorption spectrophotometer, single or dual channel, single- or double-beam instrument having a grating monochromator, photo-multiplier detector, adjustable slits, a wavelength range of 190 to 800 ηm, and provisions for simultaneous background correction and interfacing with a strip-chart recorder.

6. Chromium hollow cathode lamp, or electrodeless discharge lamp (EDL).
7. Graphite furnace. Any graphite furnace device with the appropriate temperature and timing controls.
8. Strip-chart recorder. A recorder is strongly recommended for furnace work so that there will be a permanent record and so that any problems with the analysis such as drift, incomplete atomization, losses during charring, and changes in sensitivity can easily be recognized.

4.2 Materials

1. Commercial grade available argon and nitrogen are required for furnace work.
2. Griffin beakers, 150 mL, or equivalent.
3. Volumetric flasks, class A, 1 L.
4. Volumetric flasks, class A, 100 mL.
5. Pipets. Microliter with disposable tips. Sizes can range from 5 to 100 µL, as required.

   NOTE: All glassware, polypropylene, or Teflon containers, including sample bottles, should be washed in the following sequence: detergent, tap water, 1:1 HNO_3, tap water, 1:1 HCl, tap water, and Type II water.

   NOTE: Chromic acid (H_2CrO_4) should not be used as a cleaning agent for glassware if chromium is to be included in the analytical scheme for the sample digestate.

6. Watch glass, ribbed. Watch glass should be large enough to cover the mouth of the beaker.
7. Watch glass, non-ribbed. Watch glass should be large enough to cover the mouth of the beaker.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.

2. Calcium nitrate solution ($Ca(NO_3)_2 \cdot 4H_2O$). Dissolve 11.8 g of calcium nitrate, $Ca(NO_3)_2 \cdot 4H_2O$ (analytical reagent grade), in 500 mL of Type II water. Dilute to 1 liter with Type II water.
3. Concentrated hydrochloric acid (HCl), spectrograde grade certified for AA use. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid is <MDL, then the acid can be used.
4. Hydrochloric acid solution (HCl), 1:1. Add 500 mL of concentrated HCl to 400 mL of Type II water. Dilute to 1 liter with Type II water.
5. Concentrated nitric acid (HNO_3), spectrograde grade certified for AA use. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid is <MDL, then the acid can be used.
6. Nitric acid solution (HNO_3), 1:1. Add 500 mL of concentrated HNO_3 to 400 mL of Type II water. Dilute to 1 liter with Type II water.
7. Chromium standard stock solution (1,000 mg/L). Either procure a certified aqueous standard from a supplier and verify by comparison with a second standard, or dissolve 1.923 g of chromium trioxide (CrO_3, analytical reagent grade) in Type II water. Acidify with 10 mL redistilled HNO_3. Dilute to 1 liter with Type II water.
8. Chromium working standards. Prepare dilutions of the stock solution to be used as calibration standards at the time of the analysis. To each 100 mL of standard, add 1 mL of calcium nitrate solution. The calibration standard should be prepared to contain 0.5% (v/v) HNO_3.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Water and elutriate samples should be acidified to a pH <2 with HNO_3. A holding time of 6 months is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water (see note in section 4.2). Either glass or plastic containers can be used for the storage of samples to be analyzed for chromium in aqueous samples.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The hot plate should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

Prepare a method blank and at least three standards in the appropriate concentration range to correlate chromium concentrations with the atomic absorption spectrophotometer's linear response range. Prepare standards for instrument calibration by appropriate dilution of the chromium standard stock solution. These standards should be prepared fresh on the day of use. Match the sample matrix and that of the standards as closely as possible.

Inject a suitable portion of each standard into the graphite furnace in order of increasing concentration. It is recommended that each standard solution be analyzed in triplicate in order to assess method precision. Instrument calibration curves should be composed of a minimum of a blank and three standards. A calibration curve should be prepared every day of continuous sample analysis and prior to the initiation of the project's routine sample analysis.

Construct an analytical curve by plotting the average peak absorbance or peak area for the standard solutions as a function of sample concentration on a linear graph. Prepare this graph daily when new initial calibration information is obtained. Alternatively, electronic instrument calibration can be used if the instrument is appropriately equipped.

8.0 Procedure

8.1 Sample Preparation

1. Transfer 100 mL of well mixed sample to a 150 mL Griffin beaker.
2. Add 3 mL of concentrated HNO_3.
3. Cover the beaker with a ribbed watch glass.

4. Place the beaker on a hot plate and cautiously evaporate to a low volume (approximately 5 mL) making certain that the sample does not boil and that no portion of the bottom of the beaker is allowed to go dry.
5. Cool the digested sample and add 3 mL of concentrated HNO_3.
6. Cover the beaker with a non-ribbed watch glass.
7. Place the beaker on a hot plate and increase the temperature so that a gentle reflux action occurs.
8. Continue heating, adding additional acid as necessary, until the digestion is complete.

   NOTE: Complete digestion is generally indicated by a light digestate color or if the digestate does not change in appearance with continued refluxing.

9. When the digestion is complete, cover with a ribbed watch glass and evaporate to a low volume (approximately 3 mL).

   NOTE: Do not allow any portion of the beaker bottom to go dry.

10. Add approximately 10 mL of Type II water and mix.
11. Continue warming the beaker for 10 to 15 minutes to allow additional solubilization of any residue to occur.
12. Remove beaker from hot plate and wash down the beaker walls and watch glass with Type II water.

    NOTE: It may be necessary to filter or centrifuge the sample to remove silicates and other insoluble material that may interfere with injecting the sample into the graphite furnace.

13. Adjust the final volume to 100 mL with Type II water.
14. Add 1 mL of calcium nitrate solution to each sample. The sample is now ready for analysis.

8.2 Sample Analysis

1. The 357.9 ηm wavelength line and a background correction system are required. Follow the manufacturer's suggestions for all other spectrophotometer parameters.
2. Furnace parameters suggested by the manufacturer should be employed as guidelines. Because temperature-sensing

mechanisms and temperature controllers can vary between instruments or with time, the validity of the furnace parameters must be periodically confirmed by systematically altering the furnace parameters while analyzing a standard. In this manner, losses of analyte due to overly high temperature settings or losses in sensitivity due to less than optimum settings can be minimized. Similar verification of furnace parameters may be required for complex sample matrices.

3. Inject a measured microliter (µL) aliquot of sample digest into the furnace and atomize. If the digest concentration is greater than the highest standard, or if the instrument response falls on the plateau of the calibration curve, the sample should be diluted in the same acid matrix and reanalyzed. The use of multiple injections can improve accuracy and help detect furnace pipetting errors.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for chromium in waters and elutriates is 1 µg/L.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured chromium concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standard 1643c - Water, should be run to monitor the life and performance of the graphite tube and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a water sample, prepared by spiking ASTM Type II water at the laboratory, and that has undergone multiple analyses by the analytical laboratory. The lot numbers of the As stock solution used in the creation of the LCS should be different from those used to prepare the calibration (both initial and ongoing) standards. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 15 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - chromium, to the 100 mL sample aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 3 times the estimated or actual method detection limit. The added matrix spike should have a negligible volume when compared to the routine sample volume to eliminate/control sample dilution effects. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

9.8 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values. Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition may be use (see Section 9.8.2).

9.8.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the method detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.8.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through

the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

The performance characteristics for an aqueous sample free of interferences are:

Optimum concentration range: 5-100 µg/L.
Detection limit: 1 µg/L.

Precision and accuracy data shown in Table 1 were obtained from records of state and contractor laboratories (USEPA, 1979).

11.0 Calculations and Reporting

Calculate metal concentrations by (1) the method of standard additions, (2) from a calibration curve, or (3) directly from the instrument's concentration readout. All dilution or concentration factors must be taken into account.

The method of standard additions is the preferred method for the analysis of all sediment elutriates to compensate for any sample matrix effects.

Prepare a standard curve based on the absorbance and concentration of the chromium standards. Determine the chromium concentration in each of the

sample digests by comparing the digest absorbance with the standard calibration curve.

If dilution of sample was required, the following formula is provided to account for the dilution:

$$Cr, \mu g/L = \frac{A \times (B + C)}{C}$$

where:

A = metal in diluted aliquot from calibration curve, µg/L.
B = acid blank matrix used for dilution, mL.
C = sample aliquot, mL.

Chromium concentrations in water or elutriate samples should be reported in µg/L.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition, APHA, New York, New York.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Table 1. Method Performance Data.

Number of Labs	True Value µg/L	Mean Value µg/L	Standard Deviation µg/L	Accuracy as % Bias
74	370	353	105	-4.5
76	407	380	128	-6.5
72	74	72	29	-3.1
70	93	84	35	-10.2
47	7.4	10.2	7.8	37.7
47	15.0	16.0	9.0	6.8

COPPER IN WATERS AND ELUTRIATES (ICP)

1.0 Scope and Application

Inductively coupled plasma atomic emission spectroscopy (ICP) determines copper and numerous other elements that are present in aqueous samples, such as surface water and elutriate samples. The aqueous samples must first be digested with nitric acid prior to analysis.

Detection limits, sensitivity, and optimum concentration ranges for copper, and other metals stable in a mixed standard solution with copper, will vary with the sample aliquot size, the spectrophotometer being used, and possible matrix interferences. The data shown in Table 1 provide concentration ranges for clean aqueous samples.

The method of standard addition (MSA) shall be used for the analysis of all sample digests unless either serial dilution or matrix spike addition demonstrates that it is not required (see section 9).

This method should only be used by spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

This procedure is based on SW-846 Methods 3010 (for digestion) and 6010 for quantitation (EPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Prior to analysis, the samples to be analyzed are refluxed with nitric acid in a covered beaker. This step is repeated with additional HNO_3 until the final digestate is light in color and diluted to volume.

This instrumental method measures light emitted by copper in the final digest by optical spectrometry. The digested samples are nebulized and the resulting aerosol is transported to a plasma torch. Copper-specific atomic-line emission

spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

The possibility of additional interferences named in section 3.0 should also be recognized and appropriate corrections made when necessary. The procedures that can be used for this purpose are described in section 9.

3.0 Interferences

There are three main forms of interferences that can occur during the analysis of water and elutriate samples by ICP. These three types of interferences include: (1) spectral, (2) physical, and (3) chemical interferences. Each is discussed separately in the following sections.

3.1 Spectral Interferences

Spectral interferences are caused by: (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of simultaneous multi-element instruments must verify the absence of spectral interference from an element in a sample for which there is no instrument detection channel. Potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended

as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferants can be assumed.

The magnitude of the interference effects summarized in Table 2 are expressed as analyte concentration equivalents (i.e., false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that Cu is to be determined (at 324.754 ηm) in a sample containing approximately 10 mg/L of V. According to Table 2, 100 mg/L of V would yield a false signal for Cu equivalent to approximately 0.02 mg/L. Therefore, the presence of 10 mg/L of V would result in a false signal for Cu equivalent to approximately 0.002 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, and argon flow rate.

The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.2 Physical Interferences

Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, or by using the standard additions method. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Further, it has been reported that better control of the argon flow rate improves instrument performance. Improved argon flow rate control can be accomplished with the use of mass flow controllers.

3.3 Chemical Interferences

Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant

with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, etc.), buffering of the sample, matrix matching, and standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Centrifuge and centrifuge tubes.
4. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
5. Inductively coupled argon plasma emission spectrometer.
6. Computer-controlled emission spectrometer with background correction.
7. Radio frequency generator.
8. Argon gas supply, welding grade or better.

4.2 Materials

1. Griffin beakers, 150 mL, or equivalent.
2. Watch glasses, ribbed and plain.
3. Whatman No. 41 filter paper, or equivalent.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
3. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.

4. Nitric acid (1:1). Add 500 mL concentrated HNO_3 to 400 mL Type II water. Dilute to 1 liter with Type II water.
5. Hydrochloric acid (1:1). Add 500 mL concentrated HCl to 400 mL Type II water. Dilute to 1 liter with Type II water.
6. Standard stock solutions. May be purchased or prepared from ultrahigh purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hr at 105° C, unless otherwise specified.

   CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.
7. Barium standard stock solution (100 µg/mL). Dissolve 0.1500 g $BaCl_2$ dried at 250° C for 2 hr in 10 mL Type II water with 1 mL 1:1 HCl. Add 10.0 mL 1:1 HCl. Dilute to 1 liter with Type II water.
8. Cobalt standard stock solution (100 µg/mL). Dissolve 0.1000 g of cobalt metal in a minimum amount of 1:1 HNO_3. Add 10.0 mL 1:1 HCl. Dilute to 1 liter with Type II water.
9. Copper standard stock solution (100 µg/mL). Dissolve 0.1300 g CuO in a minimum amount of 1:1 HNO_3. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
10. Iron standard stock solution (100 µg/mL). Dissolve 0.1400 g Fe_2O_3 in a warm mixture of 20 mL 1:1 HCl and 2 mL of concentrated HNO_3. Cool. Add an additional 5.0 mL of concentrated HNO_3. Dilute to 1 liter with Type II water.
11. Vanadium standard stock solution (100 µg/mL). Dissolve 0.2300 g NH_4VO_3 in a minimum amount of concentrated HNO_3. Heat to increase rate of dissolution. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
12. Mixed calibration standard solutions. Care should be taken when preparing mixed standards for ICP analysis to ensure that the elements in the final mixed standard are compatible and stable together. One set of mixed calibration standards that has been found to be useful is listed in Table 3. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interferences or the presence of impurities.

    To prepare the mixed calibration standard for copper, combine appropriate volumes of the individual stock solutions indicated in Table 3 in volumetric flask. Add 2 mL 1:1 HNO_3 and 10 mL of 1:1 HCl and dilute to 100 mL with Type II water. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be

prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see section 9.8) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should be scanned using a sequential spectrometer to verify the absence of interelement spectral interference in the recommended mixed standard solutions.

TABLE 3. MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo, and Si
IV	Al, Ca, Cr, K, Na, and Ni

NOTE: Premixed standard solutions (traceable to NIST) containing the combined elements as listed in solutions I through IV are available from a number of commercial vendors.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Aqueous samples should be acidified to a pH of <2 with HNO_3. A holding time of 6 months after sample collection is generally cited for this parameter.

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of aqueous samples to be analyzed for copper.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Prepare a calibration blank (see section 9.4.1) and at least three mixed calibration standards in the appropriate concentration range to correlate copper concentrations with the ICP's linear response range. Prepare standards for instrument calibration as instructed in section 5.13. Match the sample matrix and that of the standards as closely as possible.

Calibrate the instrument according to the instrument manufacturer's recommended procedures using typical mixed calibration standard solutions. Flush the system with the calibration blank between each standard. Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.

8.0 Procedure

1. Transfer a 100 mL representative aliquot of the well-mixed aqueous sample to a 150 mL Griffin beaker.
2. Add 3 mL of concentrated HNO_3. Cover the beaker with a ribbed watch glass.
3. Place the beaker on a hot plate and cautiously evaporate to a low volume (5 mL) making certain that the sample does not boil and that no portion of the beaker is allowed to go dry.

   NOTE: If a sample is allowed to go to dryness, low recoveries may result. Should this occur, repeat the steps 1 through 3 with a fresh sample aliquot.

4. Cool the beaker and add 3 mL of concentrated HNO_3.
5. Cover the beaker with a nonribbed watch glass and return to the hot plate. Increase the temperature of the hot plate so that a gentle reflux action occurs.
6. Continue heating, adding additional acid as necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change in appearance with continued refluxing).

7. Uncover the beaker, or use a ribbed watch glass, and evaporate to a low volume (3 mL), not allowing any portion of the bottom of the beaker to go dry. Cool the beaker.
8. Add a small quantity of 1:1 HCl (10 mL/100 of final solution) and warm the beaker for an additional 15 min to dissolve any precipitate or residue resulting from evaporation.
9. Wash down the beaker walls and watch glass with Type II water and, when necessary, filter or centrifuge the sample to remove silicates and other insoluble material that could clog the nebulizer.

   NOTE: Filtration should be done only if there is concern that insoluble materials may clog the nebulizer. This additional step can cause sample contamination unless the filter and the filtering apparatus are thoroughly cleaned and prerinsed with dilute HNO_3.

10. Adjust to the final volume of 100 mL with Type II water. The sample is now ready for analysis.
11. The analyst should follow the instructions provided by the instrument's manufacturer. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 min of operation prior to calibration). For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within instrument linear range where coordination factors are valid. The analyst must: (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.
12. Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5% (or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this conditions.
13. Flush the system with the calibration blank solution for at least 1 min before the analysis of each sample. Analyze samples.

NOTE: Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for copper in aqueous solutions is 6 µg/L.

The method detection limit specified in this method is insufficient to meet the Great Lakes Water Quality Criteria for copper (7.3 µg/L). However, this method has been presented to allow for the use of the ICP as a screening tool for copper. If the measured concentrations are greater than 7.3 µg/L, the Great Lakes Water Quality Criteria has been violated and no further analyses are needed. However, if the measured concentration is below 7.3 µg/L, copper must be quantified using the graphite furnace atomic absorption procedure presented in this appendix.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured copper concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance of the ICP and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

9.4.1 Calibration Blank

The calibration blank is prepared by diluting 2 mL of 1:1 HNO_3 and of 1:1 HCl to 100 mL with Type II water. Prepare a sufficient quantity to flush the system between standards and samples.

The calibration blank should be analyzed prior to routine sample analysis, after every 10 samples, and at the end of the analytical run. The measured concentration in the calibration blank should be less than or equal to the method detection limit.

9.4.2 Reagent Blank

The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - copper, to the 100 mL aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

9.7 Interference Check Sample

The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the method detection limit for each element. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

The interference check sample should be analyzed at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Results should be within ± 20% of the known concentration.

9.8 Calibration Control Sample

A calibration control sample should be prepared in the same acid matrix as the calibration standards at 10 times the method detection limit. This sample should be prepared in accordance with the instructions provided by the supplier. The calibration control sample is used to verify the integrity of the calibration standards on a weekly basis.

9.9 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor

negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

9.9.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the method detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.9.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.

4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 µg/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported in µg/L with up to three significant figures.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Winge, R.K., V.J. Peterson, and V.A. Fassel. 1979. Inductively coupled plasma-atomic emission spectroscopy: Prominent lines, final report, March 1977 - February 1978, Ames laboratory, Ames, IA. EPA-600/4-79-017. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Table 1. Recommended Wavelengths and Estimated Instrumental Detection Limits.

Element	Wavelength[a] (ηm)	Estimated Detection Limit[b] (µg/L)
Barium	455.403	2
Cobalt	228.616	7
Copper	324.754	6
Iron	259.940	7
Vanadium	292.402	8

a - The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see section 3.1).

b - The estimated instrumental detection limits are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

Table 2. Analyte Concentration Equivalents arising from Interference at the 100 mg/L Level.

Analyte	Wavelength (ηm)	Interferant[a,b] Al	Ca	Cr	Cu	Fe	Mg	Mn	Mo	Tl	V
Barium	455.403	-	-	-	-	-	-	-	-	-	-
Cobalt	228.616	-	-	0.03	-	0.005	-	-	0.03	0.15	-
Copper	324.754	-	-	-	-	0.003	-	-	-	0.05	0.02
Iron	259.940	-	-	-	-	-	-	0.12	-	-	-
Vanadium	292.402	-	-	0.05	-	0.005	-	-	-	0.02	-

a - Dashes indicate that no interference was observed even when interferants were introduced at the following levels:

Al - 1000 mg/L,
Ca - 1000 mg/L,
Cr - 200 mg/L,
Cu - 200 mg/L,
Fe - 1000 mg/L.
Mg - 1000 mg/L,
Mn - 200 mg/L,
Tl - 200 mg/L,
V - 200 mg/L, and

b - The figures recorded as analyte concentrations are not the actual observed concentrations; to obtain those figures, add the listed concentration to the interferant figure.

Table 4. ICP Precision and Accuracy Data[a]

Element	Sample No. 1 True Value (µg/L)	Sample No. 1 Mean Reported Value (µg/L)	Sample No. 1 Mean SD[b] (%)	Sample No. 2 True Value (µg/L)	Sample No. 2 Mean Reported Value (µg/L)	Sample No. 2 Mean SD[b] (%)	Sample No. 3 True Value (µg/L)	Sample No. 3 Mean Reported Value (µg/L)	Sample No. 3 Mean SD[b] (%)
V	750	749	1.8	70	69	2.9	170	169	1.1
Cu	250	235	5.1	11	11	40	70	67	7.9
Fe	600	594	3.0	20	19	15	180	178	6.0
Co	700	512	10	20	20	4.1	120	108	21

a - Not all elements were analyzed by all laboratories.
b - SD = standard deviation.

COPPER IN WATERS AND ELUTRIATES (GFAA)

1.0 Scope and Application

This method is an graphite furnace atomic absorption (GFAA) procedure suitable for the determination of copper in natural water samples and mobility extracts such as sediment elutriates. All samples must be subjected to an acid digestion step prior to analysis.

This procedure is based on EPA SW-846 Methods 3020 for sample digestion and 7211 for copper quantitation (USEPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

A mixture of nitric acid and the sample to be analyzed is refluxed in a covered Griffin beaker. This step is repeated with additional portions of nitric acid until the digestate is light in color or until its color has stabilized. After the digestate has been brought to a low volume, it is cooled and brought up in dilute nitric acid such that the final dilution contains 3% (v/v) HNO_3. If the sample contains suspended solids, it must be centrifuged, filtered, or allowed to settle.

Following sample digestion, a representative aliquot is slowly evaporated to dryness, charred (ashed), and atomized in a graphite furnace. The absorption of hollow cathode or EDL radiation during sample atomization is proportional to the copper concentration.

3.0 Interferences

The composition of the sample matrix can have a major effect on the analysis. It is those effects which must be determined and taken into consideration in the analysis of each different matrix encountered. To help verify the absence of matrix or chemical interference, the serial dilution technique (see section 9.8.1) may be used. Those samples which indicate the presence of interference should be treated in one or more of the following ways:

1. Successively dilute and reanalyze the samples to eliminate interferences.
2. Modify the sample matrix either to remove interferences or to stabilize the analyte. Examples are the addition of ammonium nitrate to remove alkali chlorides and the addition of ammonium phosphate to retain cadmium. The mixing of hydrogen with the inert purge gas has also been used to suppress chemical interference. The hydrogen acts as a reducing agent and aids in molecular dissociation.
3. Analyze the sample by method of standard additions while noticing the precautions and limitations of its use (see section 9.8.2).

Gases generated in the furnace during atomization may have molecular absorption bands encompassing the analytical wavelength. When this occurs, use either background correction or choose an alternate wavelength. Background correction may also compensate for nonspecific broad-band absorption interference.

Continuum background correction cannot correct for all types of background interference. When the background interference cannot be compensated for, chemically remove the analyte or use an alternate form of background correction, e.g., Zeeman background correction.

Interference from a smoke-producing sample matrix can sometimes be reduced by extending the charring time at a higher temperature or utilizing an ashing cycle in the presence of air. Care must be taken, however, to prevent loss of the analyte.

Spectral interference can occur when an absorbing wavelength of an element present in the sample but not being determined falls within the width of the absorption line of the element of interest. The results of the determination will then be erroneously high, due to the contribution of the interfering element to the atomic absorption signal. Interference can also occur when resonant energy from another element in a multielement lamp, or from a metal impurity in the lamp cathode, falls within the bandpass of the slit setting when that other metal is present in the sample. This type of interference may sometimes be reduced by narrowing the slit width.

Cross-contamination and contamination of the sample can be major sources of error because of the extreme sensitivities achieved with the furnace. The sample preparation work area should be kept scrupulously clean. All glassware should be cleaned as directed in the Materials section (section 4.2). Pipet tips are

a frequent source of contamination. If other pipet tips are suspected of causing sample contamination, they should be acid soaked with 1:5 $HN0_3$ and rinsed thoroughly with tap and Type II water. The use of a better grade of pipet tip can greatly reduce this problem.

Pyrolytic graphite, because of the production process and handling, can become contaminated. As many as five to ten high-temperature burns may be required to clean the tube before use.

Background correction may be required because nonspecific absorption and scattering can be significant at the analytical wavelength. Background correction with certain instruments may be difficult at this wavelength due to low-intensity output from hydrogen or deuterium lamps. Consult the specific instrument manufacturer's literature for details.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
4. Thermometer, 0 to 100° C range.
5. Atomic absorption spectrophotometer, single or dual channel, single- or double-beam instrument having a grating monochromator, photo-multiplier detector, adjustable slits, a wavelength range of 190 to 800 ηm, and provisions for simultaneous background correction and interfacing with a strip-chart recorder.
6. Copper hollow cathode lamp, or electrodeless discharge lamp (EDL).
7. Graphite furnace. Any graphite furnace device with the appropriate temperature and timing controls.
8. Strip-chart recorder. A recorder is strongly recommended for furnace work so that there will be a permanent record and so that any problems with the analysis such as drift, incomplete atomization, losses during charring, and changes in sensitivity can easily be recognized.

4.2 Materials

1. Commercial grade available argon and nitrogen are required for furnace work.
2. Griffin beakers, 150 mL, or equivalent.
3. Volumetric flasks, class A, 1 L.
4. Volumetric flasks, class A, 100 mL.
5. Pipets. Microliter with disposable tips. Sizes can range from 5 to 100 µL, as required.

   NOTE: All glassware, polypropylene, or Teflon containers, including sample bottles, should be washed in the following sequence: detergent, tap water, 1:1 HNO_3, tap water, 1:1 HCl, tap water, and Type II water.

   NOTE: Chromic acid (H_2CrO_4) should not be used as a cleaning agent for glassware if chromium is to be included in the analytical scheme for the sample digestate.

6. Watch glass, ribbed. Watch glass should be large enough to cover the mouth of the beaker.
7. Watch glass, non-ribbed. Watch glass should be large enough to cover the mouth of the beaker.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
3. Concentrated hydrochloric acid (HCl), spectrograde grade certified for AA use. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid is <MDL, then the acid can be used.
4. Hydrochloric acid solution (HCl), 1:1. Add 500 mL of concentrated HCl to 400 mL of Type II water. Dilute to 1 liter with Type II water.
5. Concentrated nitric acid (HNO_3), spectrograde grade certified for AA use. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid is <MDL, then the acid can be used.

6. Nitric acid solution (HNO_3), 1:1. Add 500 mL of concentrated HNO_3 to 400 mL of Type II water. Dilute to 1 liter with Type II water.
7. Copper standard stock solution (1,000 mg/L). Either procure a certified aqueous standard from a supplier and verify by comparison with a second standard, or dissolve 1.00 g of electrolytic copper, analytical reagent grade) in 5 mL of re-distilled HNO_3. Dilute to 1 liter with Type II water.
8. Copper working standards. Prepare dilutions of the stock solution to be used as calibration standards at the time of the analysis. The calibration standard should be prepared to contain 0.5% (v/v) HNO_3.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Water and elutriate samples should be acidified to a pH <2 with HNO_3. A holding time of 6 months is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water (see note in section 4.2). Either glass or plastic containers can be used for the storage of samples to be analyzed for copper in aqueous samples.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The hot plate should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

Prepare a method blank and at least three standards in the appropriate concentration range to correlate copper concentrations with the atomic absorption spectrophotometer's linear response range. Prepare standards for instrument calibration by appropriate dilution of the copper standard stock solution. These standards should be prepared fresh on the day of use. Match the sample matrix and that of the standards as closely as possible.

Inject a suitable portion of each standard into the graphite furnace in order of increasing concentration. It is recommended that each standard solution be analyzed in triplicate in order to assess method precision. Instrument calibration curves should be composed of a minimum of a blank and three standards. A calibration curve should be prepared every day of continuous sample analysis and prior to the initiation of the project's routine sample analysis.

Construct an analytical curve by plotting the average peak absorbance or peak area for the standard solutions as a function of sample concentration on a linear graph. Prepare this graph daily when new initial calibration information is obtained. Alternatively, electronic instrument calibration can be used if the instrument is appropriately equipped.

8.0 Procedure

8.1 Sample Preparation

1. Transfer 100 mL of well mixed sample to a 150 mL Griffin beaker.
2. Add 3 mL of concentrated HNO_3.
3. Cover the beaker with a ribbed watch glass.
4. Place the beaker on a hot plate and cautiously evaporate to a low volume (approximately 5 mL) making certain that the sample does not boil and that no portion of the bottom of the beaker is allowed to go dry.
5. Cool the digested sample and add 3 mL of concentrated HNO_3.
6. Cover the beaker with a non-ribbed watch glass.
7. Place the beaker on a hot plate and increase the temperature so that a gentle reflux action occurs.
8. Continue heating, adding additional acid as necessary, until the digestion is complete.

NOTE: Complete digestion is generally indicated by a light digestate color or if the digestate does not change in appearance with continued refluxing.

9. When the digestion is complete, cover with a ribbed watch glass and evaporate to a low volume (approximately 3 mL).

   NOTE: Do not allow any portion of the beaker bottom to go dry.

10. Add approximately 10 mL of Type II water and mix.
11. Continue warming the beaker for 10 to 15 minutes to allow additional solubilization of any residue to occur.
12. Remove beaker from hot plate and wash down the beaker walls and watch glass with Type II water.

    NOTE: It may be necessary to filter or centrifuge the sample to remove silicates and other insoluble material that may interfere with injecting the sample into the graphite furnace.

13. Adjust the final volume to 100 mL with Type II water.

8.2 Sample Analysis

1. The 324.7 ηm wavelength line and a background correction system are required. Follow the manufacturer's suggestions for all other spectrophotometer parameters.
2. Furnace parameters suggested by the manufacturer should be employed as guidelines. Because temperature-sensing mechanisms and temperature controllers can vary between instruments or with time, the validity of the furnace parameters must be periodically confirmed by systematically altering the furnace parameters while analyzing a standard. In this manner, losses of analyte due to overly high temperature settings or losses in sensitivity due to less than optimum settings can be minimized. Similar verification of furnace parameters may be required for complex sample matrices.
3. Inject a measured microliter (µL) aliquot of sample digest into the furnace and atomize. If the digest concentration is greater than the highest standard, or if the instrument response falls on the plateau of the calibration curve, the sample should be diluted in the same

acid matrix and reanalyzed. The use of multiple injections can improve accuracy and help detect furnace pipetting errors.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for copper in waters and elutriates is 1 µg/L.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured copper concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standard 1643c - Water, should be run to monitor the life and performance of the graphite tube and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a water sample, prepared by spiking ASTM Type II water at the laboratory, and that has undergone multiple analyses by the analytical laboratory. The lot numbers of the As stock solution used in the creation of the LCS should be different from those used to prepare the calibration (both initial and ongoing) standards. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 15 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - copper, to the 100 mL sample aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 3 times the estimated or actual method detection limit. The added matrix spike should have a negligible volume when compared to the routine sample volume to eliminate/control sample dilution effects. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

9.8 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values. Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition may be use (see Section 9.8.2).

9.8.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the method detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.8.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

The performance characteristics for an aqueous sample free of interferences are:

Optimum concentration range: 5-100 µg/L.
Detection limit: 1 µg/L.

Precision and accuracy data shown in Table 1 were obtained from records of state and contractor laboratories (USEPA, 1979).

11.0 Calculations and Reporting

Calculate metal concentrations by (1) the method of standard additions, (2) from a calibration curve, or (3) directly from the instrument's concentration readout. All dilution or concentration factors must be taken into account.

The method of standard additions is the preferred method for the analysis of all sediment elutriates to compensate for any sample matrix effects.

Prepare a standard curve based on the absorbance and concentration of the copper standards. Determine the copper concentration in each of the sample digests by comparing the digest absorbance with the standard calibration curve.

If dilution of sample was required, the following formula is provided to account for the dilution:

$$\text{Cu, µg/L} = \frac{A \times (B + C)}{C}$$

where:
A = metal in diluted aliquot from calibration curve, µg/L.
B = acid blank matrix used for dilution, mL.
C = sample aliquot, mL.

Copper concentrations in water or elutriate samples should be reported in µg/L.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition, APHA, New York, New York.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Table 1. Method Performance Data.

Number of Labs	True Value µg/L	Mean Value µg/L	Standard Deviation µg/L	Accuracy as % Bias
91	302	305	56	0.9
92	332	324	56	-2.4
86	60	64	23	7.0
84	75	76	22	1.3
66	7.5	9.7	6.1	29.7
66	12.0	13.9	9.7	15.5

MERCURY IN WATER AND ELUTRIATES (MANUAL CVAA)

1.0 Scope and Application

This method is applicable to the determination of mercury in liquid samples, such as surface waters and sediment elutriates. All samples must be subjected to an appropriate dissolution step prior to analysis.

In addition to the inorganic forms, mercury may also be present as an organic compound. Although potassium permanganate oxidizes many of these compounds, recent studies have shown that a number of organic mercurials, including phenyl mercuric acetate and methyl mercuric chloride, are only partially oxidized by this reagent. However, the use of potassium persulfate as an oxidant has been found to produce approximately 100% recovery of these compounds. Therefore, a persulfate oxidation step following the addition of the permanganate has been included to insure that organo-mercury compounds, if present, will be converted to the mercuric ion prior to sample analysis. In order to ensure conversion of methyl mercuric chloride, it is necessary to heat the samples during the pretreatment procedure.

This procedure is based on SW-846 Method 7470 (USEPA, 1986).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Mercury in a sample aliquot is treated with an oxidant to reduce it to the elemental state. The sample is attached to a cold vapor atomic absorption apparatus and the elemental mercury is flushed from the sample in a stream of air. The mercury vapor is passed through a cell positioned in the light path of an atomic absorption spectrophotometer. The mercury concentration in the sample is proportional to the absorption (peak height) of incident radiation with a wavelength of 253.7 ηm.

3.0 Interferences

Potassium permanganate is added during the sample preparation step to eliminate possible interference from sulfide. Concentrations as high as 20 mg/L of sulfide as sodium sulfide do not interfere with the recovery of added inorganic mercury from ASTM Type II water.

Although copper has also been reported to interfere with the analysis of mercury, studies suggest that copper concentrations as high as 10 mg/L had no effect on recovery of mercury from spiked samples.

Interference from certain volatile organic materials, which may absorb radiation at a wavelength of 253 ηm, is also possible but seldom encountered (USEPA, 1979). A preliminary run without reagents can be performed to identify the presence of and to correct for this matrix effect.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, 3-5 weights covering expected weight range.
3. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
4. Thermometer, 0 to 100° C range.
5. Atomic absorption spectrophotometer. Any atomic absorption unit having an open sample presentation area in which to mount the absorption cell is suitable. Instrument settings recommended by the particular manufacturer should be followed. Instruments designed specifically for the measurement of mercury using the cold vapor technique are commercially available and may be substituted for the atomic absorption spectrophotometer.
6. Mercury hollow cathode lamp or electrodeless discharge lamp.
7. Recorder. Any multi-range variable-speed recorder that is compatible with the UV detection system is suitable.
8. Absorption cell. Standard spectrophotometer cells 10 cm long with quartz end windows may be used. Suitable cells may be constructed from Plexiglas tubing, 2.54 cm O.D. x 11.43 cm. The ends are ground perpendicular to the longitudinal axis, and quartz windows (2.54 cm diameter x 0.16 cm thickness) are

cemented in place. The cell is strapped to a burner for support and aligned in the light beam by use of two 5.08 cm x 5.08 cm cards. Holes with a diameter of 2.54 cm are cut in the middle of each card. The cards are then placed over each end of the cell. The cell is then positioned and adjusted vertically and horizontally to give the maximum transmittance.

9. Air pump. Any peristaltic pump capable of delivering 1 L/min air may be used. A Masterflex pump with electronic speed control has been found to be satisfactory.
10. Flowmeter. Capable of measuring an air flow of 1 L/min.
11. Aeration tube. A straight glass frit with a coarse porosity. Tygon tubing is used for passage of the mercury vapor from the sample bottle to the absorption cell and return.
12. Drying tube, 15.2 cm X 1.90 cm diameter tube containing 20 g of magnesium perchlorate.

    NOTE: In place of the magnesium perchlorate drying tube, a small reading lamp with a 60 W bulb may be used to prevent condensation of moisture inside the cell. The lamp is positioned to shine on the absorption cell maintaining the air temperature in the cell about 10° C above ambient.

13. Cold vapor generator.

    a. The apparatus shown in Figure 1 is a closed system. An open system, in which the mercury vapor is passed through the absorption cell only once, may be used in place of the closed system.
    b. Because mercury vapor is potentially toxic, precautions must be taken to avoid inhalation of the vapor. Therefore, a bypass has been included in the analytical apparatus to either vent the mercury vapor into an exhaust hood or to pass the vapor through some absorbing medium such as:

        1. equal volumes of 0.1 M $KMnO_4$ and 10% H_2SO_4,
        2. 0.25% iodine in a 3% KI solution, or
        3. specially treated charcoal that will absorb mercury vapor.

4.2 Materials

1. BOD bottles, 300 mL, or equivalent.
2. Volumetric flasks, class A, 100 mL.
3. Graduated cylinders, various sizes up to 100 mL, or equivalent.

5.0 Reagents and Standards

1. ASTM Type II water (ASTM D1193). Water supply should be continually tested to verify that contaminants are not present at levels that will interfere with method performance.
2. Sulfuric acid (H_2SO_4), conc. reagent grade.
3. Sulfuric acid, 0.5 N. Dilute 14.0 mL of concentrated sulfuric acid to 1.0 liter with ASTM Type II water.
4. Nitric acid (HNO_3), conc. reagent grade with low mercury content. If a high reagent blank is obtained, it may be necessary to distill the nitric acid.
5. 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.

   NOTE: Stannous chloride may be used in place of stannous sulfate.

6. Sodium chloride-hydroxylamine sulfate solution. Dissolve 12 g of sodium chloride and 12 g of hydroxylamine sulfate in ASTM Type II water. Dilute to 100 mL with Type II water.

   NOTE: Hydroxylamine hydrochloride may be used in place of hydroxylamine sulfate.

7. Potassium permanganate, 5% w/v solution ($KMnO_4$). Dissolve 5 g of potassium permanganate in 100 mL of ASTM Type II water.
8. Potassium Persulfate, 5% w/v solution. Dissolve 5 g of potassium persulfate in 100 mL of ASTM Type II water.
9. Mercury stock solution. Dissolve 0.1354 g of mercuric chloride in 75 mL of ASTM Type II water. Add 10 mL of concentrated nitric acid and adjust the volume to 100 mL with ASTM Type II water. (1.0 mL = 1.0 mg Hg).
10. Mercury working 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 adding mercury stock solution.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Water and elutriate samples should be acidified to a pH <2 with HNO_3. A holding time of 28 days is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of samples to be analyzed for arsenic in sediments.

If only dissolved mercury is to be determined, the sample should be filtered through an all glass apparatus before the acid is added. If total mercury is to be determined, the filtration is omitted.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The hot plate/water bath should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

Calibration curves should be composed of a minimum of a blank and three standards. To calibrate and standardize to atomic absorption

spectrophotometer, the following steps should be used to prepare standards and prepare the instrument:

1. Transfer 0.0, 0.5, 1.0, 2.0, 5.0, and 10-mL aliquots of the mercury working standard containing 0-1.0 µg, respectively, of mercury to a series of 300-mL BOD bottles.
2. Add enough ASTM Type II water to each bottle to make a total volume of 100 mL.
3. Mix thoroughly and add 5 mL of conc. sulfuric acid and 2.5 mL of conc. nitric acid to each bottle.
4. Add 15 mL of $KMnO_4$, solution to each bottle and allow to stand at least 15 minutes.
5. Add 8 mL of potassium persulfate to each bottle and heat for 2 hours in a water bath maintained at 95° C.
6. Allow the standards to cool.
7. Add 6 mL of sodium chloride-hydroxylamine sulfate solution to reduce the excess permanganate.
8. When the solution has been decolorized, wait 30 seconds, add 5 mL of stannous sulfate solution, and immediately attach the bottle to the aeration apparatus. At this point, the sample is allowed to stand quietly without manual agitation.
9. The circulating pump, which has previously been adjusted to a rate of 1 liter per minute, is allowed to run continuously. The absorbance will increase and reach a maximum within 30 seconds. As soon as the recorder pen levels off, approximately 1 minute, open the bypass valve and continue the aeration until the absorbance returns to a minimum value. Due to the potential toxicity of these vapors, they should be properly vented through a fume hood or absorbing medium.
10. Close the bypass valve, remove the stopper and frit from the BOD bottle, and continue the aeration.
11. Repeat steps 6 through 10 for each of the standards.
12. Construct a standard curve by plotting the recorded absorbance versus the concentration of mercury in the standards.

8.0 Procedure

1. Transfer 100 mL of sample, or a suitable size aliquot diluted to 100 mL, containing not more than 1.0 μg of mercury, to a 300 mL BOD bottle.

2. Add 5 mL of conc. sulfuric acid and 2.5 mL of conc. nitric acid mixing after each addition.
3. Add 15 mL of potassium permanganate solution to each sample bottle and shake. If necessary, add additional potassium permanganate until the purple color persists for at least 15 minutes.
4. Add 8 mL of potassium persulfate to each bottle and heat for 2 hours in a water bath at 95° C.
5. Allow the samples to cool.
6. To the first sample, add 6 mL of sodium chloride-hydroxylamine sulfate to reduce the excess permanganate.
7. After a delay of at least 30 seconds, add 5 mL of stannous sulfate solution, and immediately attach the bottle to the aeration apparatus. At this point, the sample is allowed to stand quietly without manual agitation.
8. The circulating pump, which has previously been adjusted to a rate of 1 liter per minute, is allowed to run continuously. The absorbance will increase and reach a maximum within 30 seconds. As soon as the recorder pen levels off, approximately 1 minute, open the bypass valve and continue the aeration until the absorbance returns to a minimum value. Due to the potential toxicity of these vapors, they should be properly vented through a fume hood or absorbing medium.
9. Close the bypass valve, remove the stopper and frit from the BOD bottle, and continue the aeration.
10. Repeat steps 6 through 9 for each of the samples.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for mercury in waters and elutriates is 0.2 µg/L.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured arsenic concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standard 1643c - Water, should be run to monitor the life and performance of the graphite tube and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Laboratory Control Samples

A laboratory control sample (LCS) should be developed and maintained by the analytical laboratory. A laboratory control sample is a water sample, prepared by spiking ASTM Type II water at the laboratory, and that has undergone multiple analyses by the analytical laboratory. The lot numbers of the As stock solution used in the creation of the LCS should be different from those used to prepare the calibration (both initial and ongoing) standards. The measured concentration of the laboratory control sample should be within ± 3 standard deviation units from the mean concentration of the LCS.

9.5 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the instrument detection limit.

9.6 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 15 samples. The ongoing calibration check sample should be a mid-calibration range standard

prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.7 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - arsenic, to the 100 mL sample aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 3 times the estimated or actual method detection limit. The added matrix spike should have a negligible volume when compared to the routine sample volume to eliminate/control sample dilution effects. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

10.0 Method Performance

The working range of this method is 0.2 µg/L to 20 µg/L although the actual range can be modified through instrument and/or recorder expansion.

In a single laboratory (EMSL-Cincinnati), using an Ohio River composite sample with a background mercury concentration of 0.35 μg/L, spiked with concentrations of 1.0, 3.0 and 4.0 μg/L, the standard deviations were ± 0.14, ± 0.10 and ± 0.08, respectively. The standard deviation at the 0.35 µg/L level was ± 0.16. Percent recoveries at the three levels were 89, 87, and 87%, respectively.

In a joint EPA/ASTM interlaboratory study of the cold vapor technique for total mercury in water, increments of organic and inorganic mercury were added to natural waters. Recoveries were determined by difference. A statistical summary of this study is presented in Table 1.

11.0 Calculations and Reporting

Calculate metal concentrations by (1) the method of standard additions, (2) from a calibration curve, or (3) directly from the instrument's concentration readout. All dilution or concentration factors must be taken into account.

Prepare a standard curve based on the absorbance and concentration of the mercury standards. Determine the mercury concentration in each of the sediment digests by comparing the digest absorbance with the standard calibration curve.

Mercury concentrations in water or elutriate samples should be reported in µg/L.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition, APHA, New York, New York.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Kopp, J.F., M.C. Longbottom, and L.B. Lobring. 1972. Cold Vapor Method for Determining Mercury. AWWA, Vol. 64.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Table 1. Method Performance Data (after Kopp et al., 1972)

Number of Labs	True Values μg/liter	Mean Value μg/liter	Standard Deviation μg/liter	Accuracy as %Bias
76	0.21	0.349	0.276	66
80	0.27	0.414	0.279	53
82	0.51	0.674	0.541	32
77	0.60	0.709	0.390	18
82	3.4	3.41	1.49	0.34
79	4.1	3.81	1.12	-7.1
79	8.8	8.77	3.69	-0.4
78	9.6	9.10	3.57	-5.2

NICKEL IN WATERS AND ELUTRIATES (ICP)

1.0 Scope and Application

Inductively coupled plasma atomic emission spectroscopy (ICP) determines nickel and numerous other elements that are present in aqueous samples, such as surface water and elutriate samples. The aqueous samples must first be digested with nitric acid prior to analysis.

Detection limits, sensitivity, and optimum concentration ranges for nickel, and other metals stable in a mixed standard solution with nickel, will vary with the sample aliquot size, the spectrophotometer being used, and possible matrix interferences. The data shown in Table 1 provide concentration ranges for clean aqueous samples.

The method of standard addition (MSA) shall be used for the analysis of all sample digests unless either serial dilution or matrix spike addition demonstrates that it is not required (see section 9).

This method should only be used by spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

This procedure is based on SW-846 Methods 3010 (for digestion) and 6010 for quantitation (EPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Prior to analysis, the samples to be analyzed are refluxed with nitric acid in a covered beaker. This step is repeated with additional HNO_3 until the final digestate is light in color and diluted to volume.

This instrumental method measures light emitted by nickel in the final digest by optical spectrometry. The digested samples are nebulized and the

resulting aerosol is transported to a plasma torch. Nickel-specific atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

The possibility of additional interferences named in section 3.0 should also be recognized and appropriate corrections made when necessary. The procedures that can be used for this purpose are described in section 9.

3.0 Interferences

There are three main forms of interferences that can occur during the analysis of water and elutriate samples by ICP. These three types of interferences include: (1) spectral, (2) physical, and (3) chemical interferences. Each is discussed separately in the following sections.

3.1 Spectral Interferences

Spectral interferences are caused by: (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of simultaneous multi-element instruments must verify the absence of spectral interference from an element in a sample for which there is no

instrument detection channel. Potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferants can be assumed.

The magnitude of the interference effects summarized in Table 2 are expressed as analyte concentration equivalents (i.e., false analyte concentrations) arising from 100 mg/L of the interference element. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, and argon flow rate.

The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.2 Physical Interferences

Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, or by using the standard additions method. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Further, it has been reported that better control of the argon flow rate improves instrument performance. Improved argon flow rate control can be accomplished with the use of mass flow controllers.

3.3 Chemical Interferences

Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position,

etc.), buffering of the sample, matrix matching, and standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Centrifuge and centrifuge tubes.
4. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
5. Inductively coupled argon plasma emission spectrometer.
6. Computer-controlled emission spectrometer with background correction.
7. Radio frequency generator.
8. Argon gas supply, welding grade or better.

4.2 Materials

1. Griffin beakers, 150 mL, or equivalent.
2. Watch glasses, ribbed and plain.
3. Whatman No. 41 filter paper, or equivalent.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
3. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.

4. Nitric acid (1:1). Add 500 mL concentrated HNO_3 to 400 mL Type II water. Dilute to 1 liter with Type II water.
5. Hydrochloric acid (1:1). Add 500 mL concentrated HCl to 400 mL Type II water. Dilute to 1 liter with Type II water.
6. Standard stock solutions. May be purchased or prepared from ultrahigh purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hr at 105° C, unless otherwise specified.

   CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.
7. Aluminum standard stock solution (100 µg/mL). Dissolve 0.1000 g of aluminum metal in an acid mixture of 4 mL of 1:1 HCl and 1 mL of concentrated HNO_3 in a beaker. Warm gently to effect solution. When solution is complete, transfer quantitatively to a liter flask, add an additional 10 mL of 1:1 HCl. Dilute to 1 liter with Type II water.
8. Calcium standard stock solution (100 µg/mL). Suspend 0.2500 g $CaCO_3$ dried at 180° C for 1 hr before weighing in Type II water and dissolve cautiously with a minimum amount of 1:1 HNO_3. Add 10.0 mL of concentrated HNO_3. Dilute to 1 liter with Type II water.
9. Chromium standard stock solution (100 µg/mL). Dissolve 0.1900 g CrO_3 in Type II water. When solution is complete, acidify with 10 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
10. Potassium standard stock solution (100 µg/mL). Dissolve 0.1900 g KCl dried at 110° C in Type II water. Dilute to 1 liter with Type II water.
11. Sodium standard stock solution (100 µg/mL). Dissolve 0.2500 g NaCl in Type II water. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
12. Nickel standard stock solution (100 µg/mL). Dissolve 0.1000 g of nickel metal in 10.0 mL hot concentrated HNO_3. Cool. Dilute to 1 liter with Type II water.
13. Mixed calibration standard solutions. Care should be taken when preparing mixed standards for ICP analysis to ensure that the elements in the final mixed standard are compatible and stable together. One set of mixed calibration standards that has been found to be useful is listed in Table 3. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interferences or the presence of impurities.

To prepare the mixed calibration standard for nickel, combine appropriate volumes of the individual stock solutions indicated in Table 3 in volumetric flask. Add 2 mL 1:1 HNO_3 and 10 mL of 1:1 HCl and dilute to 100 mL with Type II water. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see section 9.8) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should be scanned using a sequential spectrometer to verify the absence of interelement spectral interference in the recommended mixed standard solutions.

TABLE 3. MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo, and Si
IV	Al, Ca, Cr, K, Na, and Ni

NOTE: Premixed standard solutions (traceable to NIST) containing the combined elements as listed in solutions I through IV are available from a number of commercial vendors.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Aqueous samples should be acidified to a pH of <2 with HNO_3. A holding time of 6 months after sample collection is generally cited for this parameter.

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of aqueous samples to be analyzed for nickel.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Prepare a calibration blank (see section 9.4.1) and at least three mixed calibration standards in the appropriate concentration range to correlate nickel concentrations with the ICP's linear response range. Prepare standards for instrument calibration as instructed in section 5.14. Match the sample matrix and that of the standards as closely as possible.

Calibrate the instrument according to the instrument manufacturer's recommended procedures using typical mixed calibration standard solutions. Flush the system with the calibration blank between each standard. Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.

8.0 Procedure

1. Transfer a 100 mL representative aliquot of the well-mixed aqueous sample to a 150 mL Griffin beaker.
2. Add 3 mL of concentrated HNO_3. Cover the beaker with a ribbed watch glass.
3. Place the beaker on a hot plate and cautiously evaporate to a low volume (5 mL) making certain that the sample does not boil and that no portion of the beaker is allowed to go dry.

   NOTE: If a sample is allowed to go to dryness, low recoveries may result. Should this occur, repeat the steps 1 through 3 with a fresh sample aliquot.

4. Cool the beaker and add 3 mL of concentrated HNO_3.
5. Cover the beaker with a nonribbed watch glass and return to the hot plate. Increase the temperature of the hot plate so that a gentle reflux action occurs.

6. Continue heating, adding additional acid as necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change in appearance with continued refluxing).
7. Uncover the beaker, or use a ribbed watch glass, and evaporate to a low volume (3 mL), not allowing any portion of the bottom of the beaker to go dry. Cool the beaker.
8. Add a small quantity of 1:1 HCl (10 mL/100 of final solution) and warm the beaker for an additional 15 min to dissolve any precipitate or residue resulting from evaporation.
9. Wash down the beaker walls and watch glass with Type II water and, when necessary, filter or centrifuge the sample to remove silicates and other insoluble material that could clog the nebulizer.

   NOTE: Filtration should be done only if there is concern that insoluble materials may clog the nebulizer. This additional step can cause sample contamination unless the filter and the filtering apparatus are thoroughly cleaned and prerinsed with dilute HNO_3.

10. Adjust to the final volume of 100 mL with Type II water. The sample is now ready for analysis.
11. The analyst should follow the instructions provided by the instrument's manufacturer. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 min of operation prior to calibration). For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within instrument linear range where coordination factors are valid. The analyst must: (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.
12. Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5% (or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this conditions.

13. Flush the system with the calibration blank solution for at least 1 min before the analysis of each sample. Analyze samples.

    NOTE: Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for nickel in aqueous solutions is 25 µg/L.

The method detection limit specified in this method is sufficient to meet the Great Lakes Water Quality Criteria for nickel (260 µg/L).

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured nickel concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance of the ICP and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to

correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

9.4.1 Calibration Blank

The calibration blank is prepared by diluting 2 mL of 1:1 HNO_3 and of 1:1 HCl to 100 mL with Type II water. Prepare a sufficient quantity to flush the system between standards and samples.

The calibration blank should be analyzed prior to routine sample analysis, after every 10 samples, and at the end of the analytical run. The measured concentration in the calibration blank should be less than or equal to the method detection limit.

9.4.2 Reagent Blank

The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - nickel, to the 100 mL aliquot of a routine sample for which an independent estimate of the target analyte

concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of ≤ 20%.

9.7 Interference Check Sample

The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the method detection limit for each element. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

The interference check sample should be analyzed at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Results should be within ± 20% of the known concentration.

9.8 Calibration Control Sample

A calibration control sample should be prepared in the same acid matrix as the calibration standards at 10 times the method detection limit. This sample should be prepared in accordance with the instructions provided by the supplier. The calibration control sample is used to verify the integrity of the calibration standards on a weekly basis.

9.9 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests will ensure the analyst that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

9.9.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the method detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.9.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 µg/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported in µg/L with up to three significant figures.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Winge, R.K., V.J. Peterson, and V.A. Fassel. 1979. Inductively coupled plasma-atomic emission spectroscopy: Prominent lines, final report, March 1977 - February 1978, Ames laboratory, Ames, IA. EPA-600/4-79-017. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Table 1. Recommended Wavelengths and Estimated Instrumental Detection Limits.

Element	Wavelength[a] (ηm)	Estimated Detection Limit[b] (µg/L)
Aluminum	308.215	45
Chromium	267.716	7
Copper	324.754	6
Nickel	231.604	15
Potassium	766.491	See footnote c
Sodium	588.995	29

a - The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see section 3.1).
b - The estimated instrumental detection limits are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.
c - Highly dependent on operating conditions and plasma position.

Table 2. Analyte Concentration Equivalents arising from Interference at the 100 mg/L Level.

Analyte	Wavelength (ηm)	Interferant[a,b] Al	Ca	Cr	Cu	Fe	Mg	Mn	Mo	Tl	V
Aluminum	308.215	-	-	-	-	-	-	0.21	-	-	1.4
Calcium	317.933	-	-	0.08	-	0.01	0.01	0.04	-	0.03	0.03
Chromium	267.716	-	-	-	-	0.003	-	0.04	-	-	0.04
Nickel	231.604	-	-	-	-	-	-	-	-	-	-
Sodium	588.995	0.30	-	-	-	-	-	-	-	-	-

a - Dashes indicate that no interference was observed even when interferants were introduced at the following levels:

Al - 1000 mg/L,
Ca - 1000 mg/L,
Cr - 200 mg/L,
Cu - 200 mg/L,
Fe - 1000 mg/L.
Mg - 1000 mg/L,
Mn - 200 mg/L,
Tl - 200 mg/L,
V - 200 mg/L, and

b - The figures recorded as analyte concentrations are not the actual observed concentrations; to obtain those figures, add the listed concentration to the interferant figure.

Table 4. ICP Precision and Accuracy Data[a]

Element	Sample No. 1 True Value (µg/L)	Sample No. 1 Mean Reported Value (µg/L)	Sample No. 1 Mean SD[b] (%)	Sample No. 2 True Value (µg/L)	Sample No. 2 Mean Reported Value (µg/L)	Sample No. 2 Mean SD[b] (%)	Sample No. 3 True Value (µg/L)	Sample No. 3 Mean Reported Value (µg/L)	Sample No. 3 Mean SD[b] (%)
Cr	150	149	3.8	10	10	18	50	50	3.3
Al	700	695	5.6	60	62	33	160	161	13
Ni	250	245	5.8	30	28	11	60	55	14

a - Not all elements were analyzed by all laboratories.
b - SD = standard deviation.

LEAD IN WATERS AND ELUTRIATES (ICP)

1.0 Scope and Application

Inductively coupled plasma atomic emission spectroscopy (ICP) determines lead and numerous other elements that are present in aqueous samples, such as surface water and elutriate samples. The aqueous samples must first be digested with nitric acid prior to analysis.

Detection limits, sensitivity, and optimum concentration ranges for lead, and other metals stable in a mixed standard solution with lead, will vary with the sample aliquot size, the spectrophotometer being used, and possible matrix interferences. The data shown in Table 1 provide concentration ranges for clean aqueous samples.

The method of standard addition (MSA) shall be used for the analysis of all sample digests unless either serial dilution or matrix spike addition demonstrates that it is not required (see section 9).

This method should only be used by spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

This procedure is based on SW-846 Methods 3010 (for digestion) and 6010 for quantitation (EPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Prior to analysis, the samples to be analyzed are refluxed with nitric acid in a covered beaker. This step is repeated with additional HNO_3 until the final digestate is light in color and diluted to volume.

This instrumental method measures light emitted by lead in the final digest by optical spectrometry. The digested samples are nebulized and the

resulting aerosol is transported to a plasma torch. Lead-specific atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

The possibility of additional interferences named in section 3.0 should also be recognized and appropriate corrections made when necessary. The procedures that can be used for this purpose are described in section 9.

3.0 Interferences

There are three main forms of interferences that can occur during the analysis of water and elutriate samples by ICP. These three types of interferences include: (1) spectral, (2) physical, and (3) chemical interferences. Each is discussed separately in the following sections.

3.1 Spectral Interferences

Spectral interferences are caused by: (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of simultaneous multi-element instruments must verify the absence of spectral interference from an element in a sample for which there is no

instrument detection channel. Potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferants can be assumed.

The magnitude of the interference effects summarized in Table 2 are expressed as analyte concentration equivalents (i.e., false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that Pb is to be determined (at 220.353 ηm) in a sample containing approximately 10 mg/L of Al. According to Table 2, 100 mg/L of Al would yield a false signal for Pb equivalent to approximately 0.17 mg/L. Therefore, the presence of 10 mg/L of Al would result in a false signal for Pb equivalent to approximately 0.017 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, and argon flow rate.

The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.2 Physical Interferences

Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, or by using the standard additions method. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Further, it has been reported that better control of the argon flow rate improves instrument performance. Improved argon flow rate control can be accomplished with the use of mass flow controllers.

3.3 Chemical Interferences

Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, etc.), buffering of the sample, matrix matching, and standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Centrifuge and centrifuge tubes.
4. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
5. Inductively coupled argon plasma emission spectrometer.
6. Computer-controlled emission spectrometer with background correction.
7. Radio frequency generator.
8. Argon gas supply, welding grade or better.

4.2 Materials

1. Griffin beakers, 150 mL, or equivalent.
2. Watch glasses, ribbed and plain.
3. Whatman No. 41 filter paper, or equivalent.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will

interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.

3. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
4. Nitric acid (1:1). Add 500 mL concentrated HNO_3 to 400 mL Type II water. Dilute to 1 liter with Type II water.
5. Hydrochloric acid (1:1). Add 500 mL concentrated HCl to 400 mL Type II water. Dilute to 1 liter with Type II water.
6. Standard stock solutions. May be purchased or prepared from ultrahigh purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hr at 105° C, unless otherwise specified.

   CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

7. Beryllium standard stock solution (100 µg/mL). Dissolve 1.970 g $BeSO_4 \cdot 4H_2O$ (analytical reagent grade, undried), in 100 mL of Type II water, add 10.0 ml concentrated HNO_3. Dilute to 1 liter with Type II water.
8. Cadmium standard stock solution (100 µg/mL). Dissolve 0.1100 g CdO (analytical reagent grade) in a minimum amount of 1:1 HNO_3. Heat to increase rate of dissolution. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
9. Lead standard stock solution (100 µg/mL). Dissolve 0.1600 g $Pb(NO_3)_2$ (analytical reagent grade) in a minimum amount of 1:1 HNO_3. Add 10 mL 1:1 HNO_3. Add 10 mL 1:1 HNO_3. Dilute to 1 liter with Type II water.
10. Manganese standard stock solution (100 µg/mL). Dissolve 0.1000 g of manganese metal, in an acid mixture of 10 mL concentrated HCl and 1 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
11. Selenium standard stock solution (100 µg/mL). Dissolve 0.1700 g H_2SeO_3 (analytical reagent grade, undried) in 100 mL of Type II water. Dilute to 1 liter with Type II water.
12. Zinc standard stock solution (100 µg/mL). Dissolve 0.1200 g ZnO in a minimum amount of 1:1 HNO_3. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
13. Mixed calibration standard solutions. Care should be taken when preparing mixed standards for ICP analysis to ensure that the elements in the final mixed standard are compatible and stable

together. One set of mixed calibration standards that has been found to be useful is listed in Table 3. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interferences or the presence of impurities.

To prepare the mixed calibration standard for lead, combine appropriate volumes of the individual stock solutions indicated in Table 3 in volumetric flask. Add 2 mL 1:1 HNO_3 and 10 mL of 1:1 HCl and dilute to 100 mL with Type II water. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see section 9.8) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should be scanned using a sequential spectrometer to verify the absence of interelement spectral interference in the recommended mixed standard solutions.

TABLE 3. MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo, and Si
IV	Al, Ca, Cr, K, Na, and Ni

NOTE: Premixed standard solutions (traceable to NIST) containing the combined elements as listed in solutions I through IV are available from a number of commercial vendors.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Aqueous samples should be acidified to a pH of <2 with HNO_3. A holding time of 6 months after sample collection is generally cited for this parameter.

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of aqueous samples to be analyzed for lead.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Prepare a calibration blank (see section 9.4.1) and at least three mixed calibration standards in the appropriate concentration range to correlate lead concentrations with the ICP's linear response range. Prepare standards for instrument calibration as instructed in section 5.13. Match the sample matrix and that of the standards as closely as possible.

Calibrate the instrument according to the instrument manufacturer's recommended procedures using typical mixed calibration standard solutions. Flush the system with the calibration blank between each standard. Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.

8.0 Procedure

1. Transfer a 100 mL representative aliquot of the well-mixed aqueous sample to a 150 mL Griffin beaker.
2. Add 3 mL of concentrated HNO_3. Cover the beaker with a ribbed watch glass.
3. Place the beaker on a hot plate and cautiously evaporate to a low volume (5 mL) making certain that the sample does not boil and that no portion of the beaker is allowed to go dry.

   NOTE: If a sample is allowed to go to dryness, low recoveries may result. Should this occur, repeat the steps 1 through 3 with a fresh sample aliquot.

4. Cool the beaker and add 3 mL of concentrated HNO_3.

5. Cover the beaker with a nonribbed watch glass and return to the hot plate. Increase the temperature of the hot plate so that a gentle reflux action occurs.
6. Continue heating, adding additional acid as necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change in appearance with continued refluxing).
7. Uncover the beaker, or use a ribbed watch glass, and evaporate to a low volume (3 mL), not allowing any portion of the bottom of the beaker to go dry. Cool the beaker.
8. Add a small quantity of 1:1 HCl (10 mL/100 of final solution) and warm the beaker for an additional 15 min to dissolve any precipitate or residue resulting from evaporation.
9. Wash down the beaker walls and watch glass with Type II water and, when necessary, filter or centrifuge the sample to remove silicates and other insoluble material that could clog the nebulizer.

   NOTE: Filtration should be done only if there is concern that insoluble materials may clog the nebulizer. This additional step can cause sample contamination unless the filter and the filtering apparatus are thoroughly cleaned and prerinsed with dilute HNO_3.

10. Adjust to the final volume of 100 mL with Type II water. The sample is now ready for analysis.
11. The analyst should follow the instructions provided by the instrument's manufacturer. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 min of operation prior to calibration). For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within instrument linear range where coordination factors are valid. The analyst must: (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.
12. Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5%

(or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this conditions.

13. Flush the system with the calibration blank solution for at least 1 min before the analysis of each sample. Analyze samples.

    NOTE: Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for lead in aqueous solutions is 50 µg/L.

The method detection limit specified in this method is sufficient to meet the Great Lakes Water Quality Criteria for lead (82 µg/L).

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured lead concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance of the ICP and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

9.4.1 Calibration Blank

The calibration blank is prepared by diluting 2 mL of 1:1 HNO_3 and of 1:1 HCl to 100 mL with Type II water. Prepare a sufficient quantity to flush the system between standards and samples.

The calibration blank should be analyzed prior to routine sample analysis, after every 10 samples, and at the end of the analytical run. The measured concentration in the calibration blank should be less than or equal to the method detection limit.

9.4.2 Reagent Blank

The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - lead, to the 100 mL aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

9.7 Interference Check Sample

The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the method detection limit for each element. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

The interference check sample should be analyzed at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Results should be within ± 20% of the known concentration.

9.8 Calibration Control Sample

A calibration control sample should be prepared in the same acid matrix as the calibration standards at 10 times the method detection limit. This sample should be prepared in accordance with the instructions provided by the supplier. The calibration control sample is used to verify the integrity of the calibration standards on a weekly basis.

9.9 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration

data for analyte elements. These tests will ensure the analyst that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

9.9.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the method detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.9.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.

3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 µg/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported in µg/L with up to three significant figures.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Winge, R.K., V.J. Peterson, and V.A. Fassel. 1979. Inductively coupled plasma-atomic emission spectroscopy: Prominent lines, final report, March 1977 - February 1978, Ames laboratory, Ames, IA. EPA-600/4-79-017. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Table 1. Recommended Wavelengths and Estimated Instrumental Detection Limits.

Element	Wavelength[a] (ηm)	Estimated Detection Limit[b] (µg/L)
Beryllium	313.042	0.3
Cadmium	226.502	4
Lead	220.353	42
Manganese	257.610	2
Selenium	196.026	75
Zinc	213.856	2

a - The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see section 3.1).
b - The estimated instrumental detection limits are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

Table 2. Analyte Concentration Equivalents arising from Interference at the 100 mg/L Level.

Analyte	Wavelength (ηm)	Interferant[a,b] Al	Ca	Cr	Cu	Fe	Mg	Mn	Mi	Tl	V
Beryllium	313.042	-	-	-	-	-	-	-	-	0.04	0.05
Cadmium	226.502	-	-	-	-	0.03	-	-	0.02	-	-
Lead	220.353	0.17	-	-	-	-	-	-	-	-	-
Manganese	257.610	0.005	-	0.01	-	0.002	0.002	-	-	-	-
Selenium	196.026	0.23	-	-	-	0.09	-	-	-	-	-
Zinc	213.856	-	-	-	0.14	-	-	-	0.29	-	-

a - Dashes indicate that no interference was observed even when interferants were introduced at the following levels:

Al - 1000 mg/L,
Ca - 1000 mg/L,
Cr - 200 mg/L,
Cu - 200 mg/L,
Fe - 1000 mg/L.
Mg - 1000 mg/L,
Mn - 200 mg/L,
Tl - 200 mg/L,
V - 200 mg/L, and

b - The figures recorded as analyte concentrations are not the actual observed concentrations. To obtain those figures, add the listed concentration to the interferant figure.

Table 4. ICP Precision and Accuracy Data[a]

Element	Sample No. 1 True Value (µg/L)	Sample No. 1 Mean Reported Value (µg/L)	Sample No. 1 Mean SD[b] (%)	Sample No. 2 True Value (µg/L)	Sample No. 2 Mean Reported Value (µg/L)	Sample No. 2 Mean SD[b] (%)	Sample No. 3 True Value (µg/L)	Sample No. 3 Mean Reported Value (µg/L)	Sample No. 3 Mean SD[b] (%)
Be	750	733	6.2	20	20	9.8	180	176	5.2
Mn	350	345	2.7	15	15	6.7	100	99	3.3
Cd	50	48	12	2.5	2.9	16	14	13	16
Pb	250	236	16	24	30	32	80	80	14
Zn	200	201	5.6	16	19	45	80	82	9.4
Se[c]	40	32	21.9	6	8.5	42	1`0	8.5	8.3

a - Not all elements were analyzed by all laboratories.
b - SD = standard deviation.
c - Results for Se are from two laboratories.

ZINC IN WATERS AND ELUTRIATES (ICP)

1.0 Scope and Application

Inductively coupled plasma atomic emission spectroscopy (ICP) determines zinc and numerous other elements that are present in aqueous samples, such as surface water and elutriate samples. The aqueous samples must first be digested with nitric acid prior to analysis.

Detection limits, sensitivity, and optimum concentration ranges for zinc, and other metals stable in a mixed standard solution with zinc, will vary with the sample aliquot size, the spectrophotometer being used, and possible matrix interferences. The data shown in Table 1 provide concentration ranges for clean aqueous samples.

The method of standard addition (MSA) shall be used for the analysis of all sample digests unless either serial dilution or matrix spike addition demonstrates that it is not required (see section 9).

This method should only be used by spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

This procedure is based on SW-846 Methods 3010 (for digestion) and 6010 for quantitation (EPA, 1986).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Prior to analysis, the samples to be analyzed are refluxed with nitric acid in a covered beaker. This step is repeated with additional HNO_3 until the final digestate is light in color and diluted to volume.

This instrumental method measures light emitted by zinc in the final digest by optical spectrometry. The digested samples are nebulized and the resulting

aerosol is transported to a plasma torch. Zinc-specific atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes.

Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result.

The possibility of additional interferences named in section 3.0 should also be recognized and appropriate corrections made when necessary. The procedures that can be used for this purpose are described in section 9.

3.0 Interferences

There are three main forms of interferences that can occur during the analysis of water and elutriate samples by ICP. These three types of interferences include: (1) spectral, (2) physical, and (3) chemical interferences. Each is discussed separately in the following sections.

3.1 Spectral Interferences

Spectral interferences are caused by: (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of simultaneous multi-element instruments must verify the absence of spectral interference from an element in a sample for which there is no

instrument detection channel. Potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferants can be assumed.

The magnitude of the interference effects summarized in Table 2 are expressed as analyte concentration equivalents (i.e., false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that Zn is to be determined (at 213.856 ηm) in a sample containing approximately 10 mg/L of Cu. According to Table 2, 100 mg/L of Cu would yield a false signal for Zn equivalent to approximately 0.14 mg/L. Therefore, the presence of 10 mg/L of Cu would result in a false signal for Zn equivalent to approximately 0.014 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, and argon flow rate.

The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.2 Physical Interferences

Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample, by using a peristaltic pump, or by using the standard additions method. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Further, it has been reported that better control of the argon flow rate improves instrument performance. Improved argon flow rate control can be accomplished with the use of mass flow controllers.

3.3 Chemical Interferences

Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, etc.), buffering of the sample, matrix matching, and standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Centrifuge and centrifuge tubes.
4. Electric hot plate, adjustable and capable of maintaining a temperature of 90-95° C.
5. Inductively coupled argon plasma emission spectrometer.
6. Computer-controlled emission spectrometer with background correction.
7. Radio frequency generator.
8. Argon gas supply, welding grade or better.

4.2 Materials

1. Griffin beakers, 150 mL, or equivalent.
2. Watch glasses, ribbed and plain.
3. Whatman No. 41 filter paper, or equivalent.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will

interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.

3. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
4. Nitric acid (1:1). Add 500 mL concentrated HNO_3 to 400 mL Type II water. Dilute to 1 liter with Type II water.
5. Hydrochloric acid (1:1). Add 500 mL concentrated HCl to 400 mL Type II water. Dilute to 1 liter with Type II water.
6. Standard stock solutions. May be purchased or prepared from ultrahigh purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hr at 105° C, unless otherwise specified.

   CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

7. Beryllium standard stock solution (100 µg/mL). Dissolve 1.970 g $BeSO_4 \cdot 4H_2O$ (analytical reagent grade, undried), in 100 mL of Type II water, add 10.0 ml concentrated HNO_3. Dilute to 1 liter with Type II water.
8. Cadmium standard stock solution (100 µg/mL). Dissolve 0.1100 g CdO (analytical reagent grade) in a minimum amount of 1:1 HNO_3. Heat to increase rate of dissolution. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
9. Lead standard stock solution (100 µg/mL). Dissolve 0.1600 g $Pb(NO_3)_2$ (analytical reagent grade) in a minimum amount of 1:1 HNO_3. Add 10 mL 1:1 HNO_3. Add 10 mL 1:1 HNO_3. Dilute to 1 liter with Type II water.
10. Manganese standard stock solution (100 µg/mL). Dissolve 0.1000 g of manganese metal, in an acid mixture of 10 mL concentrated HCl and 1 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
11. Selenium standard stock solution (100 µg/mL). Dissolve 0.1700 g H_2SeO_3 (analytical reagent grade, undried) in 100 mL of Type II water. Dilute to 1 liter with Type II water.
12. Zinc standard stock solution (100 µg/mL). Dissolve 0.1200 g ZnO in a minimum amount of 1:1 HNO_3. Add 10.0 mL concentrated HNO_3. Dilute to 1 liter with Type II water.
13. Mixed calibration standard solutions. Care should be taken when preparing mixed standards for ICP analysis to ensure that the elements in the final mixed standard are compatible and stable

together. One set of mixed calibration standards that has been found to be useful is listed in Table 3. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interferences or the presence of impurities.

To prepare the mixed calibration standard for zinc, combine appropriate volumes of the individual stock solutions indicated in Table 3 in volumetric flask. Add 2 mL 1:1 HNO_3 and 10 mL of 1:1 HCl and dilute to 100 mL with Type II water. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see section 9.8) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should be scanned using a sequential spectrometer to verify the absence of interelement spectral interference in the recommended mixed standard solutions.

TABLE 3. MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo, and Si
IV	Al, Ca, Cr, K, Na, and Ni

NOTE: Premixed standard solutions (traceable to NIST) containing the combined elements as listed in solutions I through IV are available from a number of commercial vendors.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Aqueous samples should be acidified to a pH of <2 with HNO_3. A holding time of 6 months after sample collection is generally cited for this parameter.

All sample containers must be prewashed with detergents, acids, and Type II water. Either glass or plastic containers can be used for the storage of aqueous samples to be analyzed for zinc.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Prepare a calibration blank (see section 9.4.1) and at least three mixed calibration standards in the appropriate concentration range to correlate zinc concentrations with the ICP's linear response range. Prepare standards for instrument calibration as instructed in section 5.13. Match the sample matrix and that of the standards as closely as possible.

Calibrate the instrument according to the instrument manufacturer's recommended procedures using typical mixed calibration standard solutions. Flush the system with the calibration blank between each standard. Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.

8.0 Procedure

1. Transfer a 100 mL representative aliquot of the well-mixed aqueous sample to a 150 mL Griffin beaker.
2. Add 3 mL of concentrated HNO_3. Cover the beaker with a ribbed watch glass.
3. Place the beaker on a hot plate and cautiously evaporate to a low volume (5 mL) making certain that the sample does not boil and that no portion of the beaker is allowed to go dry.

   NOTE: If a sample is allowed to go to dryness, low recoveries may result. Should this occur, repeat the steps 1 through 3 with a fresh sample aliquot.

4. Cool the beaker and add 3 mL of concentrated HNO_3.

5. Cover the beaker with a nonribbed watch glass and return to the hot plate. Increase the temperature of the hot plate so that a gentle reflux action occurs.
6. Continue heating, adding additional acid as necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change in appearance with continued refluxing).
7. Uncover the beaker, or use a ribbed watch glass, and evaporate to a low volume (3 mL), not allowing any portion of the bottom of the beaker to go dry. Cool the beaker.
8. Add a small quantity of 1:1 HCl (10 mL/100 of final solution) and warm the beaker for an additional 15 min to dissolve any precipitate or residue resulting from evaporation.
9. Wash down the beaker walls and watch glass with Type II water and, when necessary, filter or centrifuge the sample to remove silicates and other insoluble material that could clog the nebulizer.

   NOTE: Filtration should be done only if there is concern that insoluble materials may clog the nebulizer. This additional step can cause sample contamination unless the filter and the filtering apparatus are thoroughly cleaned and prerinsed with dilute HNO_3.

10. Adjust to the final volume of 100 mL with Type II water. The sample is now ready for analysis.
11. The analyst should follow the instructions provided by the instrument's manufacturer. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 min of operation prior to calibration). For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within instrument linear range where coordination factors are valid. The analyst must: (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.
12. Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5%

(or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this conditions.

13. Flush the system with the calibration blank solution for at least 1 min before the analysis of each sample. Analyze samples.

    NOTE: Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for zinc in aqueous solutions is 20 µg/L.

The method detection limit specified in this method is sufficient to meet the Great Lakes Water Quality Criteria for zinc (67 µg/L).

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured zinc concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance of the ICP and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

9.4.1 Calibration Blank

The calibration blank is prepared by diluting 2 mL of 1:1 HNO_3 and of 1:1 HCl to 100 mL with Type II water. Prepare a sufficient quantity to flush the system between standards and samples.

The calibration blank should be analyzed prior to routine sample analysis, after every 10 samples, and at the end of the analytical run. The measured concentration in the calibration blank should be less than or equal to the method detection limit.

9.4.2 Reagent Blank

The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - zinc, to the 100 mL aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

9.7 Interference Check Sample

The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the method detection limit for each element. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

The interference check sample should be analyzed at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Results should be within ± 20% of the known concentration.

9.8 Calibration Control Sample

A calibration control sample should be prepared in the same acid matrix as the calibration standards at 10 times the method detection limit. This sample should be prepared in accordance with the instructions provided by the supplier. The calibration control sample is used to verify the integrity of the calibration standards on a weekly basis.

9.9 Recommended Tests

It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration

data for analyte elements. These tests will ensure the analyst that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

9.9.1 Serial Dilution

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the method detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

9.9.2 Standard Addition

The standard-addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The simplest version of this technique is the single-addition method in which two identical aliquots of the sample solution, each of a known volume (V_x), are taken. To the first aliquot (labeled A), add a small volume (V_s) of a standard analyte solution of known concentration (C_s). To the second aliquot (labeled B), add the same volume (V_s) of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration (C_x) is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results of this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same way as the analyte in the sample.

3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated back to zero absorbance, the point of interception of the abscissa is the concentration of the unknown. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate.

10.0 Method Performance

In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 µg/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported in µg/L with up to three significant figures.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

U.S. EPA. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-82-055. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Winge, R.K., V.J. Peterson, and V.A. Fassel. 1979. Inductively coupled plasma-atomic emission spectroscopy: Prominent lines, final report, March 1977 - February 1978, Ames laboratory, Ames, IA. EPA-600/4-79-017. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Table 1. Recommended Wavelengths and Estimated Instrumental Detection Limits.

Element	Wavelength[a] (ηm)	Estimated Detection Limit[b] (µg/L)
Beryllium	313.042	0.3
Cadmium	226.502	4
Lead	220.353	42
Manganese	257.610	2
Selenium	196.026	75
Zinc	213.856	2

a - The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see section 3.1).
b - The estimated instrumental detection limits are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

Table 2. Analyte Concentration Equivalents arising from Interference at the 100 mg/L Level.

Analyte	Wavelength (ηm)	Interferant[a,b] Al	Ca	Cr	Cu	Fe	Mg	Mn	Mi	Tl	V
Beryllium	313.042	-	-	-	-	-	-	-	-	0.04	0.05
Cadmium	226.502	-	-	-	-	0.03	-	-	0.02	-	-
Lead	220.353	0.17	-	-	-	-	-	-	-	-	-
Manganese	257.610	0.005	-	0.01	-	0.002	0.002	-	-	-	-
Selenium	196.026	0.23	-	-	-	0.09	-	-	-	-	-
Zinc	213.856	-	-	-	0.14	-	-	-	0.29	-	-

a - Dashes indicate that no interference was observed even when interferants were introduced at the following levels:

Al - 1000 mg/L,
Ca - 1000 mg/L,
Cr - 200 mg/L,
Cu - 200 mg/L,
Fe - 1000 mg/L.
Mg - 1000 mg/L,
Mn - 200 mg/L,
Tl - 200 mg/L,
V - 200 mg/L, and

b - The figures recorded as analyte concentrations are not the actual observed concentrations. To obtain those figures, add the listed concentration to the interferant figure.

Table 4. ICP Precision and Accuracy Data[a]

Element	Sample No. 1 True Value (µg/L)	Sample No. 1 Mean Reported Value (µg/L)	Sample No. 1 Mean SD[b] (%)	Sample No. 2 True Value (µg/L)	Sample No. 2 Mean Reported Value (µg/L)	Sample No. 2 Mean SD[b] (%)	Sample No. 3 True Value (µg/L)	Sample No. 3 Mean Reported Value (µg/L)	Sample No. 3 Mean SD[b] (%)
Be	750	733	6.2	20	20	9.8	180	176	5.2
Mn	350	345	2.7	15	15	6.7	100	99	3.3
Cd	50	48	12	2.5	2.9	16	14	13	16
Pb	250	236	16	24	30	32	80	80	14
Zn	200	201	5.6	16	19	45	80	82	9.4
Se[c]	40	32	21.9	6	8.5	42	1`0	8.5	8.3

a - Not all elements were analyzed by all laboratories.
b - SD = standard deviation.
c - Results for Se are from two laboratories.

TOTAL PETROLEUM HYDROCARBONS IN WATERS AND ELUTRIATES (SPECTROPHOTOMETRIC, INFRARED)

1.0 Scope and Application

This method is appropriate for the determination of fluorocarbon-113 extractable petroleum hydrocarbons from surface waters and sediment elutriates. It should be noted that this method will change upon identification and approval of an environmentally friendly solvent.

The method is applicable to measurement of light fuels, although loss of about half of any gasoline present during the extraction manipulations can be expected.

The method is appropriate for a total petroleum hydrocarbon concentration of 1 mg/L or less.

This method is based on EPA Method 418.1 (USEPA, 1983).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

The sample is acidified to a low pH (<2) and serially extracted with fluorocarbon-113 in a separatory funnel. Interferences that may be co-extracted with the total petroleum hydrocarbons (TPHs) are removed with silica gel adsorbent. Infrared analysis of the extract is performed at 2930 cm^{-1} and TPH concentrations determined by direct comparison with standards.

3.0 Interferences

Total petroleum hydrocarbons (TPHs) are operationally defined by the extraction procedure and the analytical technique. Petroleum fuels, from gasoline through No. 2 fuel oils, may be lost during sample preparation.

The method is not considered applicable to light hydrocarbons that volatilize below 70° C. Also, some crude oils and heavy fuel oils that are not soluble in fluorocarbon-113 will have low recoveries.

The rate and time of extraction in the Soxhlet apparatus should be strictly controlled because of varying solubilities of different greases.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Infrared spectrophotometer, scanning or fixed wavelength, for measurement around 2930 cm^{-1}.
4. Magnetic stirrer, with Teflon coated stirring bars.
5. Mortar and pestle.
6. Soxhlet extraction apparatus.
7. Vacuum pump or other source of vacuum.

4.2 Materials

1. Separatory funnel with Teflon stopcock, 2000 mL.
2. Beakers, glass, 150 mL.
3. Cells, 10 mm, 50 mm, and 100 mm pathlength, sodium chloride or infrared grade glass.
4. Extraction thimbles, paper.
5. Glass bottles with stoppers, 50 mL.
6. Glass wool or beads.
7. Volumetric flasks, class A, 200 mL.
8. Volumetric flasks, class A, 100 mL.
9. Whatman filter paper No. 40, 11 cm.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.

2. Fluorocarbon-113 (1,1,2-trichloro-1,2,2-trifluoroethane), boiling point 47° C.

   NOTE: The solvent should leave no measurable residue on evaporation. Redistill if necessary.

3. Concentrated hydrochloric acid (HCl), reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
4. Hydrochloric acid (HCl), 1:1. Mix equal volumes of concentrated HCl and Type II water.
5. 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.
6. Sodium sulfate (Na_2SO_4), anhydrous crystal.
7. Reference oil used for calibration mixtures. Pipet 15.0 mL n-hexadecane ($C_{16}H_{34}$), 15.0 mL isooctane (C_8H_{18}), and 10.0 mL chlorobenzene (C_6H_5Cl) into a 50 mL glass-stoppered bottle. Maintain the integrity of the mixture by keeping stoppered except when withdrawing aliquots.
8. Reference oil stock solution. Pipet 1.0 mL reference oil into a tared 200 mL volumetric flask and immediately stopper. Weigh and dilute to volume with fluorocarbon-113.
9. Reference oil working standards. Pipet appropriate volumes of stock standard into 100 mL volumetric flasks according to the cell pathlength to be used. Dilute to volume with fluorocarbon-113. Calculate concentration of standards from the stock standard.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Because losses of grease can occur on sampling equipment, the collection of composite samples is impractical for this parameter.

Samples should be preserved with 5 mL 1:1 HCl at the time of collection to a pH of <2. A holding time of 28 days after sample collection is generally cited for this parameter.

The sample should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Sample aliquots to be analyzed for TPH should be collected and stored in 1 L glass bottles.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Select appropriate working standards and cell pathlength based on the expected total petroleum hydrocarbon concentration in the aqueous solution. The following information is presented as a guide for selecting a suitable cell pathlength:

Pathlength	Range
10 mm	2-40 mg
50 mm	0.5-8 mg
100 mm	0.1-4 mg

Calibrate the instrument for the appropriate cells using a series of working standards. It is not necessary to add silica gel to the standards.

Scan the standards from 3200 to 2700 cm^{-1} using a scanning infrared spectrophotometer. Fluorocarbon-113 should be used in the reference beam of a dual beam instrument or to zero a single beam instrument. The absorbance of the 2930 cm^{-1} peak should be used to construct a standard curve.

8.0 Procedure

1. Mark the water meniscus level on the sample bottle containing the original sample for later determination of the sample volume.

2. If the sample was not acidified at time of collection, add 5 mL 1:1 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.
3. Pour the sample into a 2000 mL separatory funnel.
4. Add 30 mL fluorocarbon-113 to the sample bottle and rotate the bottle to rinse the sides.
5. Transfer the solvent into the separatory funnel.
6. Extract by shaking vigorously for 2 minutes. Allow the layers to separate.
7. Filter the solvent layer through a funnel containing solvent-moistened filter paper into a 100 mL volumetric flask.

   NOTE: An emulsion that fails to dissipate can be broken by pouring about 1 g 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.

8. Repeat steps 4 through 7 twice more with 30 mL portions of fresh solvent, combining all solvent into the volumetric flask.
9. 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.
10. Dilute the extract to 100 mL with fluorocarbon-113.

    NOTE: If the extract is known to contain greater than 100 mg of non-hydrocarbon organic material, pipet an appropriate portion of the sample to a 100 mL volumetric and dilute to volume.

11. Discard about 5-10 mL solution from the volumetric flask. Add 3 g silica gel and a stirring bar.
12. Stopper the volumetric flask and stir the solution for a minimum of 5 min on a magnetic stirrer.
13. After the silica gel has settled in the sample extract, fill a clean cell with solution and determine the absorbance of the extract.

    NOTE: If the absorbance exceeds 0.8, prepare an appropriate dilution and reanalyze the sample.

    NOTE: The possibility that the absorptive capacity of the silica gel has been exceeded can be tested at this point by adding another 3.0 g silica gel to the extract and repeating the treatment and determination.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for TPH in aqueous solutions is 100 µg/L.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured TPH concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the instrument detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte (i.e., reference oil), in this case - TPH, to the 20 g aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

10.0 Method Performance

When a sewage sample containing 12.6 mg/L oil and grease was spiked with 14.0 mg of a mixture containing No. 2 fuel oil and Wesson oil and analyzed by a single laboratory, the recovery was 93% with a standard deviation of 0.9 mg/L.

In a second study, sewage containing 17.5 mg/L oil and grease was spiked with 14 mg of a mixture containing No. 2 fuel oil and Wesson oil. The recovery was 99% and the standard deviation was 1.4 mg/L.

11.0 Calculations and Reporting

Determine the concentration of petroleum hydrocarbons in the extract by comparing the response against the calibration plot. Calculate the concentration of petroleum hydrocarbons in the original sample using the following formula:

$$\text{TPH, mg/L} = \frac{R \times D}{V}$$

where:
R = TPH as determined from the calibration plot, mg.
D = extract dilution factor, if used.
V = volume of sample, L.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Blum, K.A. and M.J. Taras. 1968. Determination of Emulsifying Oil in Industrial Wastewater" JWPCF Research Supplement 40:R404.

U.S. Environmental Protection Agency. 1983. Methods for the Chemical Analysis of Water and Wastes. EPA-600/4-79-020. U.S. Environmental Protection Agency Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

PHENOLICS IN WATERS AND ELUTRIATES (COLORIMETRIC, AUTOMATED 4-AAP)

1.0 Scope and Application

This method is applicable to the determination of phenolic compounds in surface water samples and sediment elutriates. The method is capable of measuring phenolic materials over a range of 2 to 500 µg/L in aqueous samples when using phenol as a standard.

This method is based on SW-846 Method 9066 (EPA, 1986).

NOTE: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

Phenolic compounds are separated from the original sample matrix by distillation under acidic conditions (pH <4.0). The phenolic compounds in the distillate are then reacted with alkaline ferricyanide ($K_3Fe(CN)_6$) and 4-amino-antipyrine (4-AAP) to form a red complex which is measured at 505 or 520 ηm.

3.0 Interferences

Interferences from sulfur compounds are eliminated by acidifying the sample to a pH of <4.0 with H_2SO_4 and aerating briefly by stirring.

Color and turbidity in the original sample can interfere with this colorimetric procedure. Color interference is eliminated by distilling the phenolic compounds from the original sample prior to analysis. Turbidity is removed by sample filtration prior to analysis.

Oxidizing agents, such as chlorine, detected by the liberation of iodine upon acidification in the presence of potassium iodide, are removed immediately after sampling by the addition of an excess of ferrous ammonium sulfate (see section 5.0, item 3). If chlorine is not removed, the phenolic compounds may be partially oxidized and the sample results may be biased low.

Background contamination from plastic tubing and sample containers is eliminated by filling the wash receptacle by siphon (using Kel-F tubing) and using glass tubes for the samples and standards.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.01 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Distillation apparatus, all glass, consisting of a 1-liter pyrex distillation flask and a Graham condenser.
4. pH meter.
5. Automated continuous-flow analytical instrument with:
   a. sampler equipped with continuous mixer,
   b. manifold,
   c. proportioning pump II or III,
   d. heating bath with distillation coil,
   e. distillation head,
   f. colorimeter equipped with a 50 mm flowcell and 505 or 520 ηm filter, and
   g. recorder.

4.2 Materials

1. Volumetric flasks, class A, 1 L.
2. Volumetric flasks, class A, 100 mL.

5.0 Reagents

1. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
2. 4-Aminoantipyrine (4-AAP). Dissolve 0.65 g of 4-aminoantipyrine in 800 mL of Type II water. Dilute to 1 liter with Type II water. Prepare fresh daily.
3. Ferrous ammonium sulfate ($FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$. Dissolve 1.1 g ferrous ammonium sulfate in 500 mL of Type II water containing 1 mL

concentrated H_2SO_4. Dilute to 1 liter with freshly boiled and cooled Type II water.

4. Buffered potassium ferricyanide ($K_3Fe(CN)_6$). Dissolve 2.0 g potassium ferricyanide ($K_3Fe(CN)_6$), 3.1 g boric acid (H_3BO_3), and 3.75 g potassium chloride (KCl) in 800 mL of Type II water. Adjust to pH of 10.3 with 1 N sodium hydroxide. Dilute to 1 liter with Type II water. Add 0.5 mL of Brij-35 (available from Technicon). Prepare fresh weekly.

   NOTE: Brij-35 is a wetting agent and is a proprietary Technicon product.

5. Sodium hydroxide (NaOH), 1 N. Dissolve 40 g NaOH in 500 mL of Type II water. Cool. Dilute to 1 liter with Type II water.
6. Concentrated sulfuric acid (H_2SO_4), reagent grade. Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
7. Sulfuric acid (H_2SO_4), 1 N. Add 28 mL concentrated H_2SO_4 to 900 mL of Type II water. Dilute to 1 liter with Type II water.
8. Phenol stock solution. Dissolve 1.00 g phenol (C_6H_5OH) in 500 mL of Type II water. Dilute to 1 liter with Type II water. Add 0.5 mL concentrated H_2SO_4 as preservative (1.0 mg/mL phenol).

   CAUTION: This solution is toxic.

9. Phenol standard solution A. Dilute 10.0 mL of phenol stock solution to 1 liter with Type II water (0.01 mg/mL phenol).
10. Phenol standard solution B. Dilute 100.0 mL of phenol standard solution A to 1 liter with Type II water (0.001 mg/mL phenol).
11. Phenol standard solution C. Dilute 100.00 mL of phenol standard solution B to 1 liter with Type II water (0.0001 mg/mL phenol).

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Biological degradation of phenolic compounds is inhibited by acidification to a pH <2 with H_2SO_4. A holding time of 28 days after sample collection is generally cited for this parameter.

The sample should be stored under refrigerated conditions (4° C).

All sample containers must be prewashed with detergents, acids, and Type II water. Sample aliquots to be analyzed for phenolic compounds should be collected and stored in glass bottles.

7.0 Calibration and Standardization

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

Calibration curves must be composed of a minimum of a blank and three standards. A separate calibration curve should be prepared for every hour of continuous sample analysis.

Using standard solution A, B, or C, prepare the following standards in 100 mL volumetric flasks:

Standard Solution (mL)	Concentration (µg/L)
Solution C	
1.0	1.0
2.0	2.0
3.0	3.0
5.0	5.0
Solution B	
1.0	10.0
2.0	20.0
5.0	50.0
10.0	100.0
Solution A	
2.0	200.0
3.0	300.0
5.0	500.0

Each standard should be preserved by adding 2 drops of concentrated H_2SO_4 to 100.0 mL.

Prepare a linear standard curve by plotting peak heights of standards against concentration values.

8.0 Procedure

1. Place 500 mL of sample into a 1-L pyrex distillation flask.
2. Adjust the pH of the sample to approximately 4 with the addition of 1 N sulfuric acid.
3. Attach the condenser and distill over 450 mL of distillate.
4. When boiling has ceased in the distillation flask, add 50 mL warm Type II water to the distillation flask and resume sample distillation until a total of 500 mL distillate has been collected.
5. Acidify the distillates with 2 drops concentrated H_2SO_4 per 100 mL.

   NOTE: If the sample distillate is turbid, it should be filtered through a prewashed membrane filter prior to analysis.

6. Set up a AutoAnalyzer manifold with the following flow rates:

Air	0.32 mL/min.
Sample	2.00 mL/min.
Distilling solution	0.42 mL/min.
Waste from still	0.42 mL/min.
Air	0.32 mL/min.
Resample waste	1.00 mL/min.
Resample	1.2 mL/min.
4-AAP	0.23 mL/min.
Buffered potassium ferricyanide	0.23 mL/min.
Waste from F/C	1.0 mL/min.

7. Fill the wash receptacle by siphon. Use Kel-F tubing with a fast flow (1 liter/hr).
8. Allow colorimeter and recorder to warm up for 30 min.
9. Run a baseline with all reagents feeding Type II water through the sample line.

NOTE: Use polyethylene tubing for sample line.

NOTE: When new tubing is used, about 2 hours may be required to flush residual phenol from the tubing and obtain a stable baseline.

10. Place appropriate standards in the sampler in order of decreasing concentration.
11. Complete loading of the sampler tray with unknown and quality assurance/quality control samples in glass tubes.
12. Run with sensitivity setting at full scale or 500.
13. When the baseline becomes steady, switch sample from Type II water to samples and begin analysis.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit, or of a reagent blank.

The method detection limit for phenolics in aqueous solutions is 50 µg/L.

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 20 percent or less between the measured phenolic concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 15% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the instrument detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 10% of the known concentration.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - phenolics, to the 500 mL aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted/digested and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 15% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 20%.

10.0 Method Performance

In a single laboratory using sewage samples at concentrations of 3.8, 15, 43, and 89 µg/L, the standard deviations were ± 0.5, 0.6, 0.6, and 1.0 µg/L, respectively. At concentrations of 73, 146, 299, and 447 µg/L, the standard deviations were ± 1.0, 1.8, 4.2, and 5.3 µg/L, respectively.

In a single laboratory using sewage samples at concentrations of 5.3 and 82 µg/L, the recoveries were 78% and 98%, respectively. At concentrations of 168 and 489 µg/L, the recoveries were 97% and 98%, respectively.

11.0 Calculations and Reporting

If dilutions were performed, the appropriate factors must be applied to sample values. Compute concentration of samples by comparing sample peak heights with standards. All results should be reported in µg/L with up to three significant figures.

12.0 References

American Public Health Association. 1989. Standard Methods for the Examination of Water and Wastewater. Method 510. 14th Edition, APHA, New York, New York. p. 574.

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Gales, M.E., and R. L. Booth. 1976. Automated 4-AAP Phenolic Method. AWWA 68:540.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

TOTAL POLYCHLORINATED BIPHENYLS (PCBs) AND PESTICIDES IN WATERS AND ELUTRIATES

1.0 Scope and Application

This method is suitable for the determination of chlorinated pesticides and PCB congeners in waters and elutriates. Table 1 presents the PCB congeners most commonly found in the environment while Table 2 list the pesticides of concern in the Great Lakes. All these compounds may be determined using this method.

This procedure is based on a National Oceanic and Atmospheric Administration (NOAA) method for the determination of Extractable Toxic Organic Compounds in marine sediments (NOAA, 1985) for the quantification and clean-up of the extracts and SW-846 method 8270 (USEPA, 1986) for the extraction of the waters and elutriates.

The extracts produced from this method (sections 8.1 through 8.6) can be used in the determination of PCBs, pesticides, and polynuclear aromatic hydrocarbons (PAHs).

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

The sample is extracted in a separatory funnel with methylene chloride (CH_2Cl_2). The resultant extract is cleaned-up with silica gel and alumina. Additional clean-up steps to remove biological macromolecules are performed using Sephadex LH-20. PCB congeners and pesticides are then quantified using a glass capillary column to resolve all congeners and gas chromatography/electron capture detector (GC/ECD). The concentrations of 20 congeners (Table 3) will be summed to determine the total PCB content in the sediment.

The same extract used to analyze for PCBs and pesticides can be used to analyze polynuclear aromatic hydrocarbons (PAHs) using gas chromatography/mass spectrometry (GC/MS). The method for PAH determination is provided in this methods manual.

Table 1. PCB Congeners Commonly Identified in the Great Lakes.

BZ#	Structure	BZ#	Structure
1	2-chlorobiphenyl	105	2,3,3',4,4'-pentachlorobiphenyl
3	4-chlorobiphenyl	107	2,3,3',4',5-pentachlorobiphenyl
4	2,2'-dichlorobiphenyl	115	2,3,4,4',6-pentachlorobiphenyl
5	2,3-dichlorobiphenyl	119	2,3',4,4',6-pentachlorobiphenyl
6	2,3'-dichlorobiphenyl	122	2',3,3',4,5-pentachlorobiphenyl
9	2,5-dichlorobiphenyl	123	2',3,4,4',5-pentachlorobiphenyl
12	3,4-dichlorobiphenyl	128	2,2',3,3',4,4'-hexachlorobiphenyl
15	4,4'-dichlorobiphenyl	129	2,2',3,3',4,5-hexachlorobiphenyl
16	2,2',3-trichlorobiphenyl	136	2,2',3,3',6,6'-hexachlorobiphenyl
18	2,2',5-trichlorobiphenyl	137	2,2',3,4,4',5-hexachlorobiphenyl
19	2,2',6-trichlorobiphenyl	138	2,2',3,4,4',5'-hexachlorobiphenyl
22	2,3,4'-trichlorobiphenyl	141	2,2',3,4,5,5'-hexachlorobiphenyl
25	2,3',4-trichlorobiphenyl	149	2,2',3,4',5',6-hexachlorobiphenyl
26	2,3',5-trichlorobiphenyl	151	2,2',3,5,5',6-hexachlorobiphenyl
27	2,3',6-trichlorobiphenyl	153	2,2',4,4',5,5'-hexachlorobiphenyl
28	2,4,4'-trichlorobiphenyl	157	2,3,3',4,4',5'-hexachlorobiphenyl
29	2,4,5-trichlorobiphenyl	158	2,3,3',4,4',6-hexachlorobiphenyl
31	2,4',5-trichlorobiphenyl	167	2,3',4,4',5,5'-hexachlorobiphenyl
37	3,4,4'-trichlorobiphenyl	170	2,2',3,3',4,4',5-heptachlorobiphenyl
40	2,2',3,3'-tetrachlorobiphenyl	171	2,2',3,3',4,4',6-heptachlorobiphenyl
41	2,2',3,4-tetrachlorobiphenyl	177	2,2',3,3',4,5,6-heptachlorobiphenyl
44	2,2',3,5'-tetrachlorobiphenyl	180	2,2',3,4,4',5,5'-heptachlorobiphenyl
47	2,2',4,4'-tetrachlorobiphenyl	183	2,2',3,4,4',5',6-heptachlorobiphenyl
49	2,2',4,5'-tetrachlorobiphenyl	185	2,2',3,4,5,5,6'-heptachlorobiphenyl
52	2,2',5,5'-tetrachlorobiphenyl	187	2,2',3,4',5,5',6-heptachlorobiphenyl
53	2,2',5,6'-tetrachlorobiphenyl	189	2,3,3',4,4',5,5'-heptachlorobiphenyl
56	2,3,3',4'-tetrachlorobiphenyl	190	2,3,3',4,4',5,6-heptachlorobiphenyl
66	2,3,4,4'-tetrachlorobiphenyl	191	2,3,3',4,4',5',6-heptachlorobiphenyl
70	2,3',4',5-tetrachlorobiphenyl	193	2,3,3',4',5,5',6-heptachlorobiphenyl
75	2,4,4',6-tetrachlorobiphenyl	194	2,2',3,3',4,4',5,5'-octachlorobiphenyl
77	3,3',4,4'-tetrachlorobiphenyl	195	2,2',3,3',4,4',5,6-octachlorobiphenyl
82	2,2',3,3',4-pentachlorobiphenyl	196	2,2',3,3',4,4',5',6-octachlorobiphenyl
83	2,2',3,3',5-pentachlorobiphenyl	198	2,2',3,3',4,5,5',6-octachlorobiphenyl
84	2,2',3,3',6-pentachlorobiphenyl	199	2,2',3,3',4,5,6,6'-octachlorobiphenyl
85	2,2',3,4,4'-pentachlorobiphenyl	200	2,2',3,3',4,5',6,6'-octachlorobiphenyl
87	2,2',3,4,5'-pentachlorobiphenyl	201	2,2',3,3',4',5,5',6-octachlorobiphenyl
91	2,2',3,4',6-pentachlorobiphenyl	202	2,2',3,3',5,5',6,6'-octachlorobiphenyl
92	2,2',3,5,5'-pentachlorobiphenyl	205	2,3,3',4,4',5,5',6-octachlorobiphenyl
95	2,2',3,5',6-pentachlorobiphenyl	206	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl
97	2,2',3',4,5-pentachlorobiphenyl	207	2,2',3,3',4,4',5,6,6'-nonachlorobiphenyl
99	2,2',4,4',5-pentachlorobiphenyl	208	2,2',3,3',4,5,5',6,6'-nonachlorobiphenyl
101	2,2',4,5,5'-pentachlorobiphenyl		

Table 2. Pesticides of Concern in the Great Lakes.

aldrin	trans-nonachlor
α-chlordane	o,p'-DDE
dieldrin	p,p'-DDE
heptachlor	o,p'-DDD
heptachlor epoxide	p,p'-DDD
hexachlorobenzene	o,p'-DDT
lindane (γ-BHC)	p,p'-DDT
mirex	

Table 3. Twenty PCB Congeners to be Summed to Determine Total PCB Content[a].

BZ#	Structure	BZ#	Structure
8	2,4'-dichlorobiphenyl	126	3,3',4,4',5-pentachlorobiphenyl
18	2,2',5-trichlorobiphenyl	128	2,2',3,3',4,4'-hexachlorobiphenyl
28	2,4,4'-trichlorobiphenyl	138	2,2',3,4,4',5'-hexachlorobiphenyl
44	2,2',3,5'-tetrachlorobiphenyl	153	2,2',4,4',5,5'-hexachlorobiphenyl
52	2,2',5,5'-tetrachlorobiphenyl	169	3,3',4,4',5,5'-hexachlorobiphenyl
66	2,3,4,4'-tetrachlorobiphenyl	170	2,2',3,3',4,4',5-heptachlorobiphenyl
77	3,3',4,4'-tetrachlorobiphenyl	180	2,2',3,4,4',5,5'-heptachlorobiphenyl
101	2,2',4,5,5'-pentachlorobiphenyl	187	2,2',3,4',5,5',6-heptachlorobiphenyl
105	2,3,3',4,4'-pentachlorobiphenyl	206	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl
118	2,3',4,4',5-pentachlorobiphenyl	209	2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl

a = The selected congeners are a combination of those presented in the *Inland Testing Manual* (USEPA/USACE, 1998 and NOAA method (NOAA, 1985).

3.0 Interferences

Interferences by phthalate esters can pose a major problem in pesticide determinations when using the electron capture detector. These compounds generally appear in the chromatogram as large late-eluting peaks. Common flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalates can best be minimized by avoiding

contact with any plastic materials. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.001 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Centrifuge, capable of holding 250 mL centrifuge tubes and maintaining speeds of 1500 rpm.
4. Desiccator and desiccant. Desiccants generally used include: anhydrous calcium sulfate, silica gel, or phosphorus pentoxide. Indicating desiccants are preferable since they show when the desiccant needs to be changed or regenerated.
5. Gas chromatograph (GC) including:
   a. dual capillary column inlet system,
   b. autosampler,
   c. cartridge tape unit, and
   d. electron capture detector (ECD), two are needed.
6. Modified Kontes tube heater (block contains: Al inserts fitted to the 0.7 mL line of the Kuderna-Danish tube tip and an Al-foil shroud.
7. Molecular sieve traps (for gas cylinder)

   NOTE: One suggested source for the molecular sieve traps is Hydro-Purge model ASC-l, Coast Engineering Laboratory, Gardena, California.

8. Oxygen traps.
9. UV light source.
10. Water bath, capable of maintaining a temperature of 80 ± 2° C.

4.2 Materials

1. Beakers, 250 mL, or equivalent.
2. Centrifuge tubes, 250 mL, amber, with Teflon™ caps.
3. Chromatography column with reservoir 250 mL, 19 mm ID, 30 cm.

4. Erlenmeyer flask, 500 mL, with stopper.
5. Erlenmeyer flask, 1 L, with stopper.
6. Funnel, curved-stem (curve must be glassblown).
7. Funnel, 200 mm OD, long-stem.
8. Funnel, powder.
9. GC column, silicon-coated fused silica capillary, DB-5, 30 m x 0.25 mm I.D.
10. GC column, silicon-coated fused silica capillary, DB-17HT, 30 m × 0.25 mm I.D.
11. Graduated cylinder, 500 mL.
12. Graduated cylinder, 100 mL.
13. Graduated cylinder, 50 mL.
14. Kontes concentrator tube, 25 mL, with stopper.
15. Kuderna-Danish concentrator tube, 10 mL, graduated.
16. Kuderna-Danish evaporative flask, 500 mL.
17. pH paper, wide range, capable of determining pH from 4 to 10.
18. Separatory funnel, 2 L, with Teflon™ stopcock.
19. Snyder column, 3-ball macro.
20. Snyder column, 2-ball micro.
21. Syringe, 2000 µL.
22. Syringe, 800 µL.
23. Syringe, 400 µL.
24. Syringe, 200 µL.
25. Syringe, 100 µL.
26. Syringe, 50 µL.
27. Syringe, 10 µL.
28. Teflon wash-bottle, 500 mL (to be filled with CH_2Cl_2).
29. Transfer pipets (Pasteur style) with rubber bulbs.
30. GC vials, 2 mL.
31. GC vials, 100 µL, conical.
32. Volumetric flask, class A, 10 mL.
33. Volumetric pipet, 50 mL.

5.0 Reagents

1. Alumina, 80-200 mesh. Alumina should be activated at 120° C for 2 hr and then cooled to room temperature in a desiccator just before weighing and use.

2. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
3. Azulene, reagent grade ($C_{15}H_{18}$).
4. Helium, grade 4.5 (purified, $\geq$99.995 %).
5. Hexane, high purity (C_6H_{14}). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
6. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
7. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
8. Methanol, high purity (CH_3OH). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
9. Methylene chloride (dichloromethane), high purity (CH_2Cl_2). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
10. Pentane, high purity (C_5H_{12}). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
11. Perylene, reagent grade ($C_{20}H_{12}$).
12. Sand, Ottawa, MCB, kiln-dried, 30-40 mesh. Sand should be acid-washed (steeped in *aqua regia* (ACS grades $HN0_3$:HCl, 1:3, v:v) overnight, then washed three times each with H_2O, CH_3OH and CH_2Cl_2, dried, and stored at 120° C.
13. Sephadex LH-20, size-exclusion gel. Sephadex LH-20 should be swelled overnight in 6:4:3 solvent.
14. Silica gel, Davison Type 923 or Amicon No. 84080. Silica should be activated at 700° C for 18 hr, stored at 170° C, and cooled to room temperature in a desiccator just before weighing and use.

15. Sodium hydroxide, 10 N (NaOH). Add 20 g of NaOH to 400 mL Type II water. Dilute to 500 mL with Type II water.
16. Sodium sulfate, reagent grade, anhydrous granular (Na_2S0_4). Sodium sulfate should be CH_2Cl_2 washed, dried, stored at 120° C, and cooled to room temperature in a desiccator before weighing and use.
17. PCB/pesticide standard stock solution (100 µg/mL). From commercially available neat PCB and pesticide standards, weigh 1.00 mg of each congener and pesticide and dissolve 5 mL hexane. Dilute to 10.0 mL with hexane.

    NOTE: PCB congener standards may also be purchased commercially at concentrations of 100 µg/mL.

18. PCB/pesticide primary dilution standard solution (1 µg/mL). Accurately measure a 100 µL aliquot of the PCB and pesticide standard stock solution and dilute to 10.0 mL of hexane.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the sediments should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

A holding time of 7 days until extraction and 40 days from extraction to analysis is generally cited for this parameter.

Samples should be stored under refrigerated conditions (4° C).

NOTE: Samples can be frozen to extend the holding time for up to 1 year.

All sample containers must be prewashed with detergents, acids, and Type II water. Glass containers should be used for the storage of samples to be analyzed for PCBs in waters and elutriates. All glassware and materials contacting the solvents should be washed with CH_2Cl_2 three times prior to use.

An option to the CH_2Cl_2 washing of the glassware is to combust the glassware in a muffle oven at 400° C for 4 hours.

7.0 Calibration and Standardization

7.1 General

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The water bath and Kontes tube heater should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

7.2 Sephadex LH-20 Column Calibration

Information on preparing the Sephadex LH-20 column is presented in Attachment A.

1. Add enough azulene (approximately 10 mg/mL) and perylene (approximately 1 mg/mL) to approximately 50 mL of 6:4:3 solvent to produce a deeply colored solution.

   NOTE: Make sure that the azulene and perylene are <u>completely</u> dissolved.

2. Place a 100 mL cylinder beneath the column and using a transfer pipet, cautiously remove any excess 6:4:3 solvent from the top of the packing.
3. Using a transfer pipet, cautiously apply 2 mL of the azulene/perylene calibration solution onto the column. Use a circular motion to dispense the solution just above the packing, and drip the solution slowly down the column wall so as not to disturb the packing.
4. Open the stopcock, drain to the packing top, and close the stopcock.

5. Add approximately 0.5 mL of solvent to the top of the column. Drain to the packing top, and close the stopcock.
6. Repeat step 5 once.
7. Add 100 mL of solvent, and open the stopcock.
8. Elute the solvent until all of the perylene has emerged, using the UV light to monitor the perylene. Record the volumes at which the azulene and perylene start and finish eluting.
9. If the azulene emerges in the 50-65 mL range, and the perylene emerges in the 60-80 mL range without distinct tailing on the packing, proceed to step 10. Otherwise, recycle the packing (Attachment A).
10. Discard the eluate. Add 50 mL of solvent to the column, and flush the packing by eluting 50 mL into the cylinder. Again, discard the eluate.
11. The column is now ready for the next sample.

    NOTE: If the column is to be stored, maintain 30-50 mL of solvent in the column reservoir, and cover the top with aluminum foil. Remove the solvent if it separates into 2 phases, add 80 mL of fresh 6:4:3 solvent, and elute 50 mL.

7.3 GC Calibration

Calibration standards at a minimum of five concentration levels should be prepared. One of the calibration standards should be at a concentration near, but above, the method detection limit; the others should correspond to the range of concentrations found in real samples but should not exceed the working range of the GC/MS system. Each standard should contain each analyte for detection by this method (e.g., some or all of the compounds listed in Tables 2 and 3 may be included). All initial calibration standards should be stored at -10° C to -20° C and should be freshly prepared once a year, or sooner if check standards indicate a problem. The daily calibration standard (ongoing calibration should be prepared weekly and stored at 4° C.

Using the PCB/pesticide primary dilution standard solution, prepare the following suggested standards in 10 mL volumetric flasks:

Primary Dilution Standard Solution (µL)	Concentration (µg/L)
50	5
200	20
400	40
800	80
2000	200

Each standard should be brought to volume with hexane.

Linearity of the GC is determined by calculation of the individual response factors (RF) for each standard concentration using the following formula:

RF = total peak area/mass of injected analyte.

The calibration curve will be considered linear if the %RSD is $\leq$30% for each compound.

8.0 Procedure

8.1 Water and Elutriate Extraction

1. Using a 1 liter graduated cylinder, measure 1 liter of sample and transfer to a 2 liter separatory funnel.

   NOTE: If high concentrations are anticipated, a smaller sample volume may be used and diluted to 1 liter with Type II water.

2. Check the pH of the sample with wide-range pH paper and, if necessary, adjust the pH to greater than 11 with 10 N NaOH.
3. Add 60 mL methylene chloride to the separatory funnel.
4. Add all surrogate spike solutions (see section 9.7).

   NOTE: Make certain that the solutions are placed into the CH_2Cl_2.

5. Seal and shake the separatory funnel vigorously for 1-2 minutes with periodic venting to release excess pressure.

NOTE: Methylene chloride creates excessive pressure very rapidly; therefore, initial venting should be done immediately after the separatory funnel has been sealed and shaken once. The separatory funnel should be vented into a hood to prevent unnecessary exposure of the analyst to the organic vapor.

6. Allow the organic layer to separate from the water phase for a minimum of 10 minutes.

   NOTE: If the emulsion interface between layers is more than one-third the size of the solvent layer, a mechanical technique should be used to complete the phase separation. The optimum technique depends upon the sample and may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical method.

7. Collect the solvent extract in an Erlenmeyer flask.

   NOTE: If the emulsion cannot be broken (recovery of <80% of the methylene chloride, corrected for the water solubility of methylene chloride), transfer the sample, solvent, and emulsion into the extraction chamber of a continuous extractor and follow procedures given in SW-846 Method 3520 (USEPA, 1986).

8. Repeat steps 4 through 7 two more times using fresh 60 mL portions of methylene chloride.
9. Combine the three extracts.

8.2 Extract Concentration

1. Dry the extract by passing it through a drying column containing about 10 cm anhydrous sodium sulfate.
2. Collect the dried extract in a K-D concentrator.
3. Rinse the Erlenmeyer flask, which contained the original solvent extract, with 20-30 mL methylene chloride and add it to the drying column to complete a quantitative transfer of the sample extract.
4. Add one or two clean boiling chips to the evaporation flask.
5. Attach a three-ball Snyder column and prewet the Snyder column by adding approximately 1 mL methylene chloride to the top of the column.

6. Place the K-D apparatus on a hot water bath (80-90° C) so that the concentrator is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor.

   NOTE: The Snyder column may need to be rewetted with 1 mL of CH_2Cl_2 if the extract solution is not boiling when the Snyder column is initially wetted in step 5.

7. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 10-20 minutes.

   NOTE: At the proper rate of distillation, the balls of the chamber will actively chatter but the chambers will not flood. If the chambers flood, lightly tap the Snyder column with a soft blunt object.

8. When the apparent volume of the liquid reaches 1 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.
9. Remove the Snyder column and rinse both the flask and its lower joints into the concentrator tube with 1-2 mL methylene chloride.
10. Add a clean boiling chip to the concentrator tube.
11. Attach a two-ball micro-Snyder column and prewet the column by adding 0.5 mL methylene chloride to the top of the column.
12. Place the K-D apparatus in a hot water bath so that the concentrator tube is partially immersed in the hot water.
13. Adjust the vertical position of the apparatus and the water temperature, as required, to complete the concentration in 5-10 minutes.

    NOTE: At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. If the chambers flood, lightly tap the Snyder column with a soft blunt object.

14. When the apparent volume of extract reaches 0.5 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

15. Remove the Snyder column and rinse both the flask and its lower joints into the concentrator tube with 0.2 mL methylene chloride twice.
16. Adjust the final volume to 1.0 mL with methylene chloride.

    NOTE: If cleanup of the extract will not be performed immediately, stopper the concentrator tube and store under refrigerated conditions (4° C).

    NOTE: If the extract will be stored longer than 2 days, it should be transferred to a vial with a Teflon-lined screw cap, and appropriately labeled.

8.3 Silica Gel/Alumina Chromatography

NOTE: The laboratory temperature must be <80° F (27° C). On warm days proceed more slowly to avoid vapor bubbles.

NOTE: Columns should be prepared just prior to use.

1. Add 100 mL of CH_2Cl_2 and between 5 and 15 mm glass wool plug to a 19 mm ID column with a stopcock. Tamp the plug well to remove any bubbles.
2. Add the 10 g alumina to a beaker and slowly add 20 mL of CH_2Cl_2. Gently swirl the beaker for 30 sec, and let it stand for 5 min (to remove all air bubbles).
3. Add the 20 g silica gel to a 2nd beaker. Slowly add 40 mL of CH_2Cl_2 to the beaker. Gently swirl the beaker for 30 sec, and let it stand for 5 min (to remove all air bubbles).
4. Place a curved-stem funnel into the column reservoir so that the funnel tip hangs well off-center. Swirl the beaker to resuspend the alumina from step 2, and pour the slurry into the column.
5. Wash the beaker with approximately 5 mL of CH_2Cl_2, and add the washings to the column. Repeat the wash twice.
6. After the particles settle, open the stopcock for 30 sec to allow the alumina to pack more tightly, then close the stopcock.

    NOTE: Gentle tapping of the column while the stopcock is open will assist in the settling of the alumina and silica gel.

7. Add the silica gel from step 3 to the column, as in steps 4 and 5.
8. After the particles settle, open the stopcock. While the solvent still drains, add 1 mL of sand through the powder funnel.
9. Drain CH_2Cl_2 to the packing top, then close the stopcock.
10. Add 30 mL of 1:1 CH_2Cl_2:pentane to the column. Drain to the packing top, then close the stopcock. Discard the eluates.
11. With a transfer pipet, cautiously transfer the extract to the top of the packing. Drain to the packing top, then close the stopcock.
12. Wash down the extract tube with 0.5 mL of 1:1 CH_2Cl_2:pentane, and add the washings to the top of the packing. Drain to the packing top, then close the stopcock.
13. Repeat step 12 three times.
14. Add 200 mL of 1:1 CH_2Cl_2:pentane, and continue eluting at approximately 3 mL/min.
15. Collect 20 mL of eluate, then close the stopcock, and discard the contents of the cylinder.
16. Replace the cylinder with a labeled flask and collect eluate until the column runs dry.

8.4 Concentration of Extract

1. Add 3-4 boiling chips to the flask from step 15, section 8.3.
2. Attach a Snyder column and concentrate the fraction in a 60° C water bath to 10-15 mL, and transfer it to a concentrator tube.

   NOTE: It is necessary to wet the Snyder column by adding CH_2Cl_2 to the top of the column prior to sample boiling.

3. Wash down the flask with 3-4 mL of CH_2Cl_2, and add the washings to the tube.
4. Repeat step 3 once.
5. Add one boiling chip to the tube and concentrate the fraction to between 0.9 and 1.0 mL.
6. Add 2 mL of hexane to the tube and concentrate to between 0.9 and 1.0 mL.
7. Add approximately 0.7 mL of CH_3OH and 0.5 mL of CH_2Cl_2.

   NOTE: This step results in a final solution ratio of 6:4:3 hexane:CH_3OH:CH_2Cl_2 (v:v:v).

NOTE: The extract must be dissolved in the solvent (no layers), with the total volume $\leq$ 2.3 mL.

8.5 Sephadex LH-20 Chromatography

NOTE: It is important to check column calibration on a monthly basis.
NOTE: During column storage, maintain 30-50 mL of the solvent in the column reservoir and cover the top with aluminum foil to minimize evaporation. If the solvent in the reservoir separates into 2 phases, remove it and replace it with >80 mL of fresh 6:4:3 solvent, then elute 50 mL.

1. Remove the excess solvent from the top of the column using a transfer pipet.
2. Add 10 mL of the 6:4:3 solvent to the column. Drain to the packing top, and close the stopcock. Discard the eluate.
3. Wash the column top with 2 mL of CH_2Cl_2, and place the 50-mL cylinder under the column.
4. Using a transfer pipet, carefully apply the extract from step 7, section 8.4 to the column.
5. Use a circular motion to dispense the sample immediately above the packing, dripping it slowly down the column wall so as not to disturb the packing.
6. Drain to the packing top, and close the stopcock.
7. Wash down the tube with 0.5 mL of 6:4:3 solvent, and apply the washings to the column. Drain to the packing top, and close the stopcock.
8. Repeat step 7 once.
9. Wash down the column wall with approximately 3 mL of 6:4:3 solvent, applied above the base of the reservoir. Drain to the packing top, and close the stopcock.
10. Repeat step 9 once.
11. Cautiously add approximately 150 mL of 6:4:3 solvent to the column without disturbing the packing.
12. Collect 25 mL of eluate in the 50 mL cylinder. Close the stopcock, and discard this eluate.
13. Replace the cylinder with a concentrator tube. Open the stopcock, collect approximately 15 to 20 mL of eluate (the amount calibrated in Section 7.2 steps 8 and 9 from just before

where azulene first emerges from the column), then close the stopcock.

14. Archive this fraction.

    NOTE: This fraction is archived in case early eluting compounds are not identified in the next fraction. If early eluting compounds are not identified in the next fraction, analyze the archived fraction for these compounds. If the compounds are identified in the archived fraction, a re-calibration of the Sephadex LH-20 column is necessary.

15. Place a 100 mL cylinder under the column. Open the stopcock, and collect approximately 50 to 55 mL of eluate (the amount calibrated in Section 7.2, steps 8 and 9 from 5 mL after the last perylene has eluted). Close the stopcock, and transfer the eluate to a flask.
16. Wash down the cylinder with 3 to 4 mL of CH_2Cl_2, and add the washings to the flask.
17. Repeat step 16 once.
18. Replace the 100 mL cylinder with a waste cylinder, and elute to the top of the packing. Discard this eluate. Add 50 mL of solvent and cap. The column is now ready for the next sample.

8.6 Concentration of Sephadex LH-20 Fraction

1. Add 3-4 boiling chips to the flask from step 17 section 8.5, and attach a Snyder column.

   NOTE: It is necessary to wet the Snyder column by adding CH_2Cl_2 to the top of the column prior to sample boiling.

2. Concentrate the fraction in a 75° C water bath to 10-15 mL, and transfer it to a concentrator tube.
3. Wash down the flask with 3-4 mL of CH_2Cl_2, and add the washings to the tube.
4. Repeat step 3 once.
5. Add one boiling chip to the tube and concentrate the fraction to between 0.9 and 1.0 mL.
6. Add 7 mL of hexane to the tube and concentrate to between 0.9 and 1.0 mL.

8.7 GC/ECD Analysis

The analyst should follow the instructions provided by the instrument's manufacturer for GC operation and maintenance. The following machine operating conditions are required for the proper determination and separation of the PCB congeners and pesticides:

Machine Operating Conditions

Helium carrier	1.2 mL/min
Initial oven temperature	100° C
Initial hold time	1 min
First ramp rate	5°/min
First ramp final temperature	140°
Second hold time	1 min
Second ramp rate	1.5°/min
Second ramp final temperature	250° C
Third hold time	1 min
Third ramp rate	10°/min
Final temperature	300° C
Final hold time	5 min
ECD temperature	325° C
Injector port temperature	275° C

The primary quantification column should be a DB-5 0.25 mm ID column with a 30 m length. The secondary confirmation column should be a DB-17HT 0.25 mm ID column with a 30 m length.

When a PCB congener or pesticide is identified on the quantification column, the chromatogram of the confirmation column should also be checked to verify the identification of the analyte. If, however, the area of the confirmation column is lower than that of the quantification column, the area of the analyte in the confirmation column should be used to calculate the concentration of the analyte (along with the areas of the surrogates from the confirmation column).

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit.

The method detection limit for PCBs in water and elutriate matrices is 0.01 µg/L per congener.

The method detection limits for pesticides in water and elutriate matrices are 0.1 µg/L per compound.

NOTE: Method detection limits can be lowered by extracting larger amounts of waters or elutriates or by further concentrating the final extract volume (<1 mL).

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 25 percent or less between the measured total PCB or pesticide concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 30% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 30% of the mean RF from the initial calibration curve.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - PCBs or pesticides, to the 1 L aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 30% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 25%.

9.7 Surrogate Spikes

A surrogate spike is defined as the addition of an organic compound which is similar to analytes of interest in chemical composition, extraction, and chromatography, but which are not normally found in the environmental sample (USEPA, 1986). These compounds are spiked into all blanks, standards, samples, and spiked samples prior to extraction. Surrogate spikes should be spiked at between 50 and 100 times the method detection limit. Surrogate spike recoveries should be ± 30% of the known spiking concentration.

The following surrogate spike compounds are recommended:

4,4'-dibromooctafluorobiphenyl (DBOFB)
decachlorobiphenyl (DCB)
PCB congener 103
PCB congener 198

9.8 Internal Standards

An internal standard (also known as GC standard) is added immediately prior to analysis by GC (i.e., when loading the GC vials). The compound(s) added are sensitive to the detector and are a measure of analyte recovery without (or with highly reduced) matrix effects. These compounds are spiked into all blanks, standards, samples, and spiked samples. Internal standards should be spiked at between 50 and 100 times the method detection limit. Internal standard recoveries should be ± 30% of the known concentration. The recommended internal standard for this method is tetrachloro-m-xylene (TCMX).

Control charts for the internal standard recoveries, with ± 2 and 3 σ values as warning and action limits, respectively, will be required to be created and updated after each day of analysis to control any systematic bias that may be adding to the overall measurement uncertainty for a given parameter. A value outside the control limits is considered unacceptable, hence, the instrument should be recalibrated and the samples in that batch should be reanalyzed. If bias for a given analysis is indicated, i.e., at least seven successive points occurring on one side of the cumulative means, sample analysis should cease until an explanation is found and the system is brought under control.

10.0 Method Performance

Precision and accuracy information are not available at this time.

11.0 Calculations and Reporting

Identify the analyte peaks in the chromatograms of the extract fractions by comparing them with the analyte retention times obtained from the chromatogram of the ongoing calibration standard.

> **NOTE**: When a PCB congener or pesticide is identified on the quantification column, the chromatogram of the confirmation column should also be checked to verify the identification of the analyte. If, however, the area of the confirmation column is lower than that of the quantification column, the area of the analyte in the confirmation column should be used to calculate the concentration of the analyte (along with the areas of the surrogates from the confirmation column).

The concentration of an analyte in the sediment sample, dry weight basis:

$$\text{PCB/pesticide, ηg/L} = \frac{R_1 \times R_2 \times SS}{R_3 \times V_w}$$

where:

R_1 = $\frac{\text{analyte peak area from the sample}}{\text{surrogate spike peak area from the sample}}$

R_2 = $\frac{\text{analyte concentration in the ongoing calibration standard (ηg/µL)}}{\text{surrogate spike concentration in the ongoing calibration standard (ηg/µL)}}$

R_3 = $\frac{\text{analyte peak area from the ongoing calibration standard}}{\text{surrogate spike peak area from the ongoing calibration standard}}$

SS = surrogate spike concentration added to sample (ηg)
V_s = volume of water or elutriate extracted (L).

The concentration of the total PCBs in the sediment sample is calculated by summation of the 20 congeners (Table 3) as follows:

$$\text{Total PCBs, ηg/L} = \Sigma \text{ congener concentrations}$$

If the congener concentration is < method detection limit, then a "0" value should be used during summation (i.e., do <u>not</u> add the method detection limit for non-identified congeners).

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

Brown, D.W., A.J. Friedman, D.G. Burrows, G.R. Snyder, B.G. Patten, W.E. Ames, L.S. Ramos, P.G. Prohaska, D.D. Gennero, D.D. Dungan, M.Y. Uyeda, and W.D. MacLeod, Jr. 1979. Investigation of petroleum in the marine environments of the Strait of Juan de Fuca and Northern Puget Sound. EPA-600/7-79/164. U.S. Environmental Protection Agency, Washington D.C.

Federal Register. 1979. Guidelines establishing test procedures for the analysis of pollutants; proposed regulations. Federal Register 44(233):69464-69575.

Horwitz, W., L.P. Kamps, and K.W. Boyer. 1980. Quality assurance in the analysis of foods for trace constituents. J. Assoc. Off. Anal. Chem. 63:1344-1354.

MacLeod, W.D., Jr., D.W. Brown, R.G. Jenkins, L.S. Ramos, and Y.D. Henry. 1977. A pilot study on the design of a petroleum hydrocarbon baseline investigation for Northern Puget Sound and the Strait of Juan de Fuca. EPA-600/7-77-098. U.S. Environmental Protection Agency, Washington, D.C.

MacLeod, W.D., Jr., L.S. Ramos, A.J. Friedman, D.G. Burrows, P.G. Prohaska, D.L. Fisher, and D.W. Brown. 1981. Analysis of residual chlorinated hydrocarbons, aromatic hydrocarbons and related compounds in selected sources, sinks, and biota of the New York Bight. U.S. Dep. Commerce. NOAA Tech. Memo. OMPA-6. 128 p.

MacLeod, W.D., Jr., P.G. Prohaska, D.D. Gennero, and D.W. Brown. 1982. Interlaboratory comparisons of selected trace hydrocarbons from marine sediments. Anal. Chem. 54:386-392.

Malins, D.C., B.B. McCain, D.W. Brown, A.K. Sparks, and H.O. Hodgins. 1980. Chemical contaminants in Central and Southern Puget Sound. U.S. Dep. Commerce. NOAA Tech. Memo. OMPA-2. 295 p.

Malins, D.C., B.B. McCain, D.W. Brown, A.K. Sparks, H.O. Hodgins, and S.L. Chan. 1982. Chemical contaminants and abnormalities in fish and Invertebrates from Puget Sound. U.S. Dep. Commerce. NOAA Tech. Memo. OMPA-19.

Malins, D.C., B.B. McCain, D.W. Brown, S.L. Chan, M.S. Myers, J.T. Landahl, P.G. Prohaska, A.J. Friedman, L.D. Rhodes, D.G. Burrows, W.D. Gronlund, and H.O. Hodgins. 1984. Chemical pollutants in sediments and diseases of bottom-dwelling fish in Puget Sound, Washington. Environ. Sci. Technol. 18:705-713.

National Oceanic and Atmospheric Administration. 1985. Standard Analytical Procedures of the NOAA National Analytical Facility 1985-1985: Extractable toxic organic compounds. 2nd ed. NOAA Tech. Memo. NMFS F/NWC-92.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA/U.S. Army Corps of Engineers. 1998. Evaluation of material proposed for discharge to waters of the United States: Testing manual (Inland Testing Manual). EPA-823-98-004, Office of Water, Washington, D.C.

Attachment A - Sephadex LH-20 Column Packing and Recycling

A.1 Column Packing

1. Fit a 19 mm ID column with a stopcock, add 10 mL of 6:4:3 solvent and between 5 and 10 mm glass wool plug. Tamp the plug to remove any air bubbles.
2. Add approximately 1 mL of sand to the column, and tap the column gently so that the sand forms a smooth layer on top of the glass wool.
3. Pour the swelled Sephadex gel through the funnel into the column until the gel fills the column and about 1/4 of the reservoir.
4. Allow 10 min for the Sephadex to settle. Open the stopcock, and elute 80 mL of solvent to ensure firm packing. Add more solvent as needed. Leave 30 mL of solvent in the column reservoir. Cover the top with aluminum foil, and allow the packing to settle overnight.
5. Open the stopcock, and elute 10 mL of solvent, then close the stopcock. Remove the excess Sephadex packing from the top with a transfer pipet until the height of the Sephadex is 26.5 cm.
6. Gently add approximately 1 mL of sand onto the packing so that it forms an even layer on the top. (The column may be tapped or tilted slightly to get an even layer of sand.)
7. Examine the packing for air bubbles. If bubbles are evident, elute approximately 250 mL of warm (about 35° C) solvent through the column. If the bubbles persist, recycle the packing (see section A.2).

A.2 Recycling Sephadex LH-20 Column Packing

NOTE: When the column no longer maintains its calibration with azulene/perylene, recycle the packing.

1. Decant any solvent in the column reservoir.
2. Empty the column packing into a beaker 4 times the volume of the packing.
3. Wash with CH_2Cl_2.
4. Add enough CH_2Cl_2 to float Sephadex particles in the upper half of the beaker.
5. Remove <u>all</u> glass wool with forceps (mandatory).
6. Cover the beaker and let stand for 1 to 2 hours.
7. Decant the floating particles leaving the sand in the beaker.
8. Aspirate the CH_2Cl_2 from the Sephadex particles and set them aside.
9. Swell these particles overnight in 6:4:3 solvent before reusing.

POLYNUCLEAR AROMATIC HYDROCARBONS IN WATERS AND ELUTRIATES (GC/MS, CAPILLARY COLUMN)

1.0 Scope and Application

This method is appropriate for the determination of polynuclear aromatic hydrocarbons (PAHs) in water and elutriate samples. Individual polynuclear aromatic compounds that are soluble in methylene chloride (CH_2Cl_2) and capable of being eluted without derivitization as sharp peaks from a gas chromatographic fused-silica capillary column coated with a slightly polar silicone are listed in Table 1.

This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatograph/mass spectrometers and skilled in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method.

Extraction and quantification techniques are based on SW-846 Method 8270 (USEPA, 1986). The extract clean-up procedures are based on a National Oceanic and Atmospheric Administration (NOAA) method for the determination of Extractable Toxic Organic Compounds in marine sediments (NOAA, 1985).

The extracts produced from this method (sections 8.1 through 8.6) can be used in the determination of polychlorinated biphenyls (PCBs), pesticides, and PAHs.

> **NOTE**: The methods presented in this appendix have been prepared to analyze "clean" waters and elutriates. These methods are not intended for use on highly contaminated waters, elutriates, or sediments.

2.0 Summary of Method

A measured volume of sample, 1 liter, is serially extracted with methylene chloride in a separatory funnel. The resultant extract is cleaned-up with silica gel and alumina. Additional clean-up steps to remove biological macromolecules are performed using Sephadex LH-20. The final sample extract is injected into a gas chromatograph/mass spectrometer system using a

capillary column for separation, identification, and quantification of the individual PAHs present in the sample.

The same extract used to analyze for PAHs can be used to analyze for PCBs and pesticides using gas chromatography with electron capture detection (GC/ECD). The method for PCB and pesticide determination is provided in this methods manual.

3.0 Interferences

Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences during the analysis of samples. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing reagent blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

Contamination by carryover can occur whenever high-level and low-level samples are sequentially analyzed. To reduce carryover, the sample syringe must be rinsed out between samples with solvent. Whenever an unusually concentrated sample is encountered, it should be followed by the analysis of solvent to check for cross contamination.

4.0 Apparatus and Materials

4.1 Apparatus

1. Analytical balance, capable of weighing to 0.001 g.
2. Analytical balance calibration weights, Class S, 3-5 weights covering expected weight range.
3. Centrifuge, capable of holding 250 mL centrifuge tubes and maintaining speeds of 1500 rpm.
4. Desiccator and desiccant. Desiccants generally used include: anhydrous calcium sulfate, silica gel, or phosphorus pentoxide. Indicating desiccants are preferable since they show when the desiccant needs to be changed or regenerated.
5. Gas chromatograph/mass spectrometer system with:
   a. gas chromatograph system complete with a temperature-programmable gas chromatograph suitable for splitless injection and all required accessories, including syringes,

analytical columns, and gases. The capillary column should be directly coupled to the source.

b. mass spectrometer capable of scanning from 35 to 500 amu every 1 sec or less, using 70 volts (nominal) electron energy in the electron impact ionization mode.
c. GC/MS interface that gives acceptable calibration points at 50 ηg per injection for each compound of interest and achieves acceptable tuning performance criteria may be used.
d. data system: A computer system must be interfaced to the mass spectrometer. The system must allow the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the ration of the chromatographic program. The computer must have software that can search any GC/MS data file for ions of a specific mass and that can plot such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software must also be available that allows integrating the abundances in any EICP between specified time or scan-number limits. The most recent version of the EPA/NIH Mass Spectral Library should also be available.

6. Modified Kontes tube heater (block contains: Al inserts fitted to the 0.7 mL line of the tube tip and an Al-foil shroud.
7. Molecular sieve traps (for gas cylinder)

   NOTE: One suggested source for the molecular sieve traps is Hydro-Purge model ASC-l, Coast Engineering Laboratory, Gardena, California.

8. Oxygen traps.
9. UV light source.
10. Water bath, capable of maintaining a temperature of 80 ± 2° C.

    NOTE: The bath should be used in a hood.

4.2 Materials

1. Beakers, 250 mL, or equivalent.
2. Centrifuge tubes, 250 mL, amber, with Teflon™ caps.

3. Chromatography column with reservoir 250 mL, 19 mm ID, 30 cm.
4. Erlenmeyer flask, 500 mL, with stopper.
5. Erlenmeyer flask, 1 L, with stopper.
6. Funnel, curved-stem (curve must be glassblown).
7. Funnel, 200 mm OD, long-stem.
8. Funnel, powder.
9. GC column, silicon-coated fused-silica capillary column, DB-5, 30 m x 0.25 mm I.D. (or 0.32 mm I.D.).
10. Graduated cylinder, 500 mL.
11. Graduated cylinder, 100 mL.
12. Graduated cylinder, 50 mL.
13. Kontes concentrator tube, 25 mL, with stopper.
14. Kuderna-Danish concentrator tube, 10 mL, graduated.
15. Kuderna-Danish evaporative flask, 500 mL.
16. pH paper, wide range, capable of determining pH from 4 to 10.
17. Separatory funnel, 2 L, with Teflon™ stopcock.
18. Snyder column, 3-ball macro.
19. Snyder column, 2-ball micro.
20. Syringe, 2000 µL.
21. Syringe, 800 µL.
22. Syringe, 400 µL.
23. Syringe, 200 µL.
24. Syringe, 100 µL.
25. Syringe, 50 µL.
26. Syringe, 10 µL.
27. Teflon wash-bottle, 500 mL (to be filled with CH_2Cl_2).
28. Transfer pipets (Pasteur style) with rubber bulbs.
29. GC vials, 2 mL.
30. GC vials, 100 µL, conical.
31. Volumetric flask, class A, 100 mL
32. Volumetric flask, class A, 50 mL
33. Volumetric flask, class A, 10 mL
34. Volumetric pipet, 50 mL.

5.0 Reagents

1. Alumina, 80-200 mesh. Alumina should be activated at 120° C for 2 hr and then cooled to room temperature in a desiccator just before weighing and use.

2. ASTM Type II water (ASTM D1193). Water should be continually monitored for the presence of contaminants to verify that they are not present at levels that will interfere with method performance.
3. Azulene, reagent grade ($C_{15}H_{18}$).
4. Copper, reagent grade, fine granular. Copper should be activated < 1 hr before use. To activate copper, cover with concentrated. HCl and stir with a glass rod. Allow to stand for 5 min followed by washing twice with CH_3OH and then 3 times with CH_2Cl_2. Leave copper covered with CH_2Cl_2 to avoid contact with air.
5. Helium, grade 4.5 (purified, $\geq$99.995 %).
6. Hexane, high purity (C_6H_{14}). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
7. Concentrated hydrochloric acid, reagent grade (HCl). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
8. Concentrated nitric acid, reagent grade (HNO_3). Acid should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the acid has a concentration <MDL, then the acid can be used.
9. Methanol, high purity (CH_3OH). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
10. Methylene chloride (dichloromethane), high purity (CH_2Cl_2). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
11. Pentane, high purity (C_5H_{12}). Each solvent lot should be analyzed to verify that contaminants are not present at levels that will interfere with method performance. If a method blank using the solvent has a concentration <MDL, then the solvent can be used.
12. Perylene, reagent grade ($C_{20}H_{12}$).
13. Sand, Ottawa, MCB, kiln-dried, 30-40 mesh. Sand should be acid-washed (steeped in *aqua regia* (ACS grades $HN0_3$:HCl, 1:3, v:v) overnight, then washed three times each with Type II H_2O, CH_3OH and CH_2Cl_2, dried, and stored at 120° C.

14. Sephadex LH-20, size-exclusion gel. Sephadex LH-20 should be swelled overnight in 6:4:3 solvent.
15. Silica gel, Davison Type 923 or Amicon No. 84080. Silica should be activated at 700° C for 18 hr, stored at 170° C, and cooled to room temperature in a desiccator just before weighing and use.
16. Sodium hydroxide, 10 N (NaOH). Add 20 g of NaOH to 400 mL Type II water. Dilute to 500 mL with Type II water.
17. Sodium sulfate, reagent grade, anhydrous granular (Na_2S0_4). Sodium sulfate should be CH_2Cl_2 washed, dried, stored at 120° C, and cooled to room temperature in a desiccator before weighing and use.
18. PAH standard stock solution (1.00 µg/µL). PAH stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

    NOTE: Prepare stock standard solutions by accurately weighing 0.0100 g of pure material. Dissolve the material in pesticide quality acetone or other suitable solvent and dilute 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 may be used without correction to calculate the concentration of the stock standard.

    Commercially prepared stock standards may 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 4° C and protect from light. Stock standard solutions 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 1 yr, or sooner, if comparison with quality control check samples indicates a problem.

19. GC/MS tuning standard. A methylene chloride solution containing 50 ηg/µL of decafluorotriphenylphosphine (DFTPP) should be prepared. Store at 4° C or less when not being used.

6.0 Sample Collection, Preservation, and Handling

Sample collection procedures for the aqueous and sediment samples should be described in the approved sampling manual. Further information on bulk sediment collection procedures may be found in the sampling chapter. Aliquoting procedures from the bulk sediment sample are described in the General Laboratory Practices chapter.

Sample containers should be filled with care so as to prevent contamination due to any portion of the collected sample coming in contact with the sampler's gloves.

Samples should not be collected or stored in the presence of exhaust fumes.

A holding time of 7 days until extraction and 40 days from extraction to analysis is generally cited for this parameter.

The sample should be stored under refrigerated conditions (4° C) in the dark.

All sample containers must be prewashed with detergents, acids, and Type II water. Glass containers should be used for the storage of samples to be analyzed for PAHs in waters and elutriates. All glassware and materials contacting the solvents should be washed with CH_2Cl_2 three times prior to use.

An option to the CH_2Cl_2 washing of the glassware is to combust the glassware in a muffle oven at 400° C for 4 hours.

7.0 Calibration and Standardization

7.1 General

The calibration of the analytical balance is described in the General Laboratory Practices chapter. Additionally, the balance should be checked and cleaned by the manufacturer on a semi-annual or annual basis.

The thermometers should be checked periodically against a NIST certified thermometer to ensure that they are measuring temperature accurately. Thermometers should be accurate within ± 0.5° C.

The water bath and Kontes tube heater should be monitored to ensure that temperature fluctuations do not exceed ± 2° C.

7.2 Sephadex LH-20 Column Calibration

Information on preparing the Sephadex LH-20 column is presented in Attachment A.

1. Add enough azulene (approximately 10 mg/mL) and perylene (approximately 1 mg/mL) to approximately 50 mL of 6:4:3 solvent to produce a deeply colored solution.

   NOTE: Make sure that the azulene and perylene are <u>completely</u> dissolved.

2. Place a 100 mL cylinder beneath the column and using a transfer pipet, cautiously remove any excess 6:4:3 solvent from the top of the packing.
3. Using a transfer pipet, cautiously apply 2 mL of the azulene/perylene calibration solution onto the column. Use a circular motion to dispense the solution just above the packing, and drip the solution slowly down the column wall so as not to disturb the packing.
4. Open the stopcock, drain to the packing top, and close the stopcock.
5. Add approximately 0.5 mL of solvent to the top of the column. Drain to the packing top, and close the stopcock.
6. Repeat step 5 once.
7. Add 100 mL of solvent, and open the stopcock.
8. Elute the solvent until all of the perylene has emerged, using the UV light to monitor the perylene. Record the volumes at which the azulene and perylene start and finish eluting.
9. If the azulene emerges in the 50-65 mL range, and the perylene emerges in the 60-80 mL range without distinct tailing on the packing, proceed to step 10. Otherwise, recycle the packing (Attachment A).
10. Discard the eluate. Add 50 mL of solvent to the column, and flush the packing by eluting 50 mL into the cylinder. Again, discard the eluate.
11. The column is now ready for the next sample.

NOTE: If the column is to be stored, maintain 30-50 mL of solvent in the column reservoir, and cover the top with aluminum foil. Remove the solvent if it separates into 2 phases, add 80 mL of fresh 6:4:3 solvent, and elute 50 mL.

7.3 GC Calibration

Calibration standards at a minimum of five concentration levels should be prepared. One of the calibration standards should be at a concentration near, but above, the method detection limit; the others should correspond to the range of concentrations found in real samples but should not exceed the working range of the GC/MS system. Each standard should contain each analyte for detection by this method. All initial calibration standards should be stored at -10° to -20° C and should be freshly prepared once a year, or sooner if check standards indicate a problem. The daily calibration standard (ongoing calibration standard) should be prepared weekly and stored at 4° C.

Each GC/MS system must be hardware-tuned to meet the criteria in Table 2 for the GC/MS tuning standard. Analyses should not begin until all these criteria are met. Background subtraction should be straightforward and designed only to eliminate column bleed or instrument background ions. The GC/MS tuning standard should also be used to assess GC column performance and injection port inertness. If chromatogram peak degradation is excessive and/or poor chromatography is noted, the injection port may require cleaning. It may also be necessary to break off the first 6 to 12 in. of the capillary column.

Analyze each calibration standard (1 µL containing internal standards) and tabulate the area of the primary characteristic ion against concentration for each compound (Table 1). Calculate response factors (RFs) for each compound as follows:

$$RF = (A_x C_{is})/(A_{is} C_x)$$

where:
A_x = area of the characteristic ion for the compound being measured.
A_{is} = area of the characteristic ion for the specific internal standard.
C_x = concentration of the compound being measured (ηg/µL).
C_{is} = concentration of the specific internal standard (ηg/µL).

The average RF should be calculated for each compound. The percent relative standard deviation (%RSD) should also be calculated for each compound. The %RSD should be less than 30% for each compound. The relative retention times of each compound in each calibration run should agree within 0.06 relative retention time units.

NOTE: Late eluting compounds usually have much better agreement.

8.0 Procedure

8.1 Water and Elutriate Extraction

1. Using a 1 liter graduated cylinder, measure 1 liter of sample and transfer to a 2 liter separatory funnel.

   NOTE: If high concentrations are anticipated, a smaller sample volume may be used and diluted to 1 liter with Type II water.

3. Check the pH of the sample with wide-range pH paper and, if necessary, adjust the pH to greater than 11 with 10 N NaOH.
4. Add 60 mL methylene chloride to the separatory funnel.

   NOTE: Be sure to add all surrogate spike solutions at this point. Make certain that the solutions are placed into the CH_2Cl_2.

5. Seal and shake the separatory funnel vigorously for 1-2 minutes with periodic venting to release excess pressure.

   NOTE: Methylene chloride creates excessive pressure very rapidly; therefore, initial venting should be done immediately after the separatory funnel has been sealed and shaken once. The separatory funnel should be vented into a hood to prevent unnecessary exposure of the analyst to the organic vapor.

6. Allow the organic layer to separate from the water phase for a minimum of 10 minutes.

   NOTE: If the emulsion interface between layers is more than one-third the size of the solvent layer, a mechanical technique should be used to complete the phase separation. The

optimum technique depends upon the sample and may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical method.

7. Collect the solvent extract in an Erlenmeyer flask.

   NOTE: If the emulsion cannot be broken (recovery of <80% of the methylene chloride, corrected for the water solubility of methylene chloride), transfer the sample, solvent, and emulsion into the extraction chamber of a continuous extractor and follow procedures given in SW-846 Method 3520 (USEPA, 1986).

8. Repeat steps 4 through 7 two more times using fresh 60 mL portions of methylene chloride.
9. Combine the three extracts.

8.2 Extract Concentration

1. Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10 mL concentrator tube to a 500 mL evaporation flask.
2. Dry the extract by passing it through a drying column containing about 10 cm anhydrous sodium sulfate.
3. Collect the dried extract in a K-D concentrator.
4. Rinse the Erlenmeyer flask, which contained the original solvent extract, with 20-30 mL methylene chloride and add it to the column to complete a quantitative transfer of the sample extract.
5. Add one or two clean boiling chips to the evaporation flask.
6. Attach a three-ball Snyder column and prewet the Snyder column by adding approximately 1 mL methylene chloride to the top of the column.
7. Place the K-D apparatus on a hot water bath (80-90° C) so that the concentrator is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor.
8. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 10-20 minutes.

   NOTE: At the proper rate of distillation, the balls of the chamber will actively chatter but the chambers will not flood.

9. When the apparent volume of the liquid reaches 1 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.
10. Remove the Snyder column and rinse both the flask and its lower joints into the concentrator tube with 1-2 mL methylene chloride.
11. Add a clean boiling chip to the concentrator tube.
12. Attach a two-ball micro-Snyder column and prewet the column by adding 0.5 mL methylene chloride to the top of the column.
13. Place the K-D apparatus in a hot water bath so that the concentrator tube is partially immersed in the hot water.
14. Adjust the vertical position of the apparatus and the water temperature, as required, to complete the concentration in 5-10 minutes.

    NOTE: At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

15. When the apparent volume of extract reaches 0.5 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.
16. Remove the Snyder column and rinse both the flask and its lower joints into the concentrator tube with 0.2 mL methylene chloride.
17. Adjust the final volume to 1.0 mL with methylene chloride.

    NOTE: If analysis of the extract will not be performed immediately, stopper the concentrator tube and store under refrigerated conditions (4° C).

    NOTE: If the extract will be stored longer than 2 days, it should be transferred to a vial with a Teflon-lined screw cap, and appropriately labeled.

8.3 Silica Gel/Alumina Chromatography

NOTE: The laboratory temperature must be <80° F (27° C). On warm days proceed more slowly to avoid vapor bubbles.

NOTE: Columns should be prepared just prior to use.

1. Add 100 mL of CH_2Cl_2 and between 5 and 15 mm glass wool plug to a 19 mm ID column with a stopcock. Tamp the plug well to remove any bubbles.
2. Add the 10 g alumina to a beaker and slowly add 20 mL of CH_2Cl_2. Gently swirl the beaker for 30 sec, and let it stand for 5 min (to remove all air bubbles).
3. Add the 20 g silica gel to a 2nd beaker. Slowly add 40 mL of CH_2Cl_2 to the beaker. Gently swirl the beaker for 30 sec, and let it stand for 5 min (to remove all air bubbles).
4. Place a curved-stem funnel into the column reservoir so that the funnel tip hangs well off-center. Swirl the beaker to resuspend the alumina from step 2, and pour the slurry into the column.
5. Wash the beaker with approximately 5 mL of CH_2Cl_2, and add the washings to the column. Repeat the wash twice.
6. After the particles settle, open the stopcock for 30 sec to allow the alumina to pack more tightly, then close the stopcock.

   NOTE: Gentle tapping of the column while the stopcock is open will assist in the settling of the alumina and silica gel.

7. Add the silica gel from step 3 to the column, as in steps 4 and 5.
8. After the particles settle, open the stopcock. While the solvent still drains, add 1 mL of sand through the powder funnel.
9. Drain CH_2Cl_2 to the packing top, then close the stopcock.
10. Add 30 mL of 1:1 CH_2Cl_2:pentane to the column. Drain to the packing top, then close the stopcock. Discard the eluates.
11. With a transfer pipet, cautiously transfer the extract to the top of the packing. Drain to the packing top, then close the stopcock.
12. Wash down the extract tube with 0.5 mL of 1:1 CH_2Cl_2:pentane, and add the washings to the top of the packing. Drain to the packing top, then close the stopcock.
13. Repeat step 12 three times.
14. Add 200 mL of 1:1 CH_2Cl_2:pentane, and continue eluting at approximately 3 mL/min.
15. Collect 20 mL of eluate, then close the stopcock, and discard the contents of the cylinder.
16. Replace the cylinder with a labeled flask and collect eluate until the column runs dry.

8.4 Concentration of Extract

1. Add 3-4 boiling chips and a few grains of activated copper to the flask from step 15, section 8.3 until no further discoloring of the copper occurs.

   NOTE: Activated copper is added to the flask to remove elemental sulfur, a potential interferant for GC/ECD analyses.

2. Attach a Snyder column and concentrate the fraction in a 60° C water bath to 10-15 mL, and transfer it to a concentrator tube.

   NOTE: It is necessary to wet the Snyder column by adding CH_2Cl_2 to the top of the column prior to sample boiling.

3. Wash down the flask with 3-4 mL of CH_2Cl_2, and add the washings to the tube.
4. Repeat step 3 once.
5. Add one boiling chip to the tube and concentrate the fraction to between 0.9 and 1.0 mL.
6. Add 2 mL of hexane to the tube and concentrate to between 0.9 and 1.0 mL.
7. Add approximately 0.7 mL of CH_3OH and 0.5 mL of CH_2Cl_2.

   NOTE: This step results in a final solution ratio of 6:4:3 hexane:CH_3OH:CH_2Cl_2 (v:v:v).

   NOTE: The extract must be dissolved in the solvent (no layers), with the total volume $\leq$ 2.3 mL.

8.5 Sephadex LH-20 Chromatography

NOTE: It is important to check column calibration on a monthly basis.
NOTE: During column storage, maintain 30-50 mL of the solvent in the column reservoir and cover the top with aluminum foil to minimize evaporation. If the solvent in the reservoir separates into 2 phases, remove it and replace it with >80 mL of fresh 6:4:3 solvent, then elute 50 mL.

1. Remove the excess solvent from the top of the column using a transfer pipet.

2. Add 10 mL of the 6:4:3 solvent to the column. Drain to the packing top, and close the stopcock. Discard the eluate.
3. Wash the column top with 2 mL of CH_2Cl_2, and place the 50-mL cylinder under the column.
4. Using a transfer pipet, carefully apply the extract from step 7, section 8.4, to the column.
5. Use a circular motion to dispense the sample immediately above the packing, dripping it slowly down the column wall so as not to disturb the packing.
6. Drain to the packing top, and close the stopcock.
7. Wash down the tube with 0.5 mL of 6:4:3 solvent, and apply the washings to the column. Drain to the packing top, and close the stopcock.
8. Repeat step 7 once.
9. Wash down the column wall with approximately 3 mL of 6:4:3 solvent, applied above the base of the reservoir. Drain to the packing top, and close the stopcock.
10. Repeat step 9 once.
11. Cautiously add approximately 150 mL of 6:4:3 solvent to the column without disturbing the packing.
12. Collect 25 mL of eluate in the 50 mL cylinder. Close the stopcock, and discard this eluate.
13. Replace the cylinder with a concentrator tube. Open the stopcock, collect approximately 15 to 20 mL of eluate (the amount calibrated in Section 7.2 steps 8 and 9 from just before where azulene first emerges from the column), then close the stopcock.
14. Archive this fraction.

    NOTE: This fraction is archived in case early eluting compounds are not identified in the next fraction. If early eluting compounds are not identified in the next fraction, analyze the archived fraction for these compounds. If the compounds are identified in the archived fraction, a re-calibration of the Sephadex LH-20 column is necessary.

15. Place a 100 mL cylinder under the column. Open the stopcock, and collect approximately 50 to 55 mL of eluate (the amount calibrated in Section 7.2, steps 8 and 9 from 5 mL after the last perylene has eluted). Close the stopcock, and transfer the eluate to a flask.

16. Wash down the cylinder with 3 to 4 mL of CH_2Cl_2, and add the washings to the flask.
17. Repeat step 16 once.
18. Replace the 100 mL cylinder with a waste cylinder, and elute to the top of the packing. Discard this eluate. Add 50 mL of solvent and cap. The column is now ready for the next sample.

8.6 Concentration of Sephadex LH-20 Fraction

1. Add 3-4 boiling chips to the flask from step 17 section 8.5, and attach a Snyder column.

   NOTE: It is necessary to wet the Snyder column by adding CH_2Cl_2 to the top of the column prior to sample boiling.

2. Concentrate the fraction in a 75° C water bath to 10-15 mL, and transfer it to a concentrator tube.
3. Wash down the flask with 3-4 mL of CH_2Cl_2, and add the washings to the tube.
4. Repeat step 3 once.
5. Add one boiling chip to the tube and concentrate the fraction to between 0.9 and 1.0 mL.
6. Add 7 mL of hexane to the tube and concentrate to between 0.9 and 1.0 mL.

8.7 GC/MS Analysis

The analyst should follow the instructions provided by the instrument's manufacturer for GC operation and maintenance. The recommended GC/MS operating conditions for PAH quantification are:

Mass Range	35-500 amu
Scan time	1 sec/scan
Initial column temperature	40° C
Initial hold time:	4 min
Column temperature program	40-270° C at 10°C/min
Final column temperature hold	270° C (until benzo[g,h,i]perylene has eluted)
Injector temperature	250-300° C
Transfer line temperature	250-300° C

Source temperature	According to manufacturer's specifications
Injector	Grob-type, splitless
Sample volume	1-2 µL
Carrier gas	Helium at 30 cm/sec.

The primary quantification column should be a DB-5 0.25 mm I.D. column with a 30 m length.

The volume to be injected should ideally contain 100 ηg of the PAHs (for a 1 µL injection).

> **NOTE**: It is highly recommended that the extract be screened on a GC with flame ionization detection (FID) or GC with photoionization detection (PID) using the same type of capillary column (DB-5 0.25 mm I.D. with a 30 m length). This will minimize contamination of the GC/MS system from unexpectedly high concentrations of organic compounds.

9.0 Quality Control

9.1 Detection Limits

The method detection limit is defined as 3 times the standard deviation of a minimum of seven replicates of a low level standard whose concentration is within a factor of 10 of the estimated method detection limit.

The method detection limit for PAHs in water and elutriate matrices is 10 µg/L.

> **NOTE**: Method detection limits can be lowered by extracting larger amounts of waters or elutriates or by further concentrating the final extract volume (<1 mL).

9.2 Replicate Samples

A minimum of one sample from each sample batch should be analyzed in duplicate. The relative percent difference (RPD) should be 25 percent or less between the measured PAH concentrations.

9.3 Standard Reference Materials

Standard reference materials, such as NIST standards, should be run to monitor the performance and assess the accuracy/bias of the measurement system. Standard reference materials should be run at a frequency of one per analytical sample batch. The acceptance criterion for the standard reference materials should be ± 30% of the known value or within the certified value provided by the supplier, whichever is larger.

9.4 Blanks

A minimum of one reagent blank per sample batch should be analyzed to determine if contamination or any memory effects are occurring. The measured concentration in the reagent blank should be less than or equal to the method detection limit.

One reagent blank should also be analyzed prior to any routine sample analyses to ensure interferences and contamination are under control.

9.5 Ongoing Calibration

The calibration of the instrument should be verified by analyzing an independently prepared ongoing check standard every 10 samples. The ongoing calibration check sample should be a mid-calibration range standard prepared from an independent stock solution. The acceptance criterion for the ongoing calibration check sample should be ± 30% of the mean RF from the initial calibration curve.

9.6 Matrix Spikes/Matrix Spike Duplicates

A matrix spike/matrix spike duplicate is a sample prepared by adding a known quantity of a target analyte, in this case - PAHs, to the 1 L aliquot of a routine sample for which an independent estimate of the target analyte concentration is available. The concentration of the matrix spike should be at the regulatory standard level or at approximately 10 times the estimated or actual method detection limit. The spiked sample is then extracted and analyzed in the same manner as any other routine sample. Matrix spike recoveries should be within ± 30% of the known spike concentration. Precision between the matrix spike and its duplicate should have an relative percent difference (RPD) of $\leq$ 30%.

9.7 Surrogate Spikes

A surrogate spike is defined as the addition of an organic compound which is similar to analytes of interest in chemical composition, extraction, and chromatography, but which are not normally found in the environmental sample (USEPA, 1986). These compounds are spiked into all blanks, standards, samples, and spiked samples prior to extraction. Surrogate spikes should be spiked at between 50 and 100 times the method detection limit. Surrogate spike recoveries should be ± 30% of the known spiking concentration.

The following surrogate spike compounds are recommended:

naphthalene-d_8
acenaphthene-d_{10}
perylene-d_{12}

Other surrogate spike compounds that are also commonly used are phenanthrene-d_{10} and chrysene-d_{12}.

Control charts for the surrogate spikes, with ± 2 and 3 σ values as warning and action limits, respectively, will be required to be created and updated after each day of analysis to control any systematic bias that may be adding to the overall measurement uncertainty for a given parameter. A value outside the control limits is considered unacceptable, hence, the instrument should be recalibrated and the samples in that batch should be reanalyzed. If bias for a given analysis is indicated, i.e., at least seven successive points occurring on one side of the cumulative means, sample analysis should cease until an explanation is found and the system is brought under control.

9.8 Internal Standards

An internal standard (also known as GC standard) is added immediately prior to analysis by GC. The compound(s) added are sensitive to the detector and are a measure of analyte recovery without (or with highly reduced) matrix effects. These compounds are spiked into all blanks, standards, samples, and spiked samples. Internal standards should be spiked at between 50 and 100 times the method detection limit. Internal standard recoveries should be ± 30% of the known concentration. The recommended internal standard for this method is tetrachloro-m-xylene (TCMX). An alternate internal standard often used is hexamethylbenzene (HMB).

Control charts for the internal standards, with ± 2 and 3 σ values as warning and action limits, respectively, will be required to be created and updated after each day of analysis to control any systematic bias that may be adding to the overall measurement uncertainty for a given parameter. A value outside the control limits is considered unacceptable, hence, the instrument should be recalibrated and the samples in that batch should be reanalyzed. If bias for a given analysis is indicated, i.e., at least seven successive points occurring on one side of the cumulative means, sample analysis should cease until an explanation is found and the system is brought under control.

9.9 Ongoing GC/MS Tuning Standard

A 50 ηg injection of the GC/MS tuning standard (DFTPP) must be made during each 12 hour shift. Acceptance criteria in the mass spectrum for DFTPP must meet the criteria given in Table 2.

10.0 Method Performance

Precision and accuracy information are not available at this time.

11.0 Calculations and Reporting

11.1 Qualitative Analysis

An analyte is identified by comparison of the sample mass spectrum with the mass spectrum of a standard of the suspected compound (standard reference spectrum). Mass spectra for the standard reference should be obtained on the GC/MS within the same 12 hours as the sample analysis. These standard reference spectra may be obtained through analysis of the calibration standards. Two criteria must be satisfied to verify identification: (1) elution of sample component at the same GC relative retention time (RRT) as the standard component; and (2) correspondence of the sample component and the standard component mass spectrum.

The sample component RRT must compare within ± 0.06 RRT units of the RRT of the standard component. If coelution of interfering components prohibits accurate assignment of the sample component RRT from the total ion chromatogram, the RRT should be assigned by using extracted ion current profiles for ions unique to the component of interest.

All ions present in the standard mass spectra at a relative intensity greater than 10% (most abundant ion in the spectrum equals 100%) must be present in the sample spectrum. The relative intensities of ions must agree within plus or minus 20% between the standard and sample spectra (i.e., an ion with an abundance of 50% in the standard spectra must have the corresponding sample abundance between 30 and 70 percent).

For samples containing components not associated with the calibration standards, a library search may be made for the purpose of tentative identification. The necessity to perform this type of identification will be determined by the type of analyses being conducted. Computer-generated library search routines should not use normalization routines that would misrepresent the library or unknown spectra when compared to each other. Only after visual comparison of sample spectra with the nearest library searches will the mass spectral interpretation specialist assign a tentative identification. Guidelines for making tentative identification are:

1. Relative intensities of major ions in the reference spectrum (ion >10% of the most abundant ion) should be present in the sample spectrum.
2. The relative intensities of the major ions should agree within ±20%. (i.e., an ion with an abundance of 50% in the standard spectrum must have the corresponding sample ion abundance between 30 and 70%).
3. Molecular ions present in the reference spectrum should be present in sample the spectrum.
4. Ions present in the sample spectrum but not in the reference spectrum should be reviewed for possible background contamination or presence of coeluting compounds.
5. Ions present in the reference spectrum but not in the sample spectrum should be reviewed for possible subtraction from the sample spectrum because of background contamination or coeluting peaks. Data system library reduction programs can sometimes create these discrepancies.

11.2 Quantitative Analysis

When a compound has been identified, the quantitation of that compound will be based on the integrated abundance from the EICP of the primary characteristic ion. Quantitation will take place using the internal standard technique. The internal standard used shall be the one nearest the retention time of that of a given analyte.

Calculate the concentration of each identified analyte in the sample as follows:

$$\text{PAH, } \mu g/L = \frac{(A_x)(I_s)(V_t)}{(A_{is})(RF)(V_o)(V_i)}$$

where:
A_x = area of characteristic ion for compound being measured.
I_s = amount of internal standard injected (ηg).
V_t = volume of total extract (µL).
A_{is} = area of characteristic ion for the internal standard.
R_F = response factor for compound being measured.
V_o = volume of water extracted (mL).
V_i = volume of extract injected (µL).

Where applicable, an estimate of concentration for noncalibrated components in the sample should be made. The formulas given above should be used with the following modifications: the areas A_x and A_{is} should be from the total ion chromatograms and the RF for the compound should be assumed to equal 1. The concentration obtained using this method should be reported indicating (1) that the value is an estimate and (2) which internal standard was used to determine concentration. Use the nearest internal standard free of interferences.

Report results without correction for recovery data in µg/L of each PAH.

12.0 References

American Society for Testing and Materials. 1984. Annual Book of ASTM Standard Specifications for Reagent Water, D-1933-77. ASTM, Philadelphia, PA.

J.W. Eichelberger, L.E. Harris, and W.L. Budde. 1975. Reference Compound to Calibrate Ion Abundance Measurement in Gas Chromatography-Mass Spectrometry. Anal. Chem. 47:995.

National Oceanic and Atmospheric Administration. 1985. Standard Analytical Procedures of the NOAA National Analytical Facility 1985-1985: Extractable toxic organic compounds. 2nd ed. NOAA Tech. Memo. NMFS F/NWC-92.

U.S. EPA. 1986. Test Methods for Evaluation of Solid Wastes, 3rd edition. Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, D.C.

Table 1. Characteristic Ions for PAHs.

Compound	Retention Time (min)	Primary Ion	Secondary Ion(s)
Acenaphthene	15.13	154	153, 152
Acenaphthene-d_{10} (SS)	15.05	164	162, 160
Acenaphthylene	14.57	152	151, 153
Anthracene	19.77	178	176, 179
Benzo(a)anthracene	27.83	228	229, 226
Benzo(b)fluoranthene	31.45	252	253, 125
Benzo(k)fluoranthene	31.55	252	253, 125
Benzo(g,h,i)perylene	41.43	276	138, 277
Benzo(a)pyrene	32.80	252	253, 125
Chrysene	27.97	228	226, 229
Dibenz(a,h)anthracene	39.82	278	139, 279
Fluoranthene	23.33	202	101, 203
Fluorene	16.70	166	165, 167
Indeno(1,2,3-cd)pyrene	39.52	276	138, 227
2-Methylnaphthalene	11.87	142	141
Naphthalene-d_8 (SS)	9.75	136	68
Perylene-d_{12} (SS)	33.05	264	260, 265
Phenanthrene	19.62	178	179, 176
Pyrene	24.02	202	200, 203
Tetrachloro-m-xylene (IS)			

IS = internal standard
SS = surrogate spike
[a]estimated retention times.

Table 2. DFTPP Key Ions and Ion Abundance Criteria[a]

Mass	Ion Abundance Criteria
51	30-60% of mass 198
68	<2% of mass 69
70	<2% of mass 69
127	40-60% of mass 198
197	<1% of mass 198
198	Base peak, 100% relative abundance
199	5-9% of mass 198
275	10-30 of mass 198
365	>1% of mass 198
441	Present but less than mass 443
442	>40% of mass 198
443	17-23% of mass 442

a = from Eichelberger et al., 1975.

Attachment A - Sephadex LH-20 Column Packing and Recycling

A.1 Column Packing

1. Fit a 19 mm ID column with a stopcock, add 10 mL of 6:4:3 solvent and between 5 and 10 mm glass wool plug. Tamp the plug to remove any air bubbles.
2. Add approximately 1 mL of sand to the column, and tap the column gently so that the sand forms a smooth layer on top of the glass wool.
3. Pour the swelled Sephadex gel through the funnel into the column until the gel fills the column and about 1/4 of the reservoir.
4. Allow 10 min for the Sephadex to settle. Open the stopcock, and elute 80 mL of solvent to ensure firm packing. Add more solvent as needed. Leave 30 mL of solvent in the column reservoir. Cover the top with aluminum foil, and allow the packing to settle overnight.
5. Open the stopcock, and elute 10 mL of solvent, then close the stopcock. Remove the excess Sephadex packing from the top with a transfer pipet until the height of the Sephadex is 26.5 cm.
6. Gently add approximately 1 mL of sand onto the packing so that it forms an even layer on the top. (The column may be tapped or tilted slightly to get an even layer of sand.)
7. Examine the packing for air bubbles. If bubbles are evident, elute approximately 250 mL of warm (about 35° C) solvent through the column. If the bubbles persist, recycle the packing (see section A.2).

A.2 Recycling Sephadex LH-20 Column Packing

NOTE: When the column no longer maintains its calibration with azulene/perylene, recycle the packing.

1. Decant any solvent in the column reservoir.
2. Empty the column packing into a beaker 4 times the volume of the packing.
3. Wash with CH_2Cl_2.
4. Add enough CH_2Cl_2 to float Sephadex particles in the upper half of the beaker.
5. Remove <u>all</u> glass wool with forceps (mandatory).
6. Cover the beaker and let stand for 1 to 2 hours.
7. Decant the floating particles leaving the sand in the beaker.
8. Aspirate the CH_2Cl_2 from the Sephadex particles and set them aside.
9. Swell these particles overnight in 6:4:3 solvent before reusing.

GREAT LAKES
DREDGED MATERIAL TESTING AND EVALUATION MANUAL

APPENDIX G
BIOLOGICAL EFFECTS TESTING PROCEDURES

Prepared by

Daniel J. Call, Larry T. Brooke
Lake Superior Research Institute
University of Wisconsin-Superior
Superior, Wisconsin

and

Gerald T. Ankley, Duane A. Benoit
Environmental Research Laboratory-Duluth
U.S. Environmental Protection Agency
Duluth, Minnesota

and

Robert A. Hoke
Science Applications International Corporation
Hackensack, New Jersey

with contributions from

Corlis West, Gary L. Phipps, Teresa Norberg-King
and Jeffrey S. Denny
Environmental Research Laboratory-Duluth
U.S. Environmental Protection Agency
Duluth, Minnesota

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ATTACHMENTS

Acid volatile sulfide - Sulfide forms present in sediments which react with divalent metallic cations and render sediment metals unavailable to the surrounding water and biota.

Acute toxicity test - A test to determine the short-term toxicity of a chemical or material to an organism. The test usually measures lethality.

Bioaccumulation - The net accumulation of chemicals by an aquatic organism via direct partitioning from the water plus ingestion of the chemicals with its food items.

Brood board - A sheet of material (e.g., styrofoam) with an array of openings designed to hold culture containers with brood stock of the test species.

Brood stock - Organisms which are or will be utilized as parents for the test organisms.

Chemical bioavailability - The potential of a chemical to be readily partitioned from water or particles into plant and animal tissue.

Chronic toxicity test - A test to determine the toxic effects of a chemical or material of sufficient duration to extend over the entire life cycle of the test organism.

Control sediment - A sediment essentially free of contaminants and compatible with the biological needs of the test organisms such that it has no discernable influence on the response being measured in the test. Performance of the test organisms in the control sediment is evaluated to determine the health of the organisms and the test acceptability.

Dilution factor - The decimal fraction that a given toxicant exposure level is multiplied by to indicate the reduction in toxicant concentration at the next lower exposure level (e.g., a 0.5 dilution factor results in a 50 percent reduction; a 0.6 dilution factor results in a 40 percent reduction, etc.).

Dilution water - Water of choice for preparing dredged material elutriate and for diluting the elutriate when necessary.

Disposal site sediment - Sediment sample representative of the surficial sediments at the proposed disposal site. Used as point of comparison for interpretation of dredged material bioassay and

bioaccumulation data for regulatory decision making.

Dredged material - Aquatic sediments that have been moved by dredging operations.

EC50 - Effect concentration at which 50 percent of the test organisms elicit the defined response (usually a sublethal response).

Elutriate - Supernatant prepared by mixing sediment or dredged material with dilution water, and used for chemical analysis and toxicity testing.

Embryo - Fertilized egg or ova.

Endpoint - A response in a toxicity test such as lethality, growth or behavioral change.

Hypothesis testing - A statistical approach designed to confirm or deny the null hypothesis that organisms in a given treatment (i.e., dredged site sediment or elutriate) are not affected differently than organisms in the disposal site sediment or elutriate.

Illumination - Amount or energy of light incident upon a unit of surface area, measured in lumens/m^2.

Imago - Adult or sexually mature stage in an insect life cycle.

Infaunal species - Benthic organism which largely dwells within the sediment, as distinguished from one that largely dwells upon the sediment.

Instars - Successive developmental stages of the larval insect between molts.

LC50 - The median lethal concentration, or the concentration of a substance that kills 50 percent of the organisms tested in a laboratory toxicity test of specified duration.

Larvae - For insects, the immature, worm-like life-stages; for fish, the immature life-stages between hatching and becoming covered with scales.

Light intensity - brightness of light at a standard distance from a source, measured in lumens, foot-candles or μE/m^2/S.

LOEC - Lowest Observable Effect Concentration; in an elutriate toxicity test, the lowest toxicant concentration (or lowest

percentage of full-strength elutriate) resulting in a statistically significant difference from the control in one or more toxicity test endpoints.

May - Word meaning "is (are) allowed to".

Must - Word expressing an absolute requirement. It is used in connection with factors that directly relate to the acceptability of a test.

Neonate - Newly born organism in its first day of life (i.e., ≤24 h of age).

"New" solutions - In a toxicity test in which solutions of toxicant are periodically renewed with fresh solutions, the test solutions immediately following solution renewal.

NOEC - No Observable Effect Concentration; in an elutriate toxicity test, the highest toxicant concentration (or highest percentage of full-strength elutriate) resulting in no statistically significant difference from the control in any of the toxicity test endpoints.

"Old" solutions - In a toxicity test in which solutions of toxicant are periodically renewed with fresh solutions, the test solutions immediately prior to solution renewal.

Parthenogenesis - Reproduction directly and solely by a female of the species without participation or contribution by a male of the species.

Point estimation techniques - Statistical approaches designed to provide a point estimate of an effect of the treatments relative to the controls via regression analysis.

Pupa - In insects which undergo complete metamorphosis, the life cycle stage during which the larval structures are rebuilt into adult form.

Pupation - Process of transforming from a larval form to an adult form of insect for those insects which undergo complete metamorphosis.

Quality control criteria - Measures that are taken before and during a toxicity test to ensure that the test results are of high quality and the interpretation of the test results is valid.

Randomized complete block design - An experimental test design in which the individual experimental chambers are randomized within

a block (row) rather than over the entire set of chambers.

Reference organism - Organism from laboratory culture that is saved and preserved for examination to verify the validity of species identification.

Reference toxicant - A chemical used periodically to monitor the sensitivity of a test organism culture to determine their suitability for testing purposes.

Sediment - Material such as sand, silt, clay or organic matter, usually suspended in or settled on the bottom of a water body. The term dredged material refers to material that has been dredged from a water body, while the term sediment refers to material in a water body prior to the dredging process.

Should - Word stating that a specified condition is recommended and ought to be met, if possible. Although a violation of one "should" is rarely a serious matter, violation of several will often render the results questionable.

Solid-phase - Solid sediment or dredged material consisting of both an inorganic mineral component and an organic component.

Static toxicity test - A toxicity test without flowing water; can be without periodic water exchange or with daily exchange of new test water.

Steady-state - An equilibrium condition for the tissue burden of a chemical when there is no net change over time (i.e., chemical influx to the organism equals the efflux from the organism).

Subchronic test - A test in which exposure to a test material is abbreviated relative to a complete life-cycle test, but which occurs over the sensitive life-stages of the test organism. The results approximate those of a full life-cycle chronic test.

Substrate - Material in which benthic organisms live, either natural (e.g., sediment) or artificial (e.g., paper pulp).

1.0. PURPOSE

This appendix provides detailed instructions for the completion of biological effects-based tests for dredged material. These protocols are intended to be used to evaluate the potential for contaminant-related impacts from proposed discharges of dredged material into the U.S. waters of the Great Lakes basin. The protocols should be used and interpreted as described in the Great Lakes Dredged Material Testing and Evaluation Manual (GLTEM). Other applications of these protocols were not intended.

This appendix contains protocols for six test organisms. These tests were designed to evaluate the potential contaminant effects of dredged material discharges on water column toxicity, benthic toxicity, and benthic bioaccumulation. The development of these protocols drew upon the accumulated knowledge and expertise of several research organizations and individuals. Information is heavily utilized from several ASTM Standards and Guides, many USEPA publications, several USACE publications and the collective experience of the authors and contributors of this appendix.

2.0. APPLICABILITY

Water column (elutriate) toxicity tests are presented for three organisms: the cladocerans, *Daphnia magna* and *Ceriodaphnia dubia*, and the fathead minnow, *Pimephales promelas*. Protocols for both short-term (acute) and long-term (chronic) exposures have been presented for each of these test species. However, the GLTEM only recommends that the acute exposures and survival endpoint be used at this time for tier 3 testing. The GLTEM further recommends that tests with any one of these species should be adequate for tier 3 evaluations. The interpretive guidance for the chronic exposures and non-survival endpoints has not been adequately developed for application in tier 3, and the protocols for the chronic exposures are presented for consideration only in the rare cases where tier 4 testing is necessary.

Benthic (solid phase) toxicity tests are presented for two test organisms, including an insect, *Chironomus tentans*, and an amphipod, *Hyalella azteca*. Protocols for the measurement of survival and growth endpoints have been presented for each test species. However, the GLTEM recommends for tier 3 testing that the survival endpoint be used with both species and the growth endpoint with *C. tentans* only. The interpretive guidance for the growth endpoint with *H. azteca* is not adequately developed for application in tier 3 testing, although the protocol for its

measurement is included herein for potential use in tier 4 testing.

A benthic bioaccumulation test is presented for the oligochaete worm, *Lumbriculus variegatus*. The GLTEM recommends this test be used in tier 3, where necessary, to evaluate bioaccumulation potential.

The protocols presented here represent a significant enhancement to the methods previously used to evaluate potential contaminant related effects of proposed dredged material discharges in the Great Lakes. The USEPA and USACE expect a sharp "learning curve" during the first few years of implementation of the GLTEM, and intend on making revisions to this manual and these protocols where appropriate, based on the experiences of their application. The USEPA and USACE invite the comments and opinions of laboratories performing these protocols, particularly any refinements to these protocols which might improve their execution or reduce costs.

3.0. GENERAL LABORATORY REQUIREMENTS

Certain requirements must be met for a laboratory to successfully perform biological evaluations of sediment or sediment elutriates. The laboratory should have (1) the necessary facilities to conduct a carefully controlled test, (2) safeguards in place for protecting the health and safety of employees working with the sediment samples, and (3) the requisite qualifications among personnel involved in the performance and evaluation of the test.

3.1. Laboratory Conditions

The organism culturing or holding facility must be sufficiently separated from the testing facility to eliminate the possibility for contamination from a test to the culture organisms, particularly of volatile chemicals. Both the culturing/holding facility and the testing facility should have the capability for accurate control of temperature and light, in addition to having a supply of clean air.

3.1.1. Laboratory Equipment

Equipment that will come into contact with sediments, overlying water, elutriates, or dilution water must be constructed of a material that will not contribute any toxicants to the culture or test system. Such equipment should also be of materials that will minimize the sorption of test materials from water. Acceptable materials include glass, type 316 stainless steel, nylon, high-density polyethylene, polycarbonate and fluorocarbon plastics (USEPA 1994). These materials should be

cleaned prior to use. The procedure for cleaning glass is given in Section 5.0. The other materials listed, with the exception of stainless steel, should be similarly cleaned. Stainless steel should not be acid-rinsed.

Cast-iron pipe, copper, brass, lead, galvanized metal, natural rubber and neoprene rubber should not come into contact with the overlying water, stock solutions, elutriates or dilution water. Concrete and high-density plastic containers may be used for holding or culturing chambers, and in the water-supply system.

3.1.2. Temperature Control

For elutriate toxicity testing, the control of temperature may be accomplished by placing the test chambers into a temperature-controlled water bath or in a temperature-controlled environmental chamber/room. For solid- phase sediment toxicity and bioaccumulation tests, temperature may be controlled in the same manner as indicated above if the tests are performed statically. If an automated water renewal system is used, additional temperature control may be provided by the renewal water itself.

The test protocols for the six species considered in this Appendix require uniform test water temperatures of from 20^{o} to 25^{o}C. For each of the test species, the overall mean water temperature should be within 1^{o}C of the selected test temperature.

3.1.3. Laboratory Water

Water used in culturing and testing should be of uniform quality. Acceptable water should allow for satisfactory survival, growth or reproduction of the test organisms. For tests with elutriates, a synthetic, reconstituted water or a diluted mineral water may be used. For tests with solid-phase sediments, the overlying water may be well water, test site water, reconstituted water or water from a municipal supply that has been specially treated to remove certain chemicals.

When deionized water is used, the water-deionizing system should provide a sufficient quantity of at least 1 mega-ohm water. If large quantities of high quality deionized water are needed, it may be advisable to supply the laboratory grade water deioinizer with preconditioned water from a mixed-bed water treatment system.

A natural water is considered to be of uniform quality if monthly ranges of the hardness, alkalinity, and specific conductance are less than 10% of their respective averages and if the monthly range of pH is less than 0.4 (USEPA 1994). Natural

waters should be obtained from an uncontaminated well or spring, if possible, or from a surface-water source. If surface water is used, the intake should be positioned to: (1) minimize fluctuations in quality and contamination, (2) maximize the concentration of dissolved oxygen, and (3) ensure low concentrations of sulfide and iron. Water that might be contaminated with facultative pathogens may be passed through a properly maintained ultraviolet sterilizer equipped with an intensity meter and flow controls or passed through a filter with a pore size of 0.45 μm or less.

Municipal-water supplies may be variable and may contain unacceptably high concentrations of materials such as copper, lead, zinc, fluoride, chlorine, or chloramines. Chlorinated water should not be used for culturing or testing because residual chlorine and chlorine-produced oxidants are toxic to many aquatic organisms. Use of tap water is discouraged unless it is dechlorinated and passed through a deionizer and carbon filter (USEPA 1993a).

If reconstituted water is used, water should be prepared by adding specified amounts of reagent-grade chemicals to high-purity distilled or deionized water (ASTM 1993a, USEPA 1993). Acceptable high-purity water can be prepared using deionization, distillation, or reverse-osmosis units (USEPA 1993). In some applications, test water can be prepared by diluting natural water with deionized water (Kemble et al. 1993).

Conductivity, pH, hardness, dissolved oxygen, and alkalinity should be measured on each batch of reconstituted water. The reconstituted water should be aerated before use to adjust pH and dissolved oxygen to the acceptable ranges. USEPA (1993) recommends using a batch of reconstituted water for no longer than two weeks.

3.1.4. Laboratory Air Supply

A supply of clean air is necessary for both the test organism culturing/holding unit and the testing laboratory. The air used for water aeration should be free of oil and fumes. Oil-free air pumps are recommended, where possible. Air line filters should be used to remove oil, water and bacteria. The culturing/holding unit and the testing facility should be well ventilated and free of fumes, as well.

3.1.5. Laboratory Lighting

Lighting should be provided by wide-spectrum fluorescent bulbs, with an intensity at the surface of the test water from approximately 300 to 1,100 lux. An automatic timer should provide a photoperiod of 16 hours of light and 8 hours of dark each day.

3.1.6. Test Organism Food

Facilities for culturing the test organism food supply are essential for tests using *Daphnia magna*, *Ceriodaphnia dubia*, fathead minnows or *Hyalella azteca*, as test organisms. *D. magna* and *C. dubia* are fed live green algae, *Selenastrum capricornutum*, in addition to a suspension of trout chow for *D. magna* and a suspension of yeast, Cerophyll® and trout chow for *C. dubia*. Fathead minnows are fed newly hatched nauplius larvae of brine shrimp. Specific instructions and required equipment items for culturing *Selenastrum capricornutum* and brine shrimp are provided in the following toxicity test protocols for *D. magna*, *C. dubia* and fathead minnows. During culturing, *H. azteca* are fed green algae (e.g., *Ankistrodesmus* sp.) in addition to a mixture of yeast, Cerophyll® and trout chow (YCT). Specific equipment needs and instructions for culturing such algae are provided in the following *Hyalella azteca* test protocol. *Chironomus tentans* culture and test organisms are fed Tetrafin® goldfish food, and *Lumbriculus variegatus* is fed commercial trout chow during culture.

3.1.7. Refrigerated Storage

The testing laboratory should have a cold storage facility of sufficient size to store sediment samples for the period of time between receipt of samples and test initiation (or successful test completion for a portion of the sample). Depending upon the volume of sediment to be tested and the frequency with which such tests are performed, the cold storage facility could range in size from one or more refrigerators to a specially designed cold storage room. The sediment is to be stored in the dark at 4^{o}C until it is used in a test.

3.1.8. Biological Decontamination

The testing laboratory should have an effluent containment facility into which the test effluents (from tests having automatic renewal of overlying water) or the renewal water (from tests with manual overlying water renewals) enter and are treated to kill any exotic, nuisance species that may have been present in the test or disposal site sediment samples. The effluent containment tanks should be of sufficient volume to retain the test effluents for 24 h or more prior to discharge. This is to allow for sufficient contact time between the nuisance organisms and the chemical or heat treatment to produce complete mortality. Chlorination of the effluent is one chemical treatment option, in which case the chlorine concentrations in the effluent holding tank should be 20 mg/L or greater. In the case of heat treatment, the effluent should be heated to 50^{o} C for 2 h before being discharged to a wastewater treatment facility (Sims et al. 1993). The actual size of the containment facility needed will vary depending upon the testing volume for each laboratory.

3.1.9. Hazardous Material Storage

A testing laboratory should have an area or facility for the safe storage of sediments or effluents that are judged to be highly hazardous. These materials should be safely contained in the storage area until they are properly disposed.

3.1.10. Computational Capability

Each testing laboratory should have the necessary computer hardware and software that will allow for tracking samples, reduction of test data, and report preparation. Specific statistical tests for each type of sediment evaluation test are described in the appendix. Several software packages for data reduction are mentioned in the protocols. Other software packages that contain the recommended statistical tests are also commercially available.

3.2. Health and Safety Precautions

Chemical contaminants in field-collected sediments may include carcinogens and mutagens, as well as infectious microorganisms. The laboratory should have an ongoing commitment to the maintenance of a work environment that will not endanger the health of the staff. Special equipment (e.g., respiratory masks, special clothing) or supply items (e.g., disposable gloves) should be present in the physical facility in which the sediments are handled and the tests are performed. Laboratory personnel should periodically receive training in appropriate safety procedures.

3.2.1. Ventilation

Sediment handling and testing should be performed only with adequate ventilation. Sediment handling may be accomplished under a hood, in a ventilated glove box, or, at a minimum, in a well-ventilated room. The testing of sediments should be performed in a system that is well-ventilated.

3.2.2. Personnel Safety

For personal safety, contact of the dredged material or overlying water with skin and eyes should be avoided. Both may be contaminated to various degrees with chemicals and infectious microorganisms. Laboratory coats, disposable gloves and safety glasses should be worn while working with dredged material. The dredged material should be mixed under a hood or, at a minimum, in a well-ventilated room. Test systems should be enclosed and under negative atmospheric pressure to avoid contamination of laboratory air. Should skin or eye contact with sediment occur, immediately wash the skin with soap and water or flush the eyes with water. If the dredged material should contact a dermal wound, wash the skin and apply a topical antibiotic. For individuals regularly involved with dredged material processing, current immunizations against infectious disease microorganisms,

including hepatitis B, tetanus, typhoid fever and polio, are recommended (USEPA 1993).

3.2.3. Hazardous Waste Disposal

For environmental safety, dredged material that is to be discarded should be disposed of in full compliance with existing state Environmental Protection Agency and Department of Transportation regulations. The method of disposal should comply with a protocol for waste disposal approved by the equivalent of an Environmental Safety Officer at the laboratory performing the test. Efforts should be made (e.g., chemical or heat treatment) to destroy any life-stages of exotic nuisance species that may be present in the sediment or associated water, such as zebra or quagga mussels (*Dreissena* sp.) and Asiatic clams (*Corbicula* sp.).

3.3. Personnel Qualifications

A laboratory that conducts biological evaluations of sediments or elutriates should have experienced personnel for culturing and/or holding test organisms, for performing the toxicity or bioaccumulation tests, for performing the requisite chemical measurements that accompany the tests, for statistically analyzing the test data, for preparing a report of the test, for performing quality assurance/quality control (QA/QC) audits and reviews of the test, and for compliance with local, state and federal laws regarding the disposal of contaminated sediment and water.

One individual may fulfill more than one of the above responsibilities. However, the QA/QC audits and review must be performed by an individual not involved with the tests. It is also necessary to either have on staff or have access to an individual that is knowledgeable regarding the identification of the different test species. This will allow for verification of the test organisms as being of a given species.

4.0. QUALITY ASSURANCE REQUIREMENTS

Quality assurance/quality control (QA/QC) of test results for dredged material toxicity tests is based upon guidance offered in the document entitled "Quality assurance/quality control (QA/QC) guidance for laboratory dredged material bioassays" (Moore et al. 1994). The document offers guidance on the subjects of data quality objectives; biological procedures; sample handling, storage and shipment; data recording, reduction, validation and reporting; internal quality control checks, and corrective action. These issues are briefly addressed in this section as they specifically pertain to the toxicity tests mentioned in this appendix.

4.1. Minimum Requirements for Managing Culture Quality

4.1.1. Test Laboratory Cultured Organisms

A laboratory that cultures organisms for dredged material toxicity testing must have a culture of organisms of a single species, and species identification should be verified by a competent taxonomist. Organisms must be disease-free and not from an unusually tolerant or intolerant genetic strain. The history of the parents of the test organisms for at least one generation should be known. Organisms used for toxicity tests should be from a minimum of three female parents. The culturing laboratory should keep records of rate of reproduction and rate of survival of offspring to demonstrate that the test organisms are within normal limits based upon a particular laboratory's recorded results. It is recommended that organisms from the culture be periodically (i.e., monthly if tests are performed routinely, or with each test if performed infrequently) subjected to a toxicity test with a reference toxicant. Suggested reference toxicants are cadmium, copper, sodium or potassium chloride. Results must be within the limits established by the laboratory as a normal response (e.g., ±2 standard deviations of the mean). A minimum of 5 reference toxicant tests with each test species is recommended to document the condition of the culture animals (USEPA 1994).

4.1.2. Purchased Test Organisms

Organisms purchased from a supplier for toxicity tests with dredged material must be disease-free and from an established culture. The supplier should provide with the organisms a record of their history for at least one generation showing no unusual survival trends. Certain physical and chemical characteristics (i.e., temperature, pH, hardness) of the water used to culture the organisms should be supplied. The organisms should be of known age and their diet described. A record of reference toxicant test results should be provided by the supplier of the test organisms, but a reference toxicant test should also be conducted by the laboratory receiving the purchased organisms. If the supplier has not conducted 5 reference toxicant tests with the test organism, the testing laboratory should perform these five tests from five different groups of organisms before starting a sediment toxicity test (USEPA 1994).

4.2. Minimum Requirements for Water and Feed Quality

4.2.1. Water Quality

Water for culturing test organisms must be of suitable quality for good health of the test organisms. The water can be from the regular water supply for the laboratory, disposal site water or reconstituted water. Physical and chemical characteristics of the water must be within the range suitable

for the good health of the test organism at all times. Water quality for the dredged material toxicity test must be similar to the quality for the culture water, insuring no adverse effects upon the organisms due to water quality differences. Specific requirements for water quality are described for each test species in its respective testing protocol.

4.2.2. Food Quality

Food used during the dredged material toxicity test should be of the same type and supply as used during the culturing of the organisms. Information supplied with the food from the supplier or received through direct contract with the supplier must be reviewed to insure that no unusual ingredients or unusually high levels of contaminants are present. If chemical assays are conducted for a chemical of concern for the toxicity test, then the food should also be assayed for this chemical. Food should be suitably stored to maintain quality. Specific food requirements and preparation are described for each test species in its respective testing protocol.

4.3. Toxicity Test Pre-treatment Criteria

4.3.1. Test Water Conditions

Dissolved oxygen concentrations in exposure chambers should exceed 90 percent of saturation before the test organisms are added, but must not exceed 110 percent. Water temperature should be within 1°C of the desired test temperature.

4.3.2. Daphnia magna Test

Individualized brood-board cultures must be analyzed for adequate survival and reproduction prior to the start of a test. Only neonates from females with production of $\geq$9 young/brood should be used in tests. Neonates from about the fourth brood are recommended for testing. Survival of adults in the stock culture must exceed 80 percent. Results of the reference toxicant test (see Section 4.3.8.) must meet the requirements to indicate adequate organism health.

4.3.3. Ceriodaphnia dubia Test

Individualized brood-board cultures must be analyzed for adequate survival and reproduction prior to the start of a test. Only neonates from a brood-board set with a history of $\geq$15 young/female in 7 days or 3 broods should be used. Survival of adults in the stock culture must exceed 80 percent. Results of the reference toxicant test (see Section 4.3.8.) must meet the requirements to indicate adequate organism health.

4.3.4. Pimephales promelas Test

Survival of adults in the brood culture must exceed 90 percent. Hatching success of embryos should exceed 80 percent.

Results of the reference toxicant test (see Section 4.3.8.) must meet the requirements to indicate adequate organism health.

4.3.5. Chironomus tentans Test

Egg mass hatching must be adequate to produce the number of larvae needed for the test. Late fourth instar larvae from the culture aquaria should be monitored on a monthly schedule for adequate dry weight. Dry weight of fourth instar culture larvae must average $\geq$0.6 mg to be acceptable. Larvae must be 8 to 12 days old (post-hatch) at the start of the test. Results of the reference toxicant test (see Section 4.3.8.) must meet the requirements to indicate adequate organism health.

4.3.6. Hyalella azteca Test

Young production by adults in the culture should be 75 to 100 young/50 adults/week. Survival of adults and young must exceed 80 percent. Results of the reference toxicant test (see Section 4.3.8.) must meet the requirements to indicate adequate organism health.

4.3.7. Lumbriculus variegatus Test

Animals must come from a healthy culture. Health is indicated by organisms having normal coloration, high level of reproduction (i.e., a doubling of population density every 10 to 14 days in a fresh culture), and normal reflexive action to stimuli. Results of the reference toxicant test (see Section 4.3.8.) must meet the requirements to indicate adequate organism health.

4.3.8. Reference Toxicant Test

It is recommended that an assessment of test organism condition be conducted by performing a toxicity test using a reference toxicant (e.g., $CdCl_2$, $CuCl_2$, KCl, NaCl). The test can be conducted concurrently with dredged material toxicity tests or on a routine basis for which a monthly test is recommended. The results of any test should be within two standard deviations of the mean of all previous reference tests to consider the animals suitable for use in a dredged material toxicity test. A minimum of 5 reference toxicant tests should be performed prior to the testing of a sediment (USEPA 1994).

The reference toxicity tests should be a minimum of 48 h in duration with five replicated control and toxicant concentrations. The test should be a static exposure and should use either the culture water or test water for exposure of the organisms. These reference toxicant tests should be conducted with careful technique, and each additional test performed requires a recalculation of the mean and standard deviation.

4.4. Toxicity Test Post-treatment Criteria

4.4.1. Test Water Conditions

Dissolved oxygen should be between 40 and 100 percent of saturation at all times during the test. Light aeration is sometimes necessary to maintain the minimum percent saturation. Water temperature must be consistent amongst all test chambers. The temperatures should be within 2°C of each other at all times, and the mean water temperature for the test should be within 1°C of the desired test temperature. Total ammonia concentrations in the water should not exceed 1.0 mg/L in any test chamber during the test, and the mean total ammonia concentration should not be greater than 0.1 mg/L.

4.4.2. Daphnia magna Test

The results of an acute test should be considered acceptable only if survival in the control chambers averages ≥90 percent. In a chronic test, survival must average ≥80 percent, and production of young by control organisms must average ≥60 young/surviving female after 21 days. For both acute and chronic tests, hardness and alkalinity of reconstituted water used to initiate a test and for renewal of test solutions must fall within the ranges of 160 to 180 mg/L (as $CaCO_3$) for hardness and 110 to 120 mg/L (as $CaCO_3$) for alkalinity. The pH of reconstituted water must be between 6.8 and 8.5. The time-weighted average measured concentration of dissolved oxygen for each test chamber must be between 50 and 100 percent of saturation for the test (ASTM 1993a).

4.4.3. Ceriodaphnia dubia Test

The results of an acute test should be considered acceptable only if mean survival in the control chambers is ≥90 percent. In a chronic test, survival must average ≥80 percent, and young production by control animals must average ≥15 young/surviving female. At least 60 percent of the surviving females in the control chambers should have produced three broods. The time-weighted average measured concentration of dissolved oxygen must be between 50 and 100 percent of saturation for the test (ASTM 1993a).

4.4.4. Pimephales promelas Test

The results of an acute test should be considered acceptable if survival in the control chambers average ≥90 percent. The results of a chronic test should be considered acceptable if survival in the control chambers averages ≥80 percent and if the dry weight of control organisms averages ≥0.25 mg per larva. Dissolved oxygen must average (time-weighted) >40 percent of saturation for all test chambers and must not fall below 40 percent in any test chamber at any time.

4.4.5. Chironomus tentans Test

The results of a test should be considered acceptable if survival in the control chambers averages ≥70 percent. Dissolved oxygen saturation should average >40 percent in all test chambers. Dry weight of the controls must average 0.6 mg for the test to be considered acceptable.

4.4.6. Hyalella azteca Test

The results of a test should be considered acceptable if survival in the control chambers averages ≥80 percent. Dissolved oxygen saturation must be >40 percent in all chambers at all times and should average (time-weighted) between 50 and 100 percent of saturation for the test.

4.4.7. Lumbriculus variegatus Test

The results of a test should be considered acceptable if a sufficient mass of organisms is available after 28 days of exposure to dredged materials. Test organisms should have been observed to burrow into the sediment at the start of the test. Dissolved oxygen should be ≥40 percent of saturation in all test chambers at all times.

4.5. Biological Test Procedures

4.5.1. Standard Operating Procedures (SOPs)

The six testing procedures described in this Appendix can be adapted for use as SOPs. The testing procedures contain checklists and schedules for each of the important aspects of test organism preparation and for conducting a test.

4.5.2. Good Laboratory Practices

Good laboratory practices should be employed when conducting a test to eliminate bias and opportunity for contamination of a test. Many potential problems can be eliminated by proper acclimation, test conduct, use of proper controls, statistical design and randomization, and reference toxicant testing.

4.5.3. Statistical Design and Randomization

The appropriate statistical design should have a minimum of five replicates for all toxicity tests. Reference toxicant tests should have two to five replicates per chemical concentration. The chemical bioaccumulation test also requires a minimum of five replicates. A power analysis should be run before increasing the number of replicates to ensure cost effectiveness of increased sensitivity. A randomized block design is recommended to remove the bias of positional effects in the test.

4.6. Sample Handling, Storage and Shipment

4.6.1. Chain of Custody

Documentation which includes dates and signatures should accompany all samples from the origin of the sample to its destination at the sample testing facility. Chain of custody documentation should continue in the laboratory as the sample is stored, processed, tested and disposed.

4.6.2. Sample Preparation

Sediment or dredged material samples must be treated in a consistent manner to avoid bias in the toxicity test. For example, all elutriate samples must be centrifuged even if they appear to lack suspended material. Disposal site sediment must be treated identically with test site sediment. All samples must be kept at all times in appropriate containers that are clean.

4.6.3. Sample Storage

Samples must be stored in appropriate containers that are full (i.e., zero or minimal headspace) and tightly covered. They must be kept in the dark at 4ºC and tested within 8 weeks. Samples must be re-homogenized prior to use in a test.

4.7. Data Recording, Reduction, Validation and Reporting

4.7.1. Use of Laboratory Notebooks

Data for a test should be recorded (in indelible ink) on data forms and stored in a bound notebook. Storage of data solely on electronic media is not acceptable. Data forms should be marked with a dash when data were purposely omitted. Erasures are not permissible. If data must be corrected, they should be lined-through and initialed by the person making the correction. All data forms should identify the person reporting the data.

4.7.2. Data Management

Standardization of data reporting and statistical analysis is very important. Standardization helps reduce bias and results in consistent interpretation of test results. Duplicate copies of all data (preferably stored at different locations) are recommended to minimize loss.

4.7.3. Unacceptable Data or Outliers

If data are immediately identified as being erroneus (e.g., instrument not properly calibrated), a new measurement can be made and recorded, replacing the initial measurement. If, however, the data are unexpected, but not obviously erroneus, a second measurement may be taken to provide verification. If unexpected or erroneus data are discovered later, they must be dealt with in a scientifically defensible manner. If the outlier

data can be explained, they can be removed from the data set. Otherwise they are either used in calculations or tested statistically for their eligibility as outliers (see numerous statistical texts for outlier tests).

4.8. Internal Quality Control

A testing laboratory should have a person (e.g., a Quality Control Officer or another person under the direction of the officer) not associated with the toxicity test conduct an audit to determine if all planned procedures and measurements were completed. Results of each audit should be submitted to the testing organization's chief officer and to the study director. Additionally, there should be a verification of the taxonomy of the test organism, and a review of the acceptability of control organism survival (and growth in some cases) in the test. The reference toxicant test results must be within acceptable limits.

4.9. Corrective Action

Deficiencies in the completeness of data records and quality of test results obtained for the sample must be addressed. Some deficiencies are less important than others such as unreported water quality measurements. Retesting of a sample is usually required when there is excessive test organism mortality in control exposures, out-of-range water quality measurements, lack of randomization, lack of required reference, control, or reference toxicant tests, and out-of-range reference toxicant results. The laboratory logbook and sample file/report should document any actions taken, the reasons for such actions and the success of the actions taken.

5.0. SAMPLE HANDLING AND PREPARATION PROCEDURES

Proper handling procedures of the sediment samples from the time of collection to the final disposition of the samples following their use in a test are very important. The samples must be properly labeled and tracked using a chain-of-custody form (Moore et al. 1994).

Samples of dredged or disposal site material should be stored in the dark at 4°C with minimal headspace above the sediment. Glass storage containers should be thoroughly pre-cleaned using the following recommended procedure (ASTM 1993d): (1) non-phosphate detergent wash, (2) triple water rinse, (3) water-miscible organic solvent wash (acetone followed by pesticide grade hexane), (4) water rinse, (5) acid wash (such as 5-10% concentrated hydrochloric acid), and (6) triple rinse with deionized-distilled water. Container cleanliness should be documented according to specific QA/QC guidelines (USEPA 1990). New polyethylene containers, if used, should similarly be

thoroughly cleaned before use. Due to the difficulty in completely cleaning polyethylene containers that have stored contaminated sediments, they should be used only once, and then discarded. Storage containers should be filled completely to minimize headspace. It is prudent to complete the testing of sediments with a minimum of storage time (probably less than 2 weeks), to minimize changes in sediment chemistry (ASTM 1993d). Sediment holding time should not exceed 8 weeks (USEPA and USACE 1994). Various standard chemical extraction methods have storage time limits ranging from less than 7 days to less than 6 months (USEPA 1985, ASTM 1993e). The maximum allowable holding time may change in the future, as more information becomes available.

The dredged, disposal site or control material should be thoroughly mixed to a homogeneous state prior to use in a toxicity test. The sediment may first be screened through a coarse-mesh screen (e.g., 5 mm or no. 5 mesh) to remove large objects, such as rocks and sticks. The removal of any materials should be carefully documented. Mixing can be accomplished by hand or with mechanical mixers, depending on the sample volume. The water should not be drained from the sediment sample, but should be mixed with the sediment as much as possible. Mixing should be sufficient to homogenize the sample without significantly elevating its temperature. Mixing may need to be minimized if there are known or suspected volatile or semi-volatile contaminants of concern. Some volatilization during mixing is unavoidable and adequate ventilation should always be provided. If the sediment has been in storage following an initial mixing, it should be re-homogenized immediately prior to being tested.

For solid-phase toxicity or bioaccumulation tests, the required volume of the homogenized sample is placed into each of the clean replicate test chambers. Overlying water is then added, and the sediment is allowed to settle for 24 h before the test organisms are added.

For elutriate toxicity tests, the elutriate should be prepared on a weekly basis for the exposures, and stored for no longer than 7 days. The 100 percent or stock elutriate is prepared in a 1:4 volume ratio of sediment to test water by mixing vigorously for 30 min with a magnetic stirrer, shaker or tumbler. At 10 min intervals, the contents are manually stirred to ensure complete mixing. After the 30 min mixing period, the mixture is allowed to settle for 1 hr, and the supernatant is decanted or siphoned off from each container. Centrifuge the supernatant at 4,000 X g in a refrigerated centrifuge for 45 min (Ankley et al. 1990) to precipitate suspended solids. This supernatant may be combined with other supernatants from the same sample to provide a sufficient volume for a test. It should be

used directly as the 100 percent test site elutriate water.

The required volumes of elutriate and dilution water for test initiation and each renewal vary with each test and are provided with the specific test protocol. The elutriate should be stored in the dark at 4°C until just prior to use in a test. It should then be equilibrated to the desired test temperature before being used in the test. This can be accomplished by placing the renewal solutions into a water bath or another temperature-controlled environment maintained at the test temperature.

6.0. *Daphnia magna* WATER COLUMN TOXICITY TESTS

Daphnia magna (Figure G-1) is a planktonic freshwater cladoceran of the family Daphniidae. Under appropriate culture conditions at 20°C, it reproduces parthenogenetically, producing its first brood at 7-10 days, and subsequent broods every 2-3 days. It is quite sensitive to some toxicants (Slooff and Canton 1983, Adams et al. 1986, Nebeker et al. 1984, 1986a, Dutka et al. 1989), and has been commonly used in toxicity studies (Knight and Waller 1987). The use of *D. magna* in the evaluation of sediment quality has involved acute and chronic exposures to elutriates, organic solvent extracts of sediment samples, whole sediments and pore waters (Hoke and Prater 1980, Laskowski-Hoke and Prater 1981, Maleug et al. 1984a,b, LeBlanc and Surprenant 1985, Nebeker et al. 1986a,b, Giesy et al. 1988, 1990, Burton et al. 1989, Hoke 1989, Larson 1989, Wiederholm and Dave 1989, Stemmer et al. 1990, Davenport and Spacie 1991). These and other studies with effluents or sediments have utilized test durations ranging from 2 to 21 days; typical test durations have included 2, 4, 7, 10, 14 or 21 d. In general, test lengths of 7 or more d have been used to provide measures of subchronic or chronic toxicity, i.e., reproductive or growth effects (Adams and Heidolph 1985, Knight and Waller 1987, Lewis and Horning 1988, Winner 1988, Gersich and Milazzo 1990). Although several test durations have been used, probably the best defined protocol is for the 21-d exposure (Biesinger et al. 1987, ASTM 1993a).

This document has adapted the standardized 21-d chronic toxicity test with chemicals in water for application to dredged material elutriates. If standardized protocols of shorter duration for estimating chronic toxicity to *D. magna* are developed, it would be appropriate to shorten the test duration of this protocol in the future.

Methods for performing a 48-h acute toxicity test either with full-strength elutriate alone or with full-strength elutriate plus several dilutions of the elutriate are presented

in this document. It is recommended that an acute toxicity test be performed prior to conducting a chronic test.

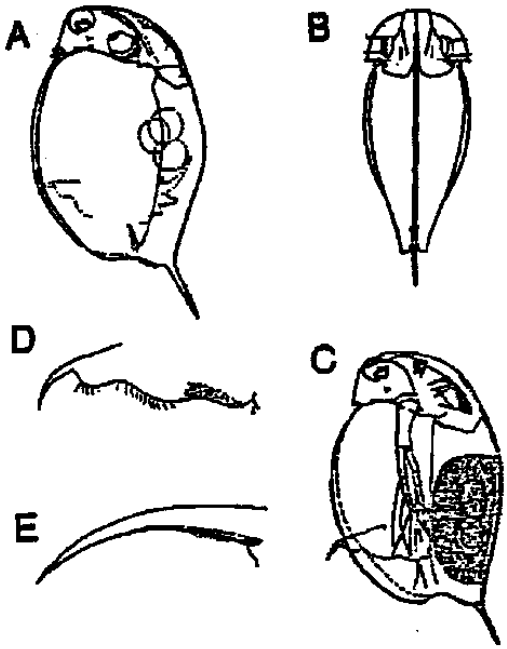


Figure G-1. *Daphnia magna* adult female (X7) and distinguishing body features. A. Lateral aspect, parthenogenetic female; B. Dorsal aspect; C. Ephippial female; D. Postabdomen showing sinuate posterior margin; E. Postabdominal claw (From Brooks 1957).

6.1. CULTURE METHODS

The culturing methods recommended are based on methods described by Biesinger et al. (1987) and ASTM (1993b). Required materials are listed in Attachment A.

6.1.1. Organism Source

Organisms for the initiation of a laboratory culture may be obtained from any source which has a culture of *D. magna* that is periodically verified by a qualified taxonomist. Brood stock organisms are available from various government laboratories and commercial sources.

6.1.2. Acclimation of New Brood Stock

Environmental stress on the daphnids in the starter culture must be minimized to facilitate normal culture growth and brood production. Measure the temperature of the water containing the

stock animals upon their arrival and gradually adjust it to the desired culture temperature. A temperature of 20°C is recommended for both culturing and testing (Biesinger et al. 1987, ASTM 1993b, Lewis and Horning 1991). Water quality in the brood stock container in which the new daphnids were transported should be gradually adjusted over a period of two or more days to meet the conditions of the water in which the organisms will be cultured. Changes in temperature >3°C in any 12-h period should be avoided (ASTM 1993b). To accomplish a gradual change, it is important to know the basic chemical characteristics (i.e., pH, hardness, alkalinity) of the water used by the laboratory from which the brood stock was received, and over a period of two or more days to dilute that water incrementally with the culture water to be used. This is continued until the water meets the requirements for the desired culture water. In preparation for a 21-d test, it is recommended that a minimum of two (and preferably five) generations be raised using the same water, food and temperature as will be used in the test (ASTM 1993b).

6.1.3. Reference Organism

It is recommended by EPA (USEPA 1989) for *Ceriodaphnia dubia* that a new laboratory culture be started with a single animal. The same is recommended here for *D. magna*. It should be killed after producing a supply of young, and definitively identified as *D. magna* using a taxonomic key. This specimen should then be permanently mounted on a slide for future reference. Procedures are available (USEPA 1989) for making slide mounts according to the method of Beckett and Lewis (1982).

Once the starter culture has arrived and neonates are being produced, adults should be separated and one offspring from a large brood (i.e., >15 young) selected as the source of the new laboratory stock culture. The remaining starter culture animals may be maintained as a back-up culture.

6.1.4. Culture Chambers

D. magna may be cultured in 2,000-mL glass beakers, each containing 1,600 mL of culture water and 20 daphnids. The beakers should be covered with glass to minimize evaporation.

6.1.5. Culture Water

Reconstituted water with a hardness of 160-180 mg/L as $CaCO_3$ and a pH between 6.8 and 8.5 is recommended as culture water (Biesinger et al. 1987); however, other types of water are acceptable provided adequate survival (i.e., >80 percent survival in 21 days) and reproduction (i.e., >4 young per female per reproductive day) of *D. magna* are documented. Methods for preparation of the reconstituted water are given in Attachment B. The culture water is renewed weekly by transferring adult daphnids to new water. Each batch of renewal water should be

monitored for temperature, dissolved oxygen, pH, conductivity, alkalinity and hardness; and the measurements recorded in a culture maintenance logbook. Renewal water temperature should be within 1°C of the culture water being renewed. Renewal should not occur until the correct temperature is attained. Dissolved oxygen, pH, conductivity, alkalinity and hardness of the renewal batch water should all be within 10 percent of the values for the same parameters for the initial batch of water. If not, adjustments should be made by aeration (in the case of low dissolved oxygen) or preparation of a new batch of renewal water. Reconstituted water more than one month old should not be used.

6.1.6. Temperature and Photoperiod

The temperature for culturing *D. magna* should be maintained at 20±2°C. The recommended photoperiod is 16 h light and 8 h dark with a light intensity of 30 to 100 foot-candles (Biesinger et al. 1987).

6.1.7. Food and Feeding

A diet that has been used successfully by USEPA to culture *D. magna* consists of trout chow and the green alga, *Selenastrum capricornutum*. Preparation methods for the trout chow and algal diets are given in Attachment C.

Each 2,000 mL culture beaker should receive volume additions of dietary ingredients resulting in final concentrations of 5 mg/L dry wt. of trout chow and 10^8 cells/L of *S. capricornutum* three times weekly (each Monday, Wednesday and Friday). The appropriate volumes of trout chow and algal cell suspensions to produce final concentrations of 5 mg/L dry wt. and 10^8 cells/L are determined as described in Attachment C.

The unused trout chow and algal concentrate should be refrigerated after use. Stored refrigerated trout chow may be used for up to one week, and *S. capricornutum* for up to 12 d (Biesinger et al. 1987).

6.1.8. Handling

A fire-polished pipet of at least 5 to 6 mm bore diameter is recommended for transferring adult daphnids (ASTM 1993b). A smaller diameter pipet may be used to transfer young, but should have an inside diameter of about 1.5 times the size of the organisms (Biesinger et al. 1987). Pipets should be stored in 100 percent methanol (which is replaced weekly), and rinsed three times with tap and distilled water prior to use. Care should be exercised to avoid injury to the daphnids during transfer and to ensure that they are gently introduced below the surface of the water in the new chambers.

6.1.9. General Culture Maintenance

Cultures should be maintained at 20±2°C in a controlled constant temperature environment (e.g., water bath, incubator, environmental chamber or room). Temperature of the culture water should be monitored daily, and a log of the temperatures maintained. Adult daphnids are transferred to new culture media weekly, and are fed each Monday, Wednesday and Friday. Young daphnids are either disposed of or used to start new cultures. Young from the second to sixth broods of the adults are used to start new cultures each week. Adults are disposed of at 4 weeks of age (Biesinger et al. 1987).

6.1.10. Pre-Test Culture Maintenance

Two weeks prior to the start of an acute or chronic test, adult brood stock about to have their second to sixth broods are placed into individual 100 mL beakers (as in the test itself) and observed. A healthy condition is indicated by the absence of floaters (i.e., animals on surface), absence of ephippia (i.e., specialized detached brood chambers with fertilized eggs, which develop under stressful conditions), large size of adults (i.e., >4 mm in total length at 21 d), dark coloration, absence of external parasites, and presence of acceptable numbers of young (four or more young per female per reproductive day). Sixty young daphnids produced from healthy adults are then transferred individually into 100 mL beakers containing new media and reared for at least two weeks. Young from these daphnids are used for the actual toxicity tests (Biesinger et al. 1987, ASTM 1993b).

6.1.11. Culture Evaluation

The general and pre-test cultures are observed daily for their condition of health and for water temperature measurements. Production of young should be at an acceptable level (i.e., 4 or more young per female per reproductive day) in both the general and pre-test cultures. A reproductive day for a given female is each day from the time of the first brood, inclusive of the day of the first brood. Daphnids should not be used to start a test if they fail one or more of the following pre-test culture criteria (ASTM 1993b):

(a) Young for a test must be from adults that appear healthy and uninjured.
(b) Young for a test must be selected from a brood later than the third brood.
(c) Young for a test must be from an adult that produced young before day 10.
(d) Young for a test must be from an adult that produced at least nine young in the previous brood.
(e) Young for a test must be from a culture which did not produce ephippia and which did not have substantial mortality in the week immediately prior to the test.

If the health of the general or pre-test culture is questionable, culturing conditions should be scrutinized and adjustments made to restore the health and increase young production. Any adjustments made may be considered to have resulted in an acceptable state of health for the culture when the pre-test culture meets the above criteria.

6.1.12. Culture Records

A separate set of record books should be kept for the culture unit. Sample culture record forms are provided in Attachment D. Records must be kept on the survival of brood organisms in both the general and pre-test cultures. In the pre-test culture, the time to first brood and the number of young produced should be recorded.

6.2. ACUTE TEST

6.2.1. Elutriate Preparation (Acute Test)

The GLTEM currently recommends that acute exposures of 48 h duration be performed for routine tier 3 testing. Chronic tests (discussed in Section 6.3) may be used for tier 4 testing, if needed. The culturing of organisms is the same for both acute and chronic tests. Animals used to start an acute test are of the same age (i.e., $\leq$24 h old) and are handled in the same way as in a chronic test. The same general test conditions of temperature, lighting and dilution water characteristics apply to both acute and chronic tests. The GLTEM recommends that an acute test first be performed with the 100 percent elutriate, and followed by a dilution series only if survival in the 100 percent elutriate is less than 50 percent. This will require 125 mL of 100 percent elutriate for the five replicate exposures. To obtain a sufficient volume of elutriate water for an acute test with a complete dilution series (i.e., about 250 mL), place 70 mL of well-mixed sediment into a clean 500 mL beaker, add 280 mL of dilution water (same as culture water), and follow the elutriate preparation procedure described in Section 5.0. The test methods follow standard procedures for measuring the acute toxicity of effluents and receiving waters (USEPA 1993). Table G-1 provides a summary of the volumes of elutriate and dilution water required in a test with five different elutriate concentrations using a 0.5 dilution factor and a water-only control.

Table G-1. Volumes (mL) of Dredged Material Elutriate and Dilution Water Required for the *Daphnia magna* 48-h Acute Toxicity Test.

Percent Elutriate	Elutriate Volume Per Replicate	Dilution Water Volume Per Replicate	Total Elutriate Volume Required	Total Dilution Water Volume Required
100	25.0	0.0	125	0
50	12.5	12.5	62.5	62.5
25	6.25	18.8	31.2	93.8
12.5	3.12	21.9	15.6	109.5
6.2	1.6	23.4	8.0	117.0
0.0	0.0	25.0	0	125

6.2.2. Acute Test Design

The basic design and conditions for performing a 48 h acute toxicity test are given in Table G-2. The exposure is static without renewal of the test solution. The test is performed with neonates (<24 h old) which have been provided with food during the holding period prior to test initiation. Newly released young should have YCT and *Selenastrum* available for a minimum of 2 h prior to their use in a test. The animals are not fed during the actual test.

Table G-2. Overview of Recommended Dredged Material Elutriate Test Conditions for the *Daphnia magna* 48-h Acute Toxicity Test.

1.	Test type	Static
2.	Temperature (°C)	20±1°C
3.	Light quality	Fluorescent bulbs (wide spectrum)
4.	Light intensity	10-20 $\mu E/m^2/s$, 540-1080 lux or 50-100 ft-c (ambient laboratory levels)
5.	Photoperiod	16 h light, 8 h dark
6.	Test chamber size	30 mL minimum
7.	Test solution volume	25 mL minimum
8.	Age of test organisms	Less than 24 h
9.	No. neonates per test chamber	5

Table G-2 (continued)

10.	No. replicate test chambers per concentration	5 minimum
11.	No. neonates per test concentration	25 minimum
12.	Feeding regime	No food provided during test. Neonates are fed once during the holding period.
13.	Aeration	None or light aeration prior to test initiation if dissolved oxygen ≤90 percent.
14.	Dilution water	Hard reconstituted water of 160-180 mg/L as $CaCO_3$ hardness and a pH range of 6.8-8.5.
15.	Elutriate concentrations	Minimum of 5 test site elutriate concentrations with a performance control (water-only).
16.	Dilution factor	≥0.5
17.	Water quality monitoring	Daily measurements of water temperature, dissolved oxygen and pH. Single measurements of hardness, alkalinity and specific conductance.
18.	Test duration	48 hr
19.	Endpoints	Survival and complete immobilization.
20.	Test acceptability	90% or greater survival in the water-only control solutions.
21.	Sample storage	Store sediment and elutriate in dark at 4ºC. Elutriate should be prepared for testing within 8 weeks of sample collection and used to initiate the test within 24 h of preparation. Elutriate should be stored for no longer than 7 d.
22.	Sediment volume required	100 mL minimum from each site sampled.

6.2.3. Organism Introduction

Fifty neonates less than 24 h old are required for an acute toxicity evaluation of 100 percent elutriate; 25 in the dredged material elutriate and 25 in the control water. A total of 150 neonates is required to start a test with five serial dilutions of the 100 percent elutriate and a control. Neonates should be randomly selected and distributed to the test chambers in a two-stage transfer process. Daphnids from the culture stock are randomly transferred into beakers containing dilution water which corresponds to each test group. The order of assignment is determined from a table of random numbers or another method of

random allocation. A second transfer is then made into beakers containing the appropriate experimental conditions. Beakers are then randomly placed in a water bath, or a controlled temperature incubator or room (Bentley et al. 1986).

6.2.4. Test Organism Monitoring

Immobilization and lethality are the endpoints in an acute test. Test organisms are observed at 24 and 48 h for complete immobilization. Complete immobilization is frequently used as an endpoint for toxicity tests with this species, resulting in an EC50 estimate. This endpoint includes those animals that are dead. Affected animals that are completely immobilized are observed to lie motionless on the bottoms of the test chambers, and do not respond to gentle prodding. Observations may be made with the use of a microscope. If survival data are desired for calculation of an LC50, the immobilized organisms should be examined for heartbeat using a dissecting microscope.

6.2.5. Water Quality Monitoring

Water quality should be carefully measured and documented for each test. Daily measurements of temperature, dissolved oxygen concentration and pH should be taken in each chamber. Hardness alkalinity and specific conductance should be measured once for the batch of water used in the test.

6.3. CHRONIC TEST

6.3.1. Elutriate Preparation (Chronic Test)

To obtain a sufficient volume of elutriate water for each week (i.e. 4.65 L) of a chronic test with *D. magna*, separately place three 500 mL subsamples of well-mixed dredged material into 3 clean 4 L containers. The dredged material may first be screened through a coarse-meshed sieve (e.g., 5 mm or No. 5 mesh) to remove large objects, such as rocks and sticks. At room temperature, add 2,000 mL of culture water (i.e., hard reconstituted water) into each container to produce a 1:4 volume ratio. Prepare the elutriate as indicated in Section 5.0. The supernatant should be centrifuged at 4,000 x g for 45 min, as in Ankley et al. (1990). Decant or siphon off the supernatant from each container and combine the supernatants for use directly as the 100 percent test site elutriate water. The required volumes of elutriate and dilution water for test initiation and each renewal of test solutions are indicated in Table G-3 for a 0.5 dilution factor. The elutriate should be stored in the dark at 4^{o}C until just prior to acclimation to test temperature and use in the test. It should not be stored for longer than 7 days.

Table G-3. Volumes (mL) of Dredged Material Elutriate and Dilution Water Required Per Renewal for the *Daphnia magna* 21-d Chronic Toxicity Test.

Percent Elutriate	Elutriate Volume Per Replicate	Dilution Water Volume Per Replicate	Total Elutriate Volume Required	Total Dilution Water Volume Required
100	80.0	0.0	800	0
50	40.0	40.0	400	400
25	20.0	60.0	200	600
12.5	10.0	70.0	100	700
6.25	5.0	75.0	50	750
0.0	0.0	80.0	0	800

6.3.2. Chronic Test Design

The basic design and conditions for performing a chronic elutriate toxicity test are given in Table G-4. The test consists of a series of 5 dilutions of dredged material elutriate and its performance control (culture water with no elutriate). Each of the experimental units in a definitive chronic test is replicated 10 times for a total of 60 exposure chambers per test site. A dilution factor of 0.5 or greater is used for determining the dilutions to be made from the full-strength (100-percent) test sediment elutriate.

Table G-4. Overview of Recommended Dredged Material Elutriate Test Conditions for the *Daphnia magna* 21-d Chronic Toxicity Test.

1.	Test type	Static with renewal
2.	Temperature (°C)	20±1°C
3.	Light quality	Fluorescent bulbs (wide spectrum)
4.	Light intensity	10-20 µE/m²/s,540-1080 lux or 50-100 ft-c (ambient laboratory levels)
5.	Photoperiod	16 h light, 8 h dark
6.	Test chamber size	100 mL
7.	Test solution volume	80 mL
8.	Renewal of test solutions	Days 2, 4, 7, 9, 11, 14, 16, and 18
9.	Age of test organisms	Less than 24 h

Table G-4 (continued)

10.	No. neonates per test chamber	1
11.	No. replicate test chambers per concentration	10
12.	No. neonates per test concentration	10
13.	Feeding regime	Feed three times weekly when solutions are renewed to result in final concentrations in each test chamber of 5 mg/L of trout chow suspension and 10^8 cells/L of *Selenastrum capricornutum*.
14.	Aeration	None or light aeration prior to test initiation if dissolved oxygen $\leq$90 percent.
15.	Dilution water	Hard reconstituted water of 160-180 mg/L as $CaCO_3$ hardness and a pH range of 6.8-8.5.
16.	Elutriate concentrations	Minimum of 5 test site elutriate concentrations with a performance control (water-only).
17.	Dilution factor	$\geq$0.5
18.	Water quality monitoring	Daily measurements of water temperature, dissolved oxygen and pH. Weekly measurements of hardness, alkalinity and specific conductance.
19.	Test duration	21 d
20.	Endpoints	Survival and reproduction.
21.	Test acceptability	80% or greater survival and an average of 60 or more young/surviving female in the water-only control solutions.
23.	Sediment volume required	4.5 L from each test site.

A randomized complete block design is used (see Figure G-2 for example). Neonates are added in a specific manner (see Section 6.3.6.), which allows for the performance of each female parent to be tracked.

A summary of daily activities prior to and during a test are presented in Attachment E. This schedule assumes that all materials are in hand, and that healthy cultures of *D. magna* and *S. capricornutum* are being maintained.

6.3.3. Test Chambers

In a chronic test, sixty 100 mL glass beakers are required for each test site including its performance control. Ten

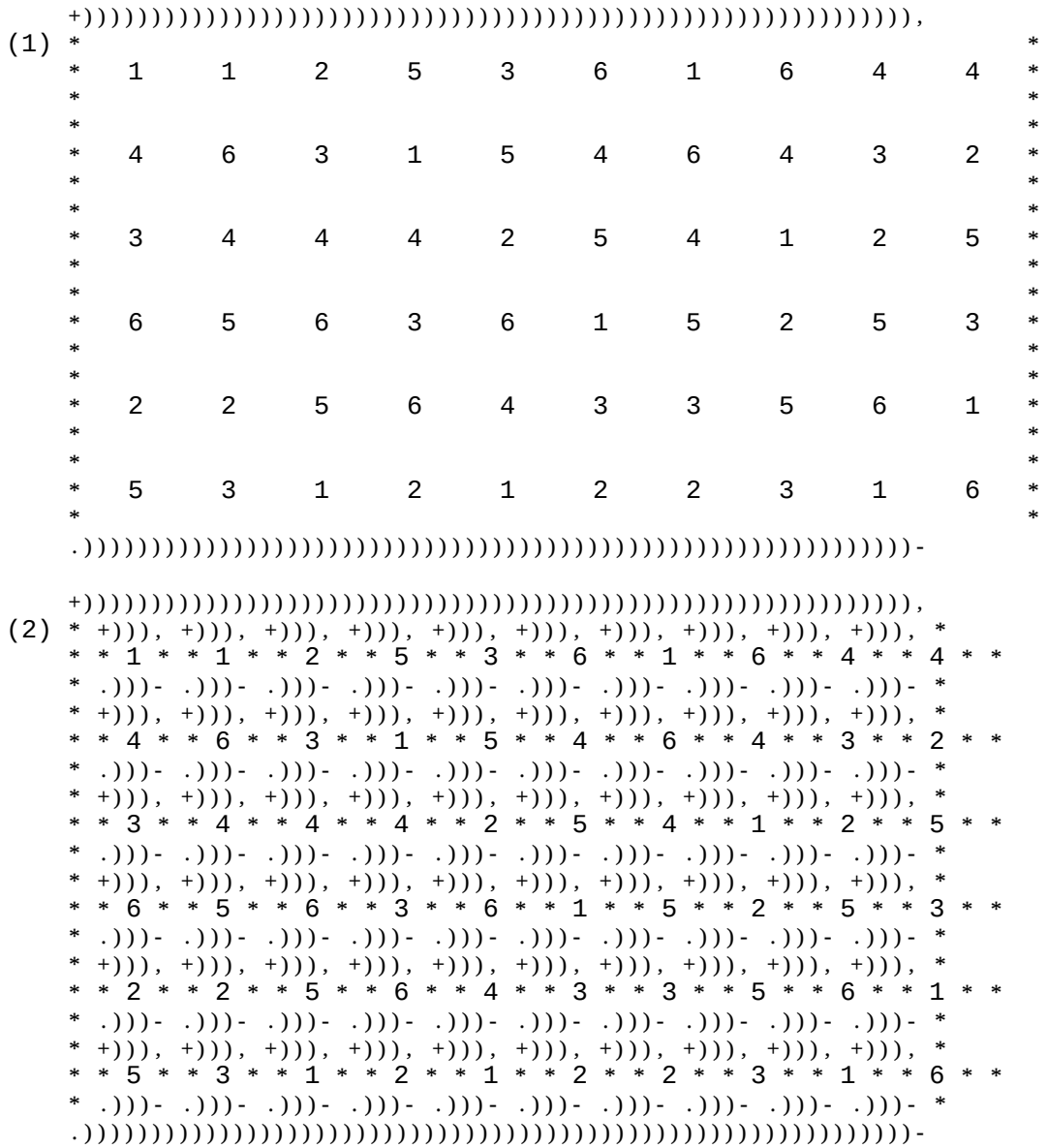


Figure G-2. Examples of a randomizing template (1) and a block-randomized arrangement of beakers for a toxicity test (2). The randomizing template is prepared from a random numbers table for each row. Each of the 10 rows contains one replicate of each treatment.

beakers are used for each of the 5 elutriate exposures and 10 for the performance control. All test beakers should be covered with a sheet of glass to reduce evaporation of water.

6.3.4. Water Renewal

All test chambers must receive a renewal of elutriate or control diluent three times weekly, or on days 0, 2, 4, 7, 9, 11, 14, 16, and 18. The renewal elutriate for the first week of the test should be made at the same time as the elutriate for test initiation, and should be stored in the dark at 4° C. Similar volumes will need to be prepared at the start of the second and third weeks. The diluent for preparation of the elutriate test solution is culture water (control diluent). Adult organisms are gently transferred by pipet to freshly prepared test solutions at each renewal. The renewal solutions should be allowed sufficient time to equilibrate to the desired test temperature prior to transfer of the animals.

6.3.5. Temperature and Photoperiod

Tests should be performed in a temperature-controlled unit at 20 ± 1°C. The daily photoperiod should be 16 L: 8 D.

6.3.6. Organism Introduction

A total of 60 neonates less than 24 h old is required to start the test. Neonates are taken from the pre-test culture adults that have 9 or more young in their fourth or subsequent broods, and are less than 21 d old. Ten brood cups, each with 9 or more young, are selected from the pre-test culture for the test. Into a block-randomized arrangement of test beakers (Figure G-2), from one beaker pipet one neonate into each of the 6 beakers representing one complete subset (i.e. performance control, and five elutriate dilutions)of the 10 replicates. It is recommended that several randomized templates be prepared in advance that can be alternated for use. Discard the additional neonates from that beaker. Repeat this process 9 times for the test site dredged material sample, using neonates from a new pre-test culture beaker for each of the 6 bioassay chambers in a replicate.

6.3.7. Food and Feeding

Three times weekly, all test chambers receive an allotment of food resulting in a final concentration 5 mg/L dry wt. of trout chow suspension and 10^8 cells/L of *S. capricornutum* (see Attachment C for food preparation).

6.3.8. Test Organism Monitoring

Test organisms are observed daily for survival and young production. Organisms also are observed for their behavior. Any abnormal behavior (e.g., rapid or slow swimming, spinning) in adults and offspring should be recorded. Observations are generally made without the aid of a microscope. A light box may be used for illuminating the young animals during examination. To aid in counting live young on renewal days, a few drops of 1N HCl added to the chamber after the adult has been transferred to

new media will kill the young and result in their settling to the bottom.

6.3.9. Water Quality Monitoring

Water temperature should be monitored daily in at least five exposure chambers (four corners and center of chamber array). At the time the water temperature is measured, the temperature reading for a continuously recording monitor in the constant temperature environment should be observed. Dissolved oxygen and pH should be measured daily in a control chamber (C) and in one of the low (L), medium (M) and high (H) exposure chambers (Attachment D, Form D2). At the time of renewal (days 2, 4, 7, 9, 11, 14, 16, and 18), these parameters should be measured in both "old" and "new" solutions. Hardness, alkalinity and specific conductance should be measured in the initial control (or culture) water and the low, medium and high elutriate concentrations at the start of the test and on days 7, 14 and 21.

Preferred ranges for water quality characteristics are presented in Table G-5. If temperature and dissolved oxygen of renewal water are outside of the preferred ranges, the water should be handled accordingly to adjust these characteristics so they fall within the preferred ranges. If pH, hardness and alkalinity of renewal dilution water are not within the preferred ranges, a new batch of renewal dilution water should be prepared. All water quality characteristics for the new batch must fall within the preferred ranges before the renewal dilution water is used.

Table G-5. Preferred Means and Ranges for Water Quality Characteristics in the *Daphnia magna* 21-d Toxicity Test.

Characteristic	Preferred Mean	Preferred Range
Temperature (°C)	20	19-21
Dissolved Oxygen (% of Saturation)	>75	50-100
pH	7.8	6.8-8.5[a]
Hardness (mg/L as $CaCO_3$)	170	160-180
Alkalinity (mg/L as $CaCO_3$)	115	110-120

[a] pH adjustments using NaOH or HCl are necessary if the test solution pH is less than 6.8 or greater than 8.5 (Biesinger 1987).

Individual measurements of water quality parameters that fall outside of the preferred ranges do not necessarily stop or invalidate the test. The test will be invalidated only if the

departures from preferred water quality values are sufficiently great or of sufficient frequency to prevent conformance with the quality assurance criteria indicated in Section 4.4.2. of this Appendix.

6.3.10. Test Termination

The test is terminated on day 21. Adult survival and young production are recorded as on previous days, and summed for the duration of the test (see Attachment D for sample data forms).

Four to six broods totaling 60 to >100 young are commonly obtained in a 21-d test at 20 ± 1°C. The first brood may be expected on d 7 to 10 with subsequent broods produced every 2-3 d. If a brood is being released at the time of transfer, and partial broods are released in each of the old and new media, consider it a single brood (USEPA 1993).

6.4. *Data Reporting and Statistical Analysis*

See Section 12.

7.0. *Ceriodaphnia dubia* **WATER COLUMN TOXICITY TESTS**

Ceriodaphnia dubia, a freshwater cladoceran in the family Daphniidae, is widely distributed in North America and Europe (Berner 1986). It is considerably smaller in size than some of the other commonly tested daphnids, such as *Daphnia magna,* and has a shorter life-cycle. Under optimal conditions, 3-4 broods may be produced parthenogenetically in one week (Berner 1986). Its utility as a test species was described by Mount and Norberg (1984), and it is routinely used in effluent biomonitoring programs (USEPA 1993). *C. dubia* has been used in acute toxicity tests to evaluate elutriates (Ankley et al. 1991b), and chronic tests with this species have been used in recent years to conduct evaluations of comparative chemical toxicity (Cowgill et al. 1985, Winner 1988, Cowgill and Milazzo 1991, Oris et al. 1991), of stream water quality (Burton et al. 1987, Nimmo et al. 1989), of the toxicity of sediment pore waters (Adams et al. 1986, Ankley et al. 1990), and of elutriates (Hoke et al. 1990). This report describes the methods used to culture *C. dubia* and to perform acute and chronic toxicity tests of dredged material elutriates in static test systems.

7.1. CULTURE METHODS

The culturing methods described below are largely taken from methods described by EPA (USEPA 1993) and ASTM (ASTM 1993c), and from Standard Operating Procedures at the USEPA National Effluent Toxicity Assessment Center, Environmental Research Laboratory-Duluth, Duluth, MN. Required materials are listed in

Attachment F.

7.1.1. Organism Source

Organisms for the initiation of a laboratory culture may be obtained from a source which has a verified culture of *Ceriodaphnia dubia*. Verification should be made by a recognized taxonomist. Starter cultures are available from either the USEPA Aquatic Biology Branch, Quality Assurance Research Division, EMSL-Cincinnati Newtown Facility, 3411 Church Street, Newtown, OH 45244 (513-533-8114); or the USEPA National Effluent Toxicity Assessment Center, Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, MN 55804 (218-720-5529).

7.1.2. Acclimation of New Brood Stock

Environmental stress on the organisms in the starter culture must be minimized to facilitate normal culture growth and brood production. The temperature of the water containing the stock animals should be measured upon their arrival and gradually adjusted to the desired culture temperature of 25±1°C. Changes in temperature >3°C per any 12-hr period should be avoided (ASTM 1993c). Instantaneous pH changes >0.5 units have been found to cause mortalities (Mount and Norberg 1984). Mount and Norberg (1984) tentatively recommended that instantaneous pH changes should not exceed 0.2 units. If the new culture medium is different from the medium used in the laboratory from which the starter culture was obtained, animals from the starter culture should be transferred to the new culture medium gradually over a period two or more days to avoid mass mortality (ASTM 1993c). To accomplish a gradual change, it is important to know the basic chemical characteristics (i.e. pH, hardness, alkalinity) of the water used by the laboratory from which the culture was received, and to incrementally dilute that water with the culture water to be employed over two or more days. This is continued until the water essentially consists of 100 percent desired culture water. In preparation for a three-brood toxicity test, it is recommended (ASTM 1993c) that two (and preferably five) generations be raised using the same water, food and temperature as will be used in the test.

7.1.3. Reference Organism

It is recommended (USEPA 1993) that a new laboratory culture be started with a single animal, which is sacrificed after producing young. It should be permanently retained on a microscope slide for future reference. Procedures for making slide mounts according to the method of Beckett and Lewis (1982) are available in USEPA (1993). Once the stock culture has arrived from the supplier and neonates are being produced, adults should be separated and one offspring from a large brood should be selected as the source of the new laboratory culture. This organism should be from a third (or later) brood of at least eight neonates, as in the selection of neonates for conducting

toxicity tests. This will indicate that the culture has originated from a genetic line capable of a high level of reproduction. The remaining starter culture animals may be maintained as a back-up mass culture.

7.1.4. Culture Chambers

Chambers for mass culturing may be large crystallizing dishes, battery jars or aquaria containing 1 to 2 L of culture water for 40 to 80 adults, respectively. Chambers for individualized culturing are new 30 mL (1 oz) polystyrene souffle cups or 30 mL glass beakers containing 15 ml of culture water. Double-strength safety glass or 6 mm plastic panels may be used as cover material for brood board or mass cultures.

7.1.5. Culture Water

Moderately hard synthetic water made with deionized or Millipore Milli-Q^{R} water and reagent grade chemicals or 20 percent diluted mineral water (DMW) are recommended as culture water. Methods for their preparation are given in Attachment G.

Culture water is renewed a minimum of three times weekly (typically Monday, Wednesday and Friday) in both mass and individual cultures. Each batch of renewal water should be monitored for temperature, dissolved oxygen, pH, conductivity, alkalinity and hardness, and the measurements recorded in a culture maintenance logbook. Water should not be used if it is more than one month old.

7.1.6. Temperature and Photoperiod

The temperature for culturing *C. dubia* should be maintained at 25±1^{o}C. A photoperiod of 16 h light and 8 h dark is recommended.

7.1.7. Food and Feeding

A diet that has been used successfully by EPA consists of a combination of yeast, Cerophyll^{R} and trout chow (i.e., the YCT diet,) supplemented with the unicellular green alga, *Selenastrum capricornutum* (USEPA 1973). Methods of preparing the YCT diet and of culturing and concentrating *Selenastrum* are given in Attachment H.

Mass cultures receive 7 mL YCT and 7 mL of algal concentrate per L of culture medium daily. Individual culture vessels receive 0.10 mL YCT and 0.10 mL of algal concentrate per 15 mL of culture medium daily. The YCT mixture must be measured for solids content. The dry weight of solids in a YCT batch should be 1.7-1.9 g/L, resulting in culture or test solution dry solid weights of 12-13 mg/L. Dry weight of solids in each batch of YCT diet is determined by oven-drying duplicate 5 mL samples of well-mixed YCT suspension in oven-dried pre-weighed weigh pans. The

mean dry weight per 5 mL is converted to a lL volume, and the resultant concentration of suspended solids is checked to see that it falls within the range of 1.7-1.9 g/L. The algal concentrate cell density must be measured, and contain between 3.0×10^7 and 3.7×10^7 cells/mL.

The unused YCT and algal concentrate should be refrigerated after use. The YCT should not be refrozen, and any unused portion of YCT should be discarded after two weeks. Algal concentrates should be discarded after one month.

7.1.8. Handling

A fire-polished pipet[1] of 2 mm bore diameter is recommended for transferring animals. Care should be exercised to avoid injury to the daphnids during transfer and to ensure that the neonates are introduced to the chambers below the surface of the water. At the time of culture medium renewal, organisms are transferred to the new medium, and food is added either immediately before or after the transfer.

7.1.9. Culture Maintenance-Mass Cultures

Animals (approximately 40 neonates <48 h old) are placed into the culture water on d 0 (typically a Friday). Culture water is renewed on d 3, 5 and 7. At the time of each water renewal, adult survival is recorded, and the offspring and old medium are discarded. On d 7 a new culture is started. The first culture is renewed on d 10 and 12 of the second week, and on d 14 the adults are discarded. A new culture is started with neonates from 7-d old adults. Mass cultures of overlapping ages are recommended to ensure the availability of animals should one or more cultures be lost due to a reduction in the quality of food or water.

7.1.10. Culture Maintenance-Brood Board Cultures

Neonates that are to be used in toxicity tests must be obtained from females individually cultured in brood boards. On d 0, one neonate *C. dubia* ($\leq$24 h old) is pipetted into each of 60 culture chambers contained in a brood board (Figure G-3). When a new brood board is started, the board should be labeled with initiation date, water type, initial animal age, and generation of adults that the young were collected from. Neonates selected for use in testing must be from the third (or later) brood of a female, and the brood should contain at least eight neonates. The medium is renewed and organisms transferred to fresh medium on d 3, 5 and 7. On d 6, chambers with no young present are

[1] Pipettes are stored in 100 percent methanol (which is replaced weekly) and are rinsed three times with tap and distilled water prior to use.

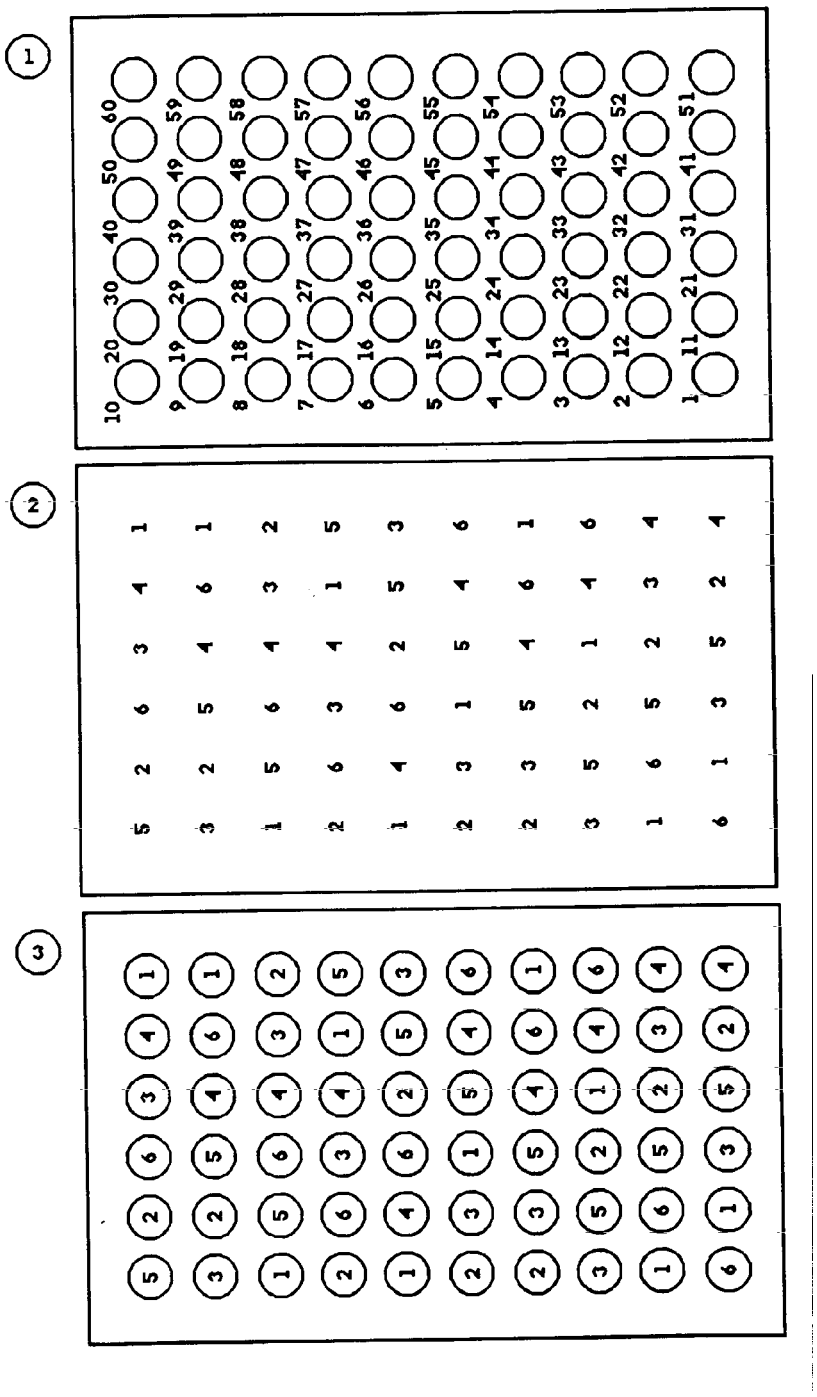


Figure G-3. Examples of a culture brood board, randomizing template, and block-randomized test board: (1) brood board with positions for six columns of ten replicate test chambers, (2) randomizing template prepared from a random numbers table for each row, and (3) randomized test board developed from (1) and (2) for the purpose of assigning treatment positions within each row on the board.

clearly marked with a black marking pen. On d 7, a new culture is started on a second brood board from broods of 10-20 neonates in the marked containers. If young are lacking in the marked cups, the second brood of young from unmarked cups may be used. If necessary, due to insufficient neonate production, new brood boards may be started with neonates from adults which produce 6-10 young in their third or fourth brood. These adults may serve as a source of neonates until they are 14 days old, at which time the adults are discarded. During the second week, the medium is renewed, adults transferred, and offspring discarded unless needed on days 10 and 12.

Two brood boards of individual cultures, each 7 days apart, should provide for a supply of organisms to initiate cultures and tests. Cultures which are well maintained and in good health will produce at least 15 young per adult in three broods (~6-8 days). A culture of this size (i.e. 60 individuals per brood board) should produce more than the minimum number of neonates needed (120) for two acute tests with five elutriate dilutions (150 neonates) or two chronic tests (120 neonates) weekly. Fifty neonates will be required for an acute toxicity test with the 100 percent elutriate only and a performacne control. Each chronic test will require 60 neonates for five elutriate concentrations of the test sediment and a performance control.

7.1.11. Culture Evaluation

Cultures are observed daily at the time of renewal and/or feeding. Test temperature is measured and recorded daily in the middle and four corner cups. Individual cultures should produce a minimum of 15 neonates each week. Production is determined monthly by randomly selecting 10 females from a brood board, and counting the young produced in the first three broods (within 7 days). If brood size and total young production in the cultures is less than 15 neonates for three broods in 60 percent of these 10 animals or overall survival is <80 percent (USEPA NETAC 1990; USEPA 1993), culturing conditions should be scrutinized and adjustments made to increase production.

Any males that may be present either in the mass cultures or the individual cultures should be removed on d 3. They are distinguished from females at this age by their smaller size, lighter color, and different body shape, having a more elliptical shape than the females (Mount and Norberg 1984). Distinguishing features of adult males include a broad cervical notch, large eye, long and cylindrical antennule with a terminal male seta 1.5 times the length of the antennule which terminates in a curved hook, and a clasper on the second thoracic appendage which is long and thin, curving to a small terminal hook (Berner 1986). Illustrations (Figure G-4) of female and male organisms are from Berner (1986).

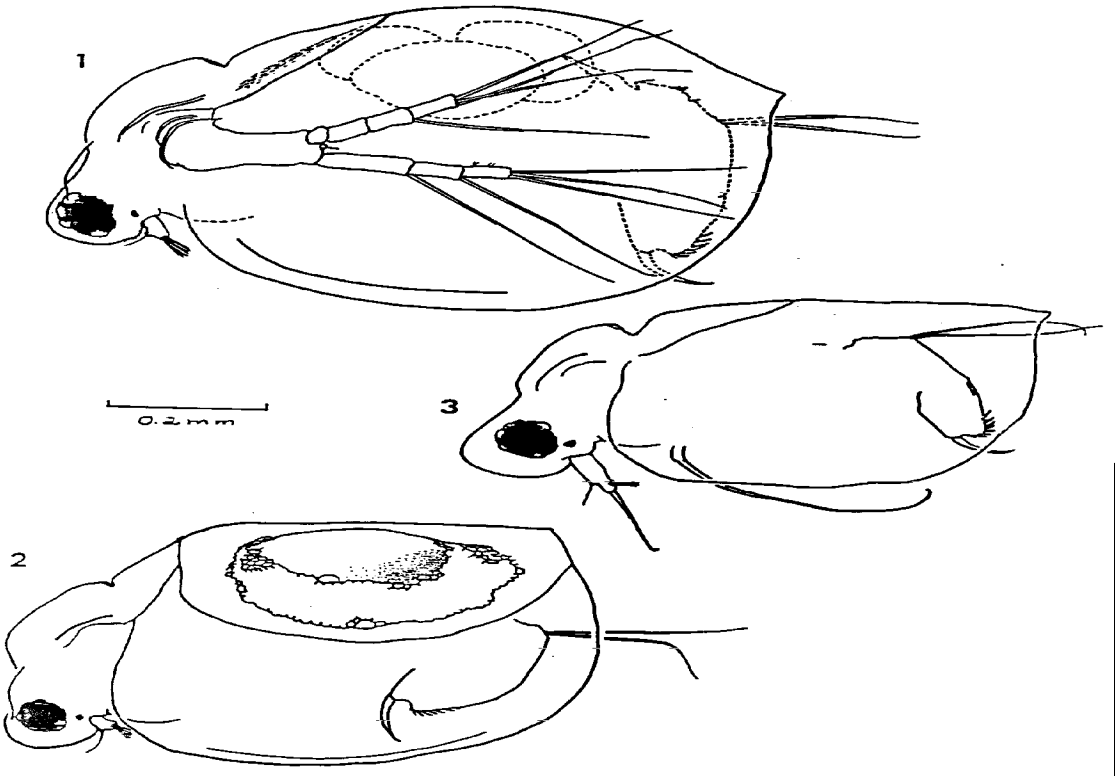


Figure G-4. *Ceriodaphnia dubia*: 1. parthenogenetic female; 2.sexual (ephippial) female; 3. male.

7.1.12. Culture Records

A separate set of record books should be maintained for the culture unit. Sample culture record forms are provided in Attachment I (Form I1). Records must be kept on the survival of brood organisms, in both the mass and individual cultures and of production of young in individual cultures. Adult mortality in excess of 20 percent or production of fewer than 15 young per three broods in 60 percent of the adults per week in a brood board are indicative of problems such as poor water or food quality. Organisms from this board should not be used in elutriate toxicity tests.

At the time of each culture medium renewal, the following information is entered into the culture record book: date, water type, culture water preparation date, preparation dates of YCT diet and *Selenastrum, Ceriodaphnia* age, animal appearance, and percent survival.

Records are maintained on each batch of culture water. Information on preparation date, pH, alkalinity, hardness, conductivity, and the last Millipore^{R} filter change is recorded in the culture log book. A record is also maintained on daily measurements of temperature in the culture unit.

7.2. ACUTE TEST

7.2.1. Elutriate Preparation (Acute Test)

The GLTEM currently recommends that acute exposures of 48 h duration be performed for routine tier 3 testing. Chronic tests (discussed in Section 7.3) may be used for tier 4 testing, if needed. The culturing of organisms is the same for both acute and chronic tests. Animals used to start an acute test are of the same age (i.e., $\leq$24 h old) and are handled the same way as in a chronic test. The same general test conditions of temperature, lighting and dilution water characteristics apply to both acute and chronic tests.

To obtain a sufficient volume of elutriate for an acute test (i.e., ~120 mL), place 40 mL of well-mixed sediment into a clean 500 mL beaker and add 160 mL of dilution water (same as culture water), and follow the elutriate preparation procedure described in section 5.0. The GLTEM recommends that the acute test first be performed with the 100 percent elutriate, and followed by a dilution series only if survival in the 100 percent elutriate is less than 50 percent. Table G-6 provides a summary of the volumes of elutriate and dilution water required in an acute test with five different elutriate concentrations and a water-only control using a 0.5 dilution level.

Table G-6. Volumes (mL) of Dredged Material Elutriate and Dilution Water Required per Renewal for the *Ceriodaphnia dubia* 7-d Acute Toxicity Test.

Percent Elutriate	Elutriate Volume Per Replicate	Dilution Water Volume Per Replicate	Total Elutriate Volume Required	Total Dilution Water Volume Required
100	15.0	0.0	75.0	0
50	7.5	7.5	37.5	37.5
25	3.8	11.2	19.0	56.0
12.5	1.9	13.1	9.5	65.5
6.25	0.95	14.1	4.8	70.5
0.0	0.0	15.0	0	75.0

7.2.2. Acute Test Design

The test methods follow standard procedures for measuring the acute toxicity of effluents and receiving waters (USEPA, 1991). The basic design and conditions for performing a 48 h acute toxicity test are given in Table G-7. The test is performed with neonates (<24 h old) which have been provided with food for a minimum of 2 h during the holding period prior to test initiation. The animals are not fed during the test.

Table G-7. Overview of Recommended Dredged Material Elutriate Test Conditions for the 48-h *Ceriodaphnia dubia* Acute Toxicity Test.

1.	Test type	Static or static-renewal
2.	Temperature (°C)	25±1°C
3.	Light quality	Fluorescent bulbs (wide spectrum)
4.	Light intensity	10-20 µE/m^2/s, 540-1080 lux or 50-100 ft-c (ambient laboratory levels)
5.	Photoperiod	16 h light, 8 h dark
6.	Test chamber size	30 mL
7.	Test solution volume	15 mL
8.	Renewal of test solutions	None (if dissolved oxygen is adequate) or at 24 h

Table G-7 (continued)

9.	Age of test organisms	Less than 24 h
10.	No. neonates per test chamber	5
11.	No. replicate test chambers per concentration	5 minimum
12.	No. neonates per test concentration	25 minimum
13.	Feeding regime	Do not feed during test. Feed 0.1 mL each of YCT and *Selenastrum capricornutum* suspension per holding chamber prior to test initiation so that young have food available for a minimum of 2 h prior to test initiation.
14.	Aeration	None during exposure; elutriate may be aerated before renewal if dissolved oxygen is low (i.e., ≤50% of saturation).
15.	Dilution water	Moderately hard synthetic water prepared using either (1) Millipore Milli-Q^{R} (or equivalent) deionized water and reagent grade chemicals or (2) 20% DMW.
16.	Elutriate concentrations	Minimum of 5 test site elutriate concentrations with a performance control (water only).
17.	Dilution factor	≥0.5
18.	Water quality monitoring	Daily measurements of water temperature, dissolved oxygen and pH. Single measurements of hardness, alkalinity and specific conductance.
19.	Test duration	48 h
20.	Endpoints	Survival and complete immobilization
21.	Test acceptability	90% or greater survival in the water-only control solutions.
22.	Sample requirements	Storage of dredged material is at 4°C in the dark. Elutriate water should be prepared for tests within 8 weeks of collection.
23.	Dredged material volume required	40 mL from each test site

7.2.3. Organism Introduction

Fifty neonates less than 24 h old are required to start an acute test with 100 percent elutriate and a performance control. A total of 150 neonates is required to start an acute test with 5 elutriate concentrations and a performance control. Neonates should be randomly selected and distributed to the test chambers as described in Section 6.2.3. for *Daphnia magna*. Neonates should be from broods of at least 8 young. Organisms should be transferred from the culture chambers to the test chambers with a widemouth pipette with an opening of approximately 4 mm diameter. The tip of the pipette should be kept under the surface of the water to prevent air from being trapped under the carapace (USEPA 1993).

7.2.4. Test Organism Monitoring

Immobilization and lethality are the endpoints in an acute test. Test organisms are observed at 24 and 48 h for complete immobilization. Complete immobilization is frequently used as an endpoint for toxicity tests with this species, resulting in an EC50 estimate. This endpoint includes those animals that are dead. Affected animals that are completely immobilized are observed to lie motionless on the bottoms of the test chambers, and do not respond to gentle prodding. A microscope may be desirable for determining this endpoint. If survival data and the calculation of an LC50 are desired, the immobilized organisms should be examined for heartbeat using a dissection microscope.

7.2.5. Water Quality Monitoring

Water quality should be carefully measured and documented for each test. Daily measurements of temperature, dissolved oxygen concentration and pH should be taken in each chamber. Hardness, alkalinity and specific conductance should be measured once for the batch of water used in the test.

7.3. CHRONIC TEST

7.3.1. Chronic Test Design

The basic design and conditions for performing a chronic toxicity test are given in Table G-8. The test consists of a series of 5 dilutions of the test site dredged material elutriate and its performance control (culture water with no elutriate). Each of these experimental units is replicated 10 times, for a total of 60 exposure chambers. A dilution factor of 0.5 or greater is used for determining the dilutions to be made from the full-strength (100 percent) test sediment elutriate. Table G-9 is an example of a 0.5 dilution factor. A randomized complete block design is used (see Figure G-3 for example of randomization of test board). Neonates are added in a specific manner (see Section 7.3.5.) which allows for the performance of each female parent to be tracked.

Table G-8. Overview of Recommended Dredged Material Elutriate Test Conditions for the *Ceriodaphnia dubia* 7-d Chronic Toxicity Test.

1.	Test type	Static renewal
2.	Temperature (°C)	25±1°C
3.	Light quality	Fluorescent bulbs (wide spectrum)
4.	Light intensity	10-20 µE/m^2/s, 540-1080 lux or 50-100 ft-c (ambient laboratory levels)
5.	Photoperiod	16 h light, 8 h dark
6.	Test chamber size	30 mL
7.	Test solution volume	15 mL
8.	Renewal of test solutions	Days 3 and 5
9.	Age of test organisms	Less than 24 h; and all released within an 8-h period
10.	No. neonates per test chamber	1
11.	No. replicate test chambers per concentration	10
12.	No. neonates per test concentration	10
13.	Feeding regime	Feed 0.1 mL each of YCT and *Selenastrum capricornutum* suspension per test chamber daily
14.	Aeration	None during exposure; elutriate may be aerated before renewal if dissolved oxygen is low (i.e., ≤50% of saturation)
15.	Dilution water	Moderately hard synthetic water prepared using either Millipore Milli-Q^R (or equivalent) deionized water and reagent grade chemicals or 20% DMW
16.	Elutriate concentrations	Minimum of 5 test site elutriate concentrations with a performance control (water only)
17.	Dilution factor	≥0.5
18.	Water quality monitoring	Daily measurements of water temperature, dissolved oxygen and pH. Hardness, alkalinity and specific conductance are measured at the beginning and end of test.

Table G-8 (continued)

19.	Test duration	Until 60% of control females have three broods (may require more or less than 7 days)
20.	Endpoints	Survival and reproduction
21.	Test acceptability	80% or greater survival and an average of 15 or more young/surviving female in the control solutions. At least 60% of surviving females in controls should have produced their third brood.
22.	Sample requirements	Storage of dredged material is at 4°C. Elutriate water should be prepared for tests within 8 weeks of collection.
23.	Dredged material volume required	300 mL from each test site

Table G-9. Volumes (mL) of Dredged Material Elutriate and Dilution Water Required per Renewal for the *Ceriodaphnia dubia* 7-d Chronic Toxicity Test.

Percent Elutriate	Elutriate Volume Per Replicate	Dilution Water Volume Per Replicate	Total Elutriate Volume Required	Total Dilution Water Volume Required
100	15.0	0.0	150	0
50	7.5	7.5	75	75
25	3.8	11.2	38	112
12.5	1.9	13.1	19	131
6.25	0.95	14.1	9	141
0.0	0.0	15.0	0	150

A summary of daily activities prior to and during a test is presented in Attachment J. This schedule assumes that all materials are in hand, and that a healthy culture of organisms is being maintained.

7.3.2. Test Chambers

For a chronic test, sixty new polystyrene 1 oz souffle cups (30 mL) or 30 mL glass beakers are required for each test site and its performance control. Test Chambers may be reused for renewals in a test, provided they are used at the same exposure levels. Polystyrene cups should be discarded after the test.

7.3.3. Water Renewal

All test chambers must receive a renewal of elutriate or

control diluent on d 3 and 5. The renewal elutriates for the test sediment should be the same as the initial elutriate, having been stored in the dark at 4°C. The diluent for preparation of elutriate test solution is culture water (control diluent). The renewal solutions should be warmed to the test temperature of 25±1°C prior to transfer of test organisms.

7.3.4. Temperature and Photoperiod

Tests should be performed in a temperature-controlled unit at 25±1°C. The daily photoperiod should be 16 L: 8 D.

7.3.5. Organism Introduction

A total of 60 neonates less than 24 h old, and all within 8 h of the same age, is required to start the test. Neonates are taken from the individual culture brood boards from adults that have 8 or more young in their third or subsequent broods.

Ten brood cups, each with 8 or more young, are selected from a brood board for the test. On a block-randomized brood board (prepare several randomized templates in advance that can be alternated for use), pipet from one cup one neonate into each of the 6 cups representing one complete subset (i.e. performance control, and five elutriate dilutions) of the 10 replicates. Discard the additional neonates from that brood cup. Repeat this process 9 times for the test site dredged material sample, using neonates from a new brood cup for each of the 6 bioassay chambers in a given row.

7.3.6. Food and Feeding

All test chambers receive a daily allotment of 100 uL of YCT diet and 100 μL of *Selenastrum* per 15 mL of medium (see Attachment I for food preparation). At the time of water renewal, (d 3 and 5), food should be added immediately prior to or after organism transfer.

7.3.7. Test Organism Monitoring

Test organisms are observed daily for survival and young production. Organisms also are observed for their behavior. Any abnormal behavior (e.g. rapid or slow swimming, spinning) in adults and offspring should be recorded. Observations are made using a dissecting microscope with substage lighting. A light box may be used for illuminating the animals during examination. To aid in counting live young on d 3 and 5, 2 drops of 1N HCl added to the chamber after the adult has been transferred to new media will kill the young and result in their settling to the bottom.

7.3.8. Water Quality Monitoring

Water temperature should be monitored daily in at least five locations on the test board (four corners and center). Dissolved

oxygen and pH should be measured daily in a control chamber (C) and in one of the low (L) medium (M) and high (H) exposure chambers (Attachment I, Form I2). At the time of renewal (d 3 and 5), these parameters should be measured in both "old" and "new" solutions. Hardness, alkalinity and specific conductance should be measured in the initial control (or culture) water and the low, medium and high elutriate concentrations at the start of the test and at termination on d 7 or 8. Preferred ranges for water quality parameters are presented in Table G-10.

7.3.9. Test Termination

The test is terminated when 60 percent of the control females have had three broods or at the end of d 8, whichever occurs first. Adult survival and young production are recorded as on previous days, and summed for the duration of the test (see Attachment I for sample data forms). Three broods totaling 20-35 young are commonly obtained in a 7-d test at 25 ± 1°C. The first brood may be expected on d 3 or 4, and typically consists of two to five young. The second and third broods are released from 36 to 48 h after the first brood, and typically consist of 8 to 20 young. If a brood is being released at the time of transfer, and partial broods are released in each of the old and new media, consider it a single brood (USEPA 1993).

Table G-10. Preferred Means and Ranges for Water Quality Parameters in the *Ceriodaphnia dubia* 7-d Chronic Toxicity Test.

Parameter	Preferred Mean	Preferred Range
Temperature (°C)	25	24-26
Dissolved Oxygen (% of saturation)	>75	50-100
pH	7.6[a], 8.1[b]	7.4-7.8[a], 7.9-8.3[b]
Hardness (mg/L as $CaC0_3$)	90	80-100
Alkalinity (mg/L as $CaC0_3$)	65	60-70

[a] Mean and range for moderately hard water prepared with reagent grade chemicals.

[b] Mean and range for moderately hard water prepared by addition of mineral water.

7.4. Data Reporting and Statistical Analysis
See Section 12.

8.0. *Pimephales promelas* **WATER COLUMN TOXICITY TESTS**

The fathead minnow, *Pimephales promelas* Rafinesque (Figure G-5), is a freshwater fish included in the family Cyprinidae. This species was chosen as a test organism for several reasons: (1) ease of culturing and testing, (2) widespread occurrence, (3) rapid growth, (4) ecological importance, and (5) sensitivity to a variety of environmental pollutants. Fathead minnows are omnivorous feeders which eat a variety of plant and animal life (Devine 1968, Held and Peterka 1974). They are tolerant of a wide range of physical and natural chemical conditions. Adults can attain a total length of 90-101 mm and mature to reproduction in 90 to 120 days under ideal conditions. Spawning may be impacted at extremes of pH but has been successful at pH values ranging from 5.9 (Mount 1973) to 9.5 (McCarraher and Thomas 1968). The species is tolerant of water temperatures ranging from 2 to 33°C (Bardach et al. 1966) and spawns successfully in the temperature range of 15.6 to 29.8°C (Brungs 1971). Fathead minnows tolerate high total alkalinity concentrations of up to 1,800 mg/L as $CaCO_3$ (McCarraher and Thomas 1968) and turbidity as high as 15,000 mg/L total solids (Rawson and Moore 1944).

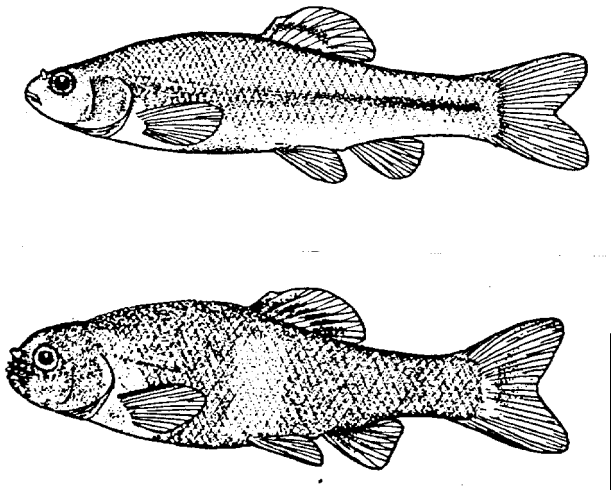


Figure G-5. Mature female and male fathead minnow, *Pimephales promelas*, approximately life-sized (From Eddy and Underhill 1974).

A large database of acute and chronic toxicity information is available for the fathead minnow (e.g., Brooke et al. 1984,

Geiger et al. 1985, 1986, 1988, 1990, Mayer and Ellersieck 1986). These studies indicate that the fathead minnow is sensitive to low levels of most industrial organic chemicals, pesticides, and metals. Fathead minnows are also extremely sensitive to ammonia (Ankley et al. 1990) and hydrogen sulfide (Smith et al. 1976), both common toxicants in sediments.

This report describes the methods used to culture fathead minnows and to perform acute and subchronic toxicity tests of larvae in static test systems with regular renewal of the elutriate. The endpoint in a 4-d acute test is survival. Endpoints in the 7-d fathead minnow toxicity test include both survival and growth.

The use of growth as a routinely monitored endpoint in chronic toxicity tests with fish was first suggested by Sprague (1971) because it serves as an integrator of all sublethal effects. Growth is the result of a suite of physiological and behavioral interactions which may be adversely affected by chemical contaminants in sediments, the water column or food (McKim 1977). If fish experience a decreased growth rate, the larval-juvenile period (the period of highest mortality for fish) will be extended. The probability of mortality due to predation also will be increased, because the period of greatest susceptibility to predation is extended (Werner and Hall 1974; Werner and Gilliam 1984; Post and Prankevicius 1987). Thus, minor reductions in growth can result in significant reductions of survival and recruitment of larval fish to adult populations, and subsequent population level impacts (May 1971; Laurence 1974; Werner and Blaxter 1980: Leiby 1984; Adams and DeAngelis 1987). With specific regard to growth as an endpoint in the 7-d fathead minnow embryo-larval test, a number of studies have demonstrated a significant correlation between growth reduction and different synoptic measures of impact upon aquatic community structure (Mount et al. 1984; Mount et al. 1985; Norberg-King and Mount 1986; Birge et al. 1989; Dickson et al. 1990).

8.1. CULTURE METHODS

The culturing methods described in this report are based on the methods recommended by USEPA (Denny 1987, USEPA 1993), and ASTM (1993a, 1993f). Required materials are listed in Attachment K. Fathead minnow embryos and larvae for use in testing are available from a variety of commercial sources.

8.1.1. Organism Source

Organisms for the initiation of a laboratory culture should be obtained from a source which has a verified culture of *P. promelas*. Fish that are adapted to laboratory conditions and free of disease must be used as the brood stock. Embryos make the best initial stock of fish because they are easiest to

transport and most likely to be free of disease. Stock from wild populations should be avoided unless cultured through at least one generation to ensure they are disease-free and of adequate vigor (Denny 1987). Starter cultures are available from the USEPA Aquatic Biology Branch, Quality Assurance Research Division, EMSL-Cincinnati Newtown Facility, 3411 Church Street, Newtown, OH 45244 (Telephone: 513-533-8114); the USEPA Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, MN 55804 (Telephone: 218-720-5500); or any of several commercial suppliers with species verified stocks.

8.1.2. Acclimation

Environmental stress on the organisms in the starter culture must be minimized to facilitate normal growth and embryo production. The temperature of the water containing the brood stock animals should be measured upon their arrival and gradually adjusted to the desired culture temperature of 25±1°C. Changes in water temperature >3°C in any 12-h period should be avoided and, in general, water temperature should not change more than 3°C in a 72-h period (ASTM 1993f). The dissolved oxygen concentration should be maintained between 60 and 100% of saturation. Gentle aeration with oil-free compressed room air is desirable. Supersaturation by dissolved gases should be avoided to prevent gas bubble disease. If the culture medium differs in hardness, alkalinity or pH from that in which the organisms were received, animals from the starter culture should be transferred to the new culture medium gradually over a period of 1 to 2 days to avoid stressing the organisms (ASTM 1993f).

Reproductively mature (≥120-d old when cultured at 25°C) individuals must be segregated into breeding groups to successfully produce embryos. When sexes can be distinguished (Denny 1987), a male is placed with one or two females and a spawning substrate. This sex ratio accelerates the onset of spawning activity. If misidentification of sexes occurs, replace with properly sexed individuals. Group spawning with 3-4 males and 10-15 females also works well.

8.1.3. Reference Organism

It is recommended that several organisms in the brood stock be examined by a biologist competent in fish taxonomy to ascertain that the brood stock are *P. promelas*. Several taxonomic references are available to distinguish members of the fish family Cyprinidae (e.g. Eddy and Underhill 1974, Scott and Crossman 1973). Verification should be documented in writing and should include the name of the individual responsible for the taxonomy, the taxonomic key used, the date of identification and the source of the individual organisms used in the identification.

8.1.4. Culture Chambers

Chambers for mass culturing of fathead embryos may be constructed of glass, fiberglass, or stainless steel, although glass is preferred. An example of a single culturing system would be a 57 L (15 gallon) glass aquarium (31 cm x 61 cm x 32 cm, WxLxH) with a standpipe drain adjusted to provide 10 to 20 cm of water depth (Denny 1987). Each aquarium can be divided into quarters to accommodate four breeding males. The divisions are made with stainless steel mesh (5 mm opening), fastened in place with silicone glue. The entire unit must be acid washed with 1N HCl or HNO_3 to remove any manufacture residues. One water inlet, drain and airstone can serve each aquarium.

White plastic dish-washing pans commonly available in stores (e.g. 53 cm x 40 cm x 12 cm, LxWxH) make good hatching trays for embryo incubation because white pans facilitate viewing the newly hatched larvae. The water temperature within this pan should be maintained at 25±1ºC. Newly hatched larvae are small (<5 mm total length) and can easily escape to a drain if flowing water is used. Therefore, static water conditions are best with water temperature controlled by a water bath, in-tank electric heaters, or constant room temperatures. A generous supply of low pressure (≈3 psi) air (compressed oil-free room air) and an airstone are needed to mix the water in the pan and aerate the water that is in contact with the developing embryos.

8.1.5. Spawning Substrates

Fathead minnows deposit their eggs on the underside of submerged or floating objects. For cultures, suitable spawning substrates have been provided with PVC (polyvinylchloride) pipe, 7.6 to 10.2 cm diameter, cut into 7 to 10 cm lengths. The pipes are then halved lengthwise, the underside roughened with a wire brush (Gale and Bunyak 1982) and placed into each breeding tank with the arch down. This creates a room-like spawning area under which fathead minnows will deposit the eggs. Other spawning substrate materials have been used successfully, such as, stainless steel shaped like the PVC pipe with sand coated to the underside with silicone glue (Benoit 1982).

8.1.6. Culture Water

An adequate supply of water such as from a spring, well or controlled surface water of consistent high quality is necessary to culture fathead minnows. Water quality parameters such as hardness, alkalinity, conductivity and pH should fall within the following ranges: hardness, 25-300 mg/L as $CaCO_3$; alkalinity, 25-300 mg/L as $CaCO_3$; conductivity, 50-500 umhos/cm; and pH, 6.5 to 9.0. Dechlorinated water can be used when dechlorinated with sodium bisulfite (sodium sulfite can be used but is less desirable) which also removes chloramines (ASTM 1993a, 1993f) or by dechlorination with aeration in an open chamber of sufficient

retention time (>24 h) to remove chlorine and chloramines. Chemical monitoring of the water for residual chlorine or chloramine concentration must be conducted to ensure concentrations of these chemicals do not exceed 3 μg/L. Municipal drinking water also often contains copper, lead, zinc and fluoride which can be removed, when excessive, by using appropriate ion exchange resins (ASTM 1993a, 1993f). The national water quality criteria for the chronic values for freshwater organisms exposed to copper, lead and zinc are 12, 3.2 and 110 μg/L, respectively, at water hardness of 100 mg/L as $CaCO_3$ (USEPA 1987). Different chronic values must be calculated if the culturing water differs from 100 mg/L as $CaCO_3$. No criterion is available for fluoride.

8.1.7. Temperature and Photoperiod

Water temperature for culturing fathead minnows should be maintained at 25±1°C. This temperature is suitable for reproduction, incubation and growth. Temperatures below 22°C and above 26°C reduce reproduction of fathead minnows (Brungs 1971).

A photoperiod of 16 h light and 8 h dark during each 24-h period is recommended. Wide spectrum fluorescent lights with intensity of 10-20 µE/m^2/s (540-1080 lux, 50-100 ft-c) at the water surface are preferred.

8.1.8. Food and Feeding

Adult fathead minnows and fish over 30-days old are fed adult brine shrimp (*Artemia* sp.). These shrimp are purchased frozen from suppliers and allowed to slightly thaw prior to feeding. It is not necessary to rinse the shrimp with culture water to remove brine before feeding. Fish are fed *ad libitum* twice daily (i.e. approximately 1 to 2 mL per feeding per breeding pair). A rule of thumb is that most of the food will be consumed in about 10 min (Denny 1987) if the amount is appropriate.

Larval fathead minnows are fed freshly hatched (<24-h old) brine shrimp nauplii a minimum of twice daily. Before feeding the nauplii, they should be concentrated on a fine Nitex® screen rinsed with culture water, and resuspended with a minimum of culture water before feeding to the fish. Feeding should begin the same day as larvae hatch. The size of the nauplii is important; they must be small enough to be ingested by the larvae. Feeding of fresh nauplii is continued until fish reach ~25-30 days old when they can be fed adult brine shrimp.

Culturing of brine shrimp nauplii is done in a 25°C brine (NaCl) incubator. Hatchery designs and necessary apparatus are simple (Denny 1987). ASTM (1993g) has published guidelines for using brine shrimp nauplii in aquatic toxicology.

Other commercial diets are acceptable. However, they must exhibit growth of larval fathead minnows comparable to the recommended diet.

8.1.9. Chamber Cleaning

Chambers containing adult breeding fish should be scraped and siphoned a maximum of once weekly. Clean only a few chambers each day so that the whole brood culture is not disturbed by the cleaning. Some algal growth in the chambers is desirable as the fish eat it to supplement their diet. Chambers containing larvae and fish less than 30-d old should have the bottom siphoned carefully each day to remove uneaten brine shrimp nauplii and feces. Dead brine shrimp promote growth of a fungal mat which may entrap and kill larval fish.

8.1.10. Handling

Care must be taken to avoid disturbance of the adults and young fish due to unnecessary movement, noise, and extraneous lighting. Fish handling should be kept at a minimum. An adult fish should be moved carefully using only nets made of soft knotless material, and must be released quickly into water to avoid stress. Fish too small (<50 mg) to net should be handled with glass pipets or tubing with fire-polished ends. Organisms that are injured or dropped during handling or that touch dry surfaces should be discarded (ASTM 1993a, 1993f).

8.1.11. Water Quality Monitoring

Water used to culture fathead minnows should be monitored for temperature (25±1°C), dissolved oxygen (5.0-8.3 mg/L), hardness (25-300 mg/L as $CaCO_3$), alkalinity (25-300 mg/L as $CaCO_3$), conductivity (50-1,000 µmhos/cm), pH (6.5-9.0 pH units), ammonia (<0.1 mg/L total ammonia), and any other characteristics [e.g. chlorine and chloramines (residual chlorine ion specific electrode method, Rigdon et al. 1978), sulfides (iodometric method, APHA 1985)] useful to indicate consistent quality. Temperature should be measured daily and dissolved oxygen twice weekly in the breeding and culturing tanks. Hardness and alkalinity should be measured weekly at the water supply source to the tanks.

8.1.12. Embryo Incubation

Visually inspect the spawning substrates daily for deposited embryos by removing them from the water with your hands or check for embryos without removing the substrates from the water by feeling with your fingers. When handling substrates, clean hands or wear latex gloves. When embryos are present, transfer the substrate to the incubation pan and replace the substrate with a clean one. Estimate the number of embryos on each substrate so that, if hatching is successful, the number of fry available on a certain date can be determined. Report this information on the

embryo production record form (Attachment L, Form L-1).

Incubate embryos on substrates in the incubation pan by standing the substrates on edge and placing an airstone near each substrate. For successful incubation, abundant air-induced circulation of the water is needed to maintain oxygen to the embryos. Check each substrate daily for white unfertilized and fungus-infected embryos. When defective embryos are located they must be removed with a forceps to prevent the spread of fungus to healthy embryos. Embryos will form dark eye-spots at about 48 h in 25ºC water and will begin hatching by 96 h. Hatched embryos may remain in the incubation pan until hatching of all viable embryos is complete by 120 h. Larvae are then counted and transferred to rearing tanks or used for tests. Larvae used for tests should be <24-h old when reared at the testing facility or must be <48-h old when larvae are received from remote sites. Larvae should be within 12 h of each other in age for use in the tests.

8.1.13. Culture Evaluation

Brood stock evaluation is based upon survival and reproductive rate of the fish. Rarely do mature fathead minnows die in the culture tanks. Any more than an occasional random death of one of the brood stock should be cause for concern. The first symptom of problems in the culture tanks is a reduction in the reproductive rate. Typical spawning rates can be as high as a spawning every 2 or 3 d with one male and two females present. Once a spawning rate has been established any decrease in rate can be attributed to a change in water or food quality, brood stock health, or "spawned out" fish. Spawning pairs usually continue to reproduce for several months.

Embryos are evaluated daily for suitability as test organisms by observing the embryos for changes in development or fungal attack. Generally, when one-half or more of the embryos on a spawning substrate show either lack of development or fungal infection, the entire group of embryos from that substrate should be discarded (Denny 1987). After hatching and until used for testing, each batch of larvae should be observed for rate of survival. When approximately 20% or more of the hatched larvae have died during this time interval, that batch of larvae should be considered unsuitable.

8.1.14. Culture Records

A separate set of records should be maintained for the culture unit. The records should show dates of spawnings by each breeding pair and the estimated number of embryos per substrate. In addition, there should be daily records (Attachment L) of water temperature, feedings, chamber cleaning, aeration, water flow, spawning substrate inspections, mortalities and hatching

success of embryos.

8.2. ELUTRIATE ACUTE TOXICITY TEST METHODS

8.2.1. Acute Test Design

The basic design and conditions for performing a 4-d dredged material elutriate toxicity test are given in Table G-11. Additionally, a daily activity schedule (Attachment M) is provided to facilitate planning and starting a toxicity test. The GLTEM recommends that the acute test first be performed with the 100 percent elutriate, and followed by a dilution series only if survival in the 100 percent elutriate is less than 50 percent. Each exposure is replicated five times. Table G-12 provides a summary of the volumes of dredged material and water required for a complete dilution series, using a 0.6 dilution factor. If more than 50% of the test organisms die at the lowest dilution treatment (10%) then another test must be conducted starting at about a 10% elutriate solution concentration and reducing the elutriate concentration by a factor that will allow the organisms to survive sufficiently to calculate an LC50.

A completely random design or a randomized complete block design can be used to arrange the test chambers. The randomized complete block design is used if the possibility exists for an effect due to test chamber placement by such things as slight differences of water temperature or lighting. The randomized complete block design requires three groupings (one for each replicate) with randomization within each grouping (Figure G-6). Larvae are introduced to the test chambers in a specific manner (see Section 8.2.6.).

Table G-11. Overview of Recommended Dredged Material Elutriate Test Conditions for the Fathead Minnow 4-d Acute Toxicity Test.

1.	Test type	Static with one renewal at 48 h
2.	Water temperature	25±1°C
3.	Illumination quality	Fluorescent bulbs (wide spectrum)
4.	Light intensity	10-20 $\mu E/M^2/s$, 540-1080 lux, or 50-100 ft-c (ambient laboratory levels)
5.	Photoperiod	16 h light, 8 h dark
6.	Test chamber size	250 mL minimum
7.	Test solution volume	200 mL minimum
8.	Renewal of test solutions	Daily
9.	Age of test organisms	24 to 48 h

Table G-11 (continued)

10.	No. of larvae per test chamber	10
11.	No. replicate test chambers per concentration	5 minimum
12.	No. of larvae per test concentration	50 minimum
13.	Feeding regimen	Feed 0.1 mL brine shrimp nauplii suspension per test chamber three times daily; or 0.15 mL twice daily
14.	Aeration	None unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min.
15.	Dilution water	Culture water, test site water, or reconstituted water
16.	Elutriate concentrations	Minimum of 5 test site elutriate concentrations, and a performance control (diluent or culture water only)
17.	Dilution factor	≥0.5
18.	Test duration	4 days
19.	Endpoint	Mortality (LC50 or NOEC)
20.	Test acceptability	Dissolved oxygen ≥40% of saturation, mean temperature of 25±1°C; 90% or greater survival in the controls and satisfactory results from reference toxicant tests
21.	Sample requirements	Storage of dredged material is at 4°C; elutriate water should be prepared and tests initiated within 8 weeks of collection
22.	Dredged material required	A minimum of 2400 mL for each test site

8.2.2. Test Chambers

Glass or non-toxic disposable plastic test chambers are required for each concentration and control. Test chambers must be clean and of 250 mL capacity or greater. To avoid potential contamination from the air and to reduce evaporation of the test solutions, the test chambers should be covered with sheets of safety plate glass or plastic (6mm, 1/4 inch thickness; USEPA 1989). If plastic sheets are used they should not be of fresh construction and should not emit chemical odors. Fresh plastic sheets should be immersed in culture water for a minimum of 24 h to reduce chemical odors.

Table G-12. Volumes (mL) of Dredged Material Elutriate and Dilution Water for Each Renewal of a Single Test Elutriate in the Fathead Minnow 4-d Acute Toxicity Test Using a 0.6 Dilution Factor.

Percent Elutriate	Elutriate Volume Per Replicate	Dilution Water Volume Per Replicate	Total Elutriate Volume Required/5 Reps	Total Dilution Water Volume Required/5 Reps
100.0	200.0	0.0	1000.0	0.0
60.0	120.0	80.0	6000.0	400.0
36.0	72.0	128.0	360.0	640.0
21.6	43.2	156.8	216.0	784.0
13.0	25.9	174.1	129.5	870.5
0.0	0.0	200.0	0.0	1000.0

8.2.3. Cleaning of Glassware

All glassware used in the tests should be thoroughly cleaned before contact with test solutions or test organisms. Glassware should be washed with detergent, rinsed three times each with tap water and deionized water, and then placed in a clean 10 percent HC1 or HNO_3 bath for approximately 4 h. This should be followed by several rinses with deionized water, an acetone rinse, and a final rinse with deionized water. Disposable plastic test chambers should need no cleaning prior to use in a toxicity test but should be rinsed with deionized water. If an oil-sheen is seen in the rinse water, discard the test chambers. Plastic test chambers must not be reused.

8.2.4. Elutriate Renewal

All test chambers must receive fresh elutriate and/or diluent water a minimum of once at 48 h. The renewal solutions must be prepared on the day of renewal with the elutriate stock solution prepared at the beginning of the test. The diluent water is either culture or site water. The renewal solutions must be prepared in advance to allow temperature adjustment to the test temperature of 25°C. As the solutions warm from the 4°C storage temperature, supersaturation of the dissolved gasses may occur. If the dissolved oxygen concentration exceeds 100% of saturation (≈8.3 mg/L), gentle aeration may be necessary to reduce the supersaturation of gases. If the dissolved oxygen in test solutions drops below 4.0 mg/L during the test, gentle aeration should be added. The aeration should be added through a disposable glass pipet tip that has been inserted to near the bottom of the test chamber and adjusted to deliver at a rate of

about 100 bubbles/min.

8.2.5. Temperature and Photoperiod

Tests should be performed in a temperature-controlled room or in a temperature-controlled water bath. The test temperature recommended for this method is 25±1°C and the photoperiod recommendation is 16 h light and 8 h dark in each 24-h period. The light quality and intensity should be at ambient laboratory levels (USEPA 1989), which is approximately 540-1080 lux, or 50-100 foot candles (ft-c) at the water surface in the test chambers.

8.2.6. Organism Introduction

A total of 300 larval fathead minnows 24-48 h old are required to start the test. Larvae are captured from a common pool of larvae with an appropriate pipet (fire-polished opening, 5 mm diameter) for young larvae and a beaker in conjunction with a small dip net for larger larvae, and placed into the exposure chambers containing test solutions equilibrated to 25°C. The test chambers are randomized before introducing the larvae. Larvae are introduced in the order the chambers have been randomized either by row or within a block (Fig. G-6). Two or three larvae are consecutively added beneath the surface of the test solutions in each test chamber until a total of ten organisms are in each. Care should be taken to count them and to add the least possible volume of culture water. The pipet may become contaminated with elutriate constituents; however, this source of potential contamination can be eliminated by rinsing the pipet in a beaker of diluent water after each larval transfer.

8.2.7. Food and Feeding

The fish in each test chamber are fed 0.1 mL (approximately 700-1,000 nauplii) of a concentrated suspension of brine shrimp nauplii. The brine shrimp must be recently hatched (less than 24-h old). One method to achieve this feeding rate is to allow the brine culture of nauplii to settle a few minutes in a 2 L separatory funnel without aeration. As soon as the nauplii are mostly near the bottom of the container, take 125 mL of them by pipette and place them in a beaker with a fine-meshed bottom and rinse one to three times with diluent water to reduce the sodium chloride concentration. Immediately back-rinse the beaker with 10 to 15 mL of diluent water into another beaker. This solution contains the appropriate density of nauplii for feeding. Feedings should be three times daily at 4-h intervals. If twice-daily feedings are used, 0.15 mL of nauplii, rather than 0.10 mL, are fed to each test chamber at 6-h intervals.

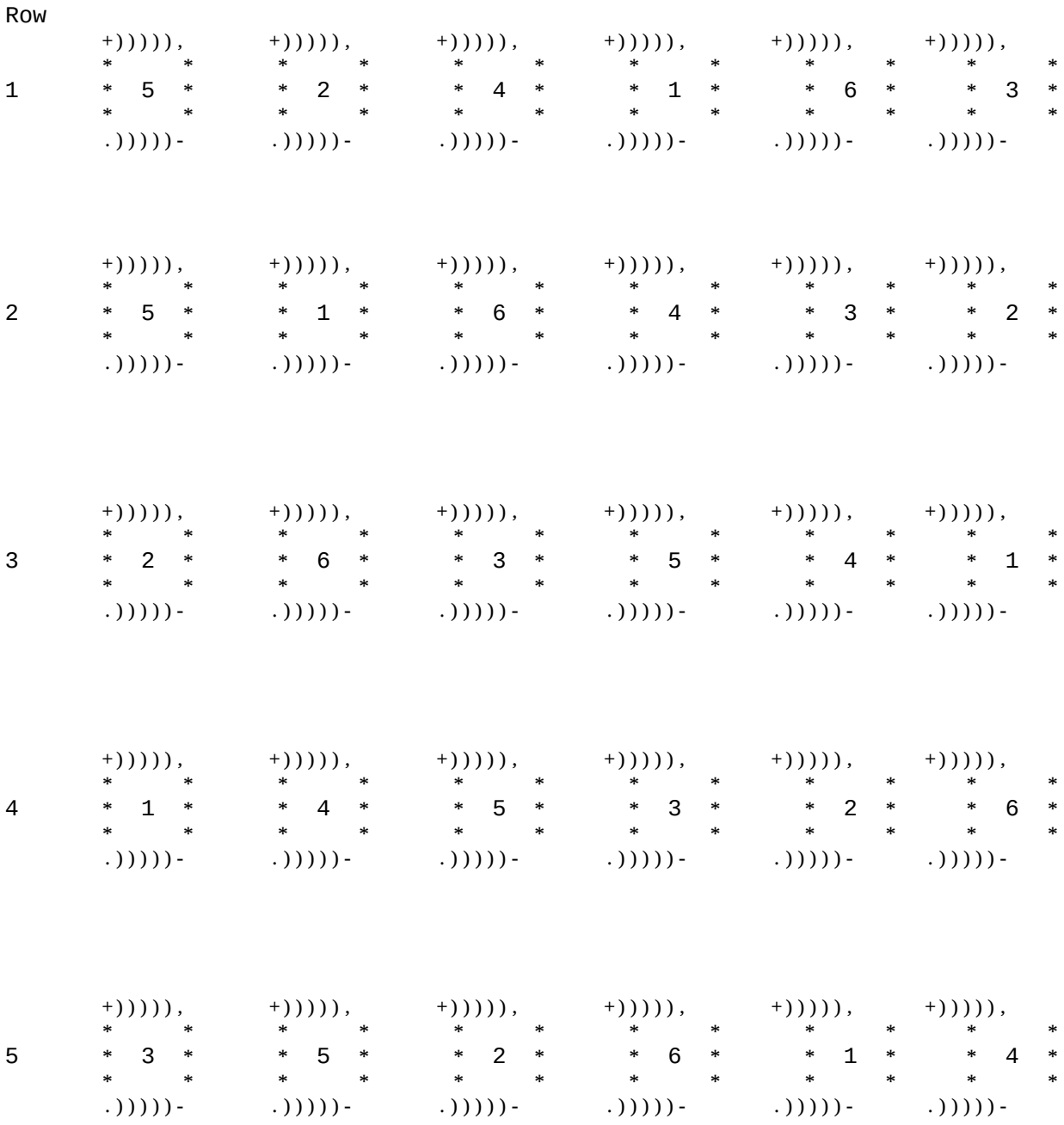


Figure G-6. Example of an exposure chamber arrangement for a randomized complete block design. Each row contains one replicate of all treatments.

The feeding schedule will be dependent on when the test solutions are renewed. If the test is initiated after 1200 PM, the larvae may be fed once or twice the first day. On following days, the larvae normally would be fed at the beginning of the work day, or a minimum of 2 h before test solution renewal,

during mid-day, and at the end of the work day after test solution renewal. However, if test solutions are renewed at the beginning of the work day, the first feeding should be after test solution renewal and the remaining feedings would be at appropriate intervals (USEPA 1989).

8.2.8. Cleaning Test Chambers

At the time of renewal of test solutions, uneaten and dead brine shrimp nauplii and other debris are removed from the bottom of the test chamber with a small siphon hose (1/8" i.d. made of Tygon® or Teflon®). Three possible techniques for cleaning are: (1) a hose is held with a loop in one hand to close the hose with a crimp if a fathead minnow larva is endangered by the siphon, (2) use a spring-loaded hose clamp to control flow, or; (3) an alternative to the siphon is a glass tube or 50 mL pipet fitted with a rubber bulb to suction the bottom. Because the larvae may be small during the test, cleaning may be facilitated by placing the test chamber on a light box during cleaning. Siphoning or discharging the suction bulb into a white plastic container is recommended to insure that no larvae escape the test chambers unnoticed. Any larvae found in the white container should be returned to the study and a record made of it in the log. The test chambers are siphoned to within 5-7 mm of the bottom leaving approximately 15-20% of the previous test solution volume in place (USEPA 1993).

8.2.9. Test Solution Renewal

Test solutions can be renewed each day but must be renewed at 48 h from the stock elutriate solution. The stock solution must be prepared the day before the test begins due to the several hours of time required to prepare the necessary elutriate volume. The test solutions are made in the same manner as at test initiation. Make certain that the temperature of the new solutions is 25°C and that supersaturation of gasses has not occurred. Immediately after siphoning the test chambers, the test solutions are renewed by adding the new solution down the inside of the test chamber being careful not to disturb the larvae with excessive turbulence.

8.2.10. Test Organism Monitoring

Test organisms are observed daily for survival and abnormal behavior. Mortalities and any abnormal behavior (e.g. rapid or slow swimming, swimming at the surface, spinning) should be recorded. Organisms can be observed without magnification. Dead organisms must be removed immediately upon discovery or a minimum of once daily.

8.2.11. Water Quality Monitoring

Water temperature should be measured daily in at least five locations in the test chamber array (four corners and center)

with an accurate thermometer precise to 0.1ºC. Dissolved oxygen and pH should be measured daily with precision to 0.1 mg/L and 0.1 pH unit, respectively, in a control chamber and in one each of the low, medium and high concentration exposure chambers. Measure ammonia once before starting the test. The same measurements should be made in the fresh renewal solutions as well as the old solutions. Hardness and alkalinity determinations should be precise to 1-2 mg/L as $CaCO_3$ and specific conductance measured with precision to 5 µmhos/cm in the control (diluent) water and the low, medium and high elutriate concentrations at the start of the test and at test termination on d 4.

8.2.12. Test Termination

The test is terminated on d 4 at the same hour of the day that the test was begun. Survival of the larvae is recorded as on previous days and summed for the duration of the test.

8.3. ELUTRIATE CHRONIC TOXICITY TEST METHODS

8.3.1. Test Design

The basic design and conditions for performing a 7-d dredged material elutriate toxicity test are given in Table G-13. Additionally, a daily activity schedule (Attachment M) has been prepared to facilitate planning and starting a toxicity test. The test consists of a series of 5 dilutions of the test site dredged material elutriate and its performance control (culture water). Each of these experimental units (treatments) is replicated a minimum of 5 times, for a total of 25 exposure chambers per dredged material elutriate, plus controls. Thus, a chronic test of one dredged material elutriate with five dilutions requires 30 exposure chambers. A dilution factor of 0.5 or greater is used to determine the dilutions to be made from the full-strength (100 percent) test elutriate (Table G-14). An alternative design that could be used if there are several test sites would be to initially test only the 100% elutriate for each site (with an appropriate performance control). Then, test with a series of dilutions could be performed only for those dredged materials exhibiting chronic toxicity in the 100% elutriate solution.

Table G-13. Overview of Recommended Dredged Material Elutriate Test Conditions for the Fathead Minnow 7-d Chronic Survival And Growth Toxicity Test.

1.	Test type	Static with daily renewal
2.	Water Temperature	25±1°C
3.	Illumination quality	fluorescent bulbs (wide spectrum)
4.	Light intensity	10-20 μE/M^2/s, 540-1080 lux, or 50-100 ft-c (ambient laboratory levels)
5.	Photoperiod	16 h light, 8 h dark
6.	Test chamber size	250 mL
7.	Test solution volume	100 mL
8.	Renewal of test solutions	Daily
9.	Age of test organisms	Less than 24 h preferred when in-house culture available; must be less than 48 h when larvae shipped from remote sites
10.	No. larvae per test chamber	10
11.	No. replicate test chambers per concentration	5 minimum
12.	No. larvae per test concentration	50 minimum
13.	Feeding regimen	Feed 0.1 mL brine shrimp nauplii suspension per test chamber three times daily; or 0.15 mL twice daily.
14.	Aeration	None, unless DO drops below 4.0; rate should not exceed 100 bubbles/min.
15.	Dilution water	Culture water, test site water, or reconstituted water.
16.	Elutriate concentrations	Minimum of 5 test site elutriate concentrations, and a performance control (diluent or culture water only)
17.	Dilution factor	≥0.5
18.	Test duration	7 d
19.	Endpoints	Survival and growth (dry weight)
20.	Test acceptability	80% or greater survival in the control solutions; mean weight per control fish ≥0.25 mg; satisfactory results from the reference toxicant test
21.	Sample requirements	Storage of dredged material is at 4°C; elutriate water should be prepared and tests initiated within 6 weeks of collection
22.	Dredged material required	A minimum of 1,800 mL for each test site

Table G-14. Volumes (mL) of Dredged Material Elutriate and Dilution Water for Each Renewal of a Single Test Elutriate in the Fathead Minnow 7-d Chronic Survival and Growth Toxicity Test Using a 0.5 Dilution Factor.

Percent Elutriate	Elutriate Volume Per Replicate	Dilution Water Volume Per Replicate	Total Elutriate Volume Required/5 Reps	Total Dilution Water Volume Required/5 Reps
100.0	100.0	0.0	500.0	0.0
50.0	50.0	50.0	250.0	250.0
25.0	25.0	75.0	125.0	375.0
12.5	12.5	87.5	62.5	437.5
6.25	6.25	93.75	31.2	468.8
0.0	0.0	100.0	0.0	500.0

A completely random design or a randomized complete block design can be used to arrange the test chambers. The randomized complete block design is used if the possibility exists for an effect due to test chamber placement by such things as slight differences of water temperature or lighting. The randomized block design requires five groupings (one for each replicate) with randomization within each grouping (Fig. G-6). Larvae are introduced to the test chambers in a specific manner (see Section 8.2.6.) to insure random selection of test organisms.

8.3.2. Test Chambers

Test chambers must be clean and of 250 mL capacity or greater. To avoid potential contamination from the air and to reduce evaporation of the test solutions, the test chambers should be covered with sheets of safety plate glass or plastic (6mm, 1/4 inch thickness; USEPA 1993). If plastic sheets are used they should not be of fresh construction and emitting chemical odors.

8.3.3. Cleaning of Glassware

See Section 8.2.3. "Cleaning of Glassware".

8.3.4. Elutriate Renewal

All test chambers must receive fresh elutriate and/or diluent water each day except d 7. The renewal solutions must be prepared daily with the elutriate stock solution prepared at the beginning of the test. The diluent water is either culture or

site water. The renewal solutions must be prepared in advance to allow temperature adjustment to the test temperature of 25°C. As the solutions warm from the 4°C storage temperature, supersaturation of the dissolved gasses may occur. If the dissolved oxygen concentration exceeds 100% of saturation (≈8.3 mg/L), gentle aeration may be necessary to reduce the supersaturation of gases. If the dissolved oxygen in test solutions drops below 4.0 mg/L during the test, gentle aeration should be added. The aeration should be added through a disposable glass pipet tip that has been inserted to near the bottom of the test chamber and adjusted to deliver at a rate of about 100 bubbles/min.

8.3.5. Temperature and Photoperiod

See Section 8.2.5. "Temperature and Photoperiod".

8.3.6. Organism Introduction

A total of 300 larval fathead minnows less than 24-h old (maximum of 48-h old when shipped from remote sources) are required to start the test. Larvae are captured from a common pool of larvae with an appropriate pipet (fire-polished opening, 5 mm diameter) and placed into the exposure chambers containing test solutions equilibrated to 25°C. The test chambers are randomized before introducing the larvae. Larvae are introduced in the order the chambers have been randomized either by row or within a block (Fig. G-6). Two or three larvae are consecutively added beneath the surface of the test solutions in each test chamber until a total of ten organisms are in each. Care should be taken to count them and to add the least possible volume of culture water. The pipet may become contaminated with elutriate constituents; however, this source of potential contamination can be eliminated by rinsing the pipet in a beaker of diluent water after each larval transfer. A randomly selected group of ten fish must be weighed in the same manner as the fish at test termination (Section 8.3.12) to measure control fish growth during the study.

8.3.7. Food and Feeding

See Section 8.2.7. "Food and Feeding".

8.3.8. Cleaning Test Chambers

See Section 8.2.8. "Cleaning Test Chambers".

8.3.9. Test Solution Renewal

Test solutions must be renewed each day except on d 7 from the stock elutriate solution. (The stock solution must be prepared the day before the test begins due to the several hours of time required to prepare the necessary elutriate volume). The test solutions are made in the same manner as at test initiation. Make certain that the temperature of the new solutions is 25°C

and that supersaturation of gasses has not occurred. Immediately after siphoning the test chambers, the test solutions are renewed by adding the new solution down the inside of the test chamber being careful not to disturb the larvae with excessive turbulence.

8.3.10. Test Organism Monitoring

See Section 8.2.10. "Test Organism Monitoring".

8.3.11. Water Quality Monitoring

Water temperature should be measured daily in at least five locations in the test chamber array (four corners and center) with an accurate thermometer precise to 0.1°C. Dissolved oxygen and pH should be measured daily with precision to 0.1 mg/L and 0.1 pH unit, respectively, in the old and new solutions in a control chamber and in one each of the low, medium and high concentration exposure chambers. Ammonia concentrations should be measured at least once in the high elutriate concentration. Hardness and alkalinity determination should be precise to 1-2 mg/L as $CaCO_3$ and specific conductance measured with precision to 5 µmhos/cm in the control (diluent) water and the low, medium and high elutriate concentrations at the start of the test and at test termination on d 7.

8.3.12. Test Termination

The test is terminated on d 7 at the same hour of the day that the test was begun. Survival of the larvae is recorded as on previous days and summed for the duration of the test. Surviving larvae are quickly killed (by overdosing with an anesthetic or freezing), and transferred to aluminum weighing boats which have been oven-dried. The fish larvae are dried in an oven at 100°C for at least 4 h (until steady weight is achieved), cooled in a dessicator, and weighed. Larvae are weighed as groups with all surviving larvae from one test chamber in one weighing boat. The weighing boats are marked with the exposure chamber code for proper identification. Weighing must be done with a balance capable of weighing to 0.01 mg (0.00001 gm). The dried weights and number of organisms weighed are reported on prepared data forms (Attachment L).

8.4. Data Reporting and Statistical Analysis

See Section 12.

9.0 *Chironomus tentans* **SOLID-PHASE TOXICITY TEST**

Chironomus tentans Fabricius (Diptera:Chironomidae) is a widely distributed (holarctic) non-biting midge (Townsend et al. 1981). It and several other species of chironomids are commonly referred to as "bloodworms" due to their red coloration during

their aquatic larval stages. *Chironomus tentans* is commonly found in eutrophic ponds and lakes (Flannagan 1971, Driver 1977), where it serves as an important dietary component for various species of fish and waterfowl (Sadler 1935, Siegfried 1973, Driver et al. 1974, McLarney et al. 1974).

Larvae of *C. tentans* prefer soft sediments, and normally inhabit the uppermost portion of the sediment. They inhabit sediments having particle sizes ranging between 0.15 mm to 2.0 mm, and were found to be adapted in British Columbia lakes to the following ranges of environmental factors (Topping 1971): temperature, 0-23.3°C; dissolved oxygen, 0.22-8.23 mg/L; pH, 8.0-9.2; conductivity, 481-4,136 μmhos; organic carbon, 1.92-15.45 percent. They were absent from lakes if the hydrogen sulfide concentration in overlying water exceeded 0.3 mg/L. Abundance of larvae was positively correlated with conductivity, pH, amount of food, percentages of particles in the 0.59-1.98 mm size range, and concentrations of sodium, potassium, magnesium, chloride, sulfate and dissolved oxygen. Other publications (e.g., Curry 1962, Oliver 1971) have extended the ranges for temperature (0-35°C) and pH (7-10) for waters which are inhabited by *C. tentans.*

The *C. tentans* life cycle is mainly aquatic. Adult females that have mated oviposit a single transparent, gelatinous egg mass directly into the water. An average egg mass contains approximately 2,300 eggs (Sadler 1935), which hatch within 2 or 3 days at 19-22°C (ASTM 1993e). Four larval instars are recognized, each lasting for about one week at a temperature of 20°C (ASTM 1993e). Larvae begin to construct minute tubes or cases on the second or third day after hatching. The cases, which are lengthened and enlarged as the larvae grow, are composed of very small particles bound together with threads from the mouths of the larvae (Sadler 1935). The larvae draw food particles, commonly algae, inside the tubes, and also feed in the immediate vicinity of either end of the open-ended tubes with their caudal extremities anchored within the tube. The larval stages are followed by a black-colored pupal stage (3 days) and emergence to a terrestrial adult (imago) stage. Larval and pupal life stages are presented in Figure G-7. The adult stage lasts for several days (3-5), during which the adults mate during flight and the females oviposit their egg masses (2-3 days post-emergence) (Sadler 1935). The complete life cycle requires about 30 days at 25°C (Adams et al. 1985).

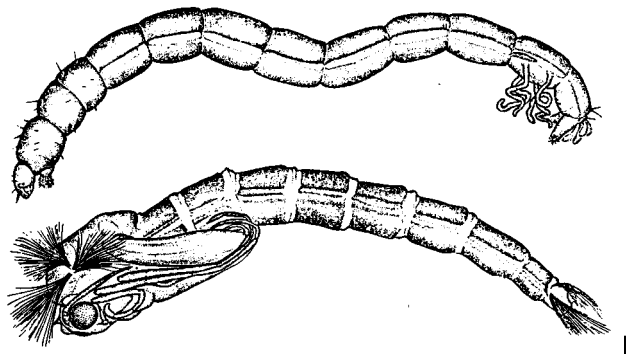


Figure G-7. *Chironomus tentans* 4th instar larvae (top) and pupa (bottom), X4. (From Johannsen and Thomsen 1937; drawing by Dr. Velma Knox).

Sexual dimorphism is readily apparent in adults. Adult males are distinguished from females by the presence of large, plumose antennae, a thinner abdomen and visible genitalia (ASTM 1993e). The male has paired genital claspers on the posterior tip of the abdomen (Townsend et al. 1981). The adult female weighs approximately twice as much as the male, with about 30 percent of the female weight contributed by the eggs.

Various laboratory and field investigations of water and sediment quality in freshwater systems have used *C. tentans*. For example, a field study of *C. tentans* distribution in a heavy metal-contaminated lake showed decreased populations in the most heavily contaminated sediments (Wentsel et al. 1977a). Laboratory evaluations of *C. tentans* survival and growth in sediments contaminated with heavy metals or organic compounds have resulted in decreased survival and/or reduced growth of larvae (e.g., Wentsel et al. 1977b, Adams et al. 1985, 1986, Giesy et al. 1988, 1990, Nebeker 1984b, 1988, Hoke et al. 1990, West et al. 1993). Decreased numbers of emergent adults have also been observed following exposure of larvae to contaminated sediments (Wentsel et al. 1978).

Chironomus tentans has a number of attributes which make it a good choice for the development of standardized laboratory test methods, including: (a) ease of culture, (b) ease of handling, (c) ecological relevance, (d) extreme sensitivity to certain classes of contaminants (e.g., pesticides), and (e) the availability of some basic culture/test conditions (e.g., ASTM 1993e). This report describes methods used to culture *C. tentans* and to perform a 10-day solid-phase sediment toxicity test with the midge. The culture methods described have been used successfully in various laboratories; however, slight

modifications to the culture methods are allowable provided that they result in a stable supply of organisms for testing.

9.1. CULTURE METHODS

The culturing methods described below are based largely on methods in use at ERL-Duluth (USEPA) and described in documents which include the standard operating procedure for culturing *Chironomus tentans* (Denny and Mead 1991, Denny et al. 1992). Required materials are listed in Attachment N.

9.1.1. Organism Source

Organisms for the initiation of a laboratory culture may be collected from the field or obtained from a laboratory (Attachment O) with a verified culture. In either case, organisms collected or received should be carefully examined by a qualified taxonomist using a key to verify the species identity. One or more organisms should be cleared to allow for improved viewing of the important characteristics of the head capsule, and then mounted to serve as reference material. Methods for clearing and mounting aquatic invertebrates are provided in Pennak (1989).

9.1.2. Acclimation of New Brood Stock

Environmental stress on the starter culture should be minimized to facilitate the development of a healthy culture. The temperature of the water containing the egg masses or larvae should be measured upon arrival and gradually adjusted to the desired culture temperature of 23° C. The water temperature change should not exceed 2°C per 24 h (ASTM 1993e). The water in which the brood stock arrived should also be gradually changed to 100% culture water from the new laboratory over at least a 4 h period.

Since culture performance fluctuates with temperature, a constant temperature of 23°C is recommended for uniformity in maturation and emergence of culture midges (Denny et al. 1992). Although toxicity tests have been performed over a temperature range of 19-23°C (e.g., Wentsel 1977b, 1978, Cairns et al. 1984, Nebeker et al. 1984a,b, 1988, Gauss et al. 1985, Adams et al. 1985, Tucker and Adams 1986, Giesy et al. 1988), we recommend testing at 23°C to maintain continuity with the culture temperature.

9.1.3. Culture Chambers

Glass aquaria (e.g., standard 19.0 L capacity, 36 cm x 21 cm x 26 cm high) are recommended for use as culture chambers. A water volume of approximately 7.5 L may be maintained in an aquarium of these dimensions by drilling an overflow hole in one end 11 cm from the bottom. The top should be covered with a mesh material to trap emergent adults. Queen-sized panty hose with

the elasticized waist positioned around the chamber top and the legs cut off and clipped shut have been used at ERL-Duluth (Denny et al. 1992). Fiberglass window screen glued to a glass strip (approximately 2-3 cm wide) rectangle placed on top of each aquarium has been used at the University of Wisconsin-Superior.

9.1.4. Substrate

Both shredded paper toweling and silica sand have been successfully used as artificial substrates. A sand substrate may facilitate ease of larval transfer at test initiation over that from paper toweling. Either substrate may be used if a healthy culture can be maintained.

The paper towel substrate is prepared according to a procedure adapted from Batac-Catalan and White (1982). Plain white kitchen paper towels are first either shredded in a paper shredder or cut into strips with scissors. A mass of the shredded or cut toweling is then loosely packed into a 2 L beaker, submersed in acetone, covered, and, in a fume hood, allowed to soak overnight to solubilize any trace organic contaminants. The acetone is discarded into a waste solvent bottle and the toweling rinsed three times with distilled water. Distilled water is again added and brought to a boil with occasional stirring to drive off the acetone vapors. The boiling and stirring process is performed three times, followed by rinses with cold distilled water. A mass of the toweling sufficient to fill a 150 mL beaker is placed into a heavy duty blender (e.g., commercial style blender) containing 1 L of distilled water, and blended for 30 seconds or until the strips are well broken apart and in the form of a pulp. The pulp is then placed into a 710 micron sieve and rinsed well with distilled water to remove the shortest fibers.

The initial mass of dry shredded paper toweling loosely packed into a 2 L beaker as described above will provide sufficient pulp substrate for about ten 19 L chambers. The toweling from the 150 mL beaker produces a mass of towel substrate that is approximately sufficient for one 19 L chamber. Several masses may be prepared separately at the same time and either stored in deionized water in a suitable container (e.g., 500 mL plastic bottle), or kept frozen for later use.

A sand substrate that has been used successfully at different laboratories consists of silica sand of 0.25 to 0.50 mm grain size (94 percent of total). The sand is rinsed with hot culture water, autoclaved, and oven-dried prior to use. One L of sand is a sufficient volume of substrate for each 19 L chamber.

9.1.5. Culture Water

Chironomus tentans can be successfully cultured in a variety

of types of overlying water. Regardless of the water type used by a given testing laboratory, it is suggested that the culture water be of the same basic qualities as the overlying water to be used in toxicity tests. Water of a quality sufficient to culture other test species such as the fathead minnow, *Pimephales promelas*, or the cladoceran, *Daphnia magna*, generally will be adequate for culturing *C.tentans*.

Chironomus tentans can be cultured under either static or renewal conditions. If a laboratory has a flow-through supply of water of high quality, a culture system which uses an automatic daily renewal of overlying water is recommended to avoid the possibility of fouling of the culture system by excess food and waste, and the resultant death of culture organisms due to oxygen depletion. Water renewal may be either intermittent or continuous. An automatic intermittent renewal for a daily three hour period is used at ERL-Duluth (Denny et al. 1992). A continuous renewal over a 24 h period is used by the University of Wisconsin-Superior.

If a static system is used, the overlying water may be derived from different sources. Untreated well water or dechlorinated water from a municipal supply may be used, or culture water of desired characteristics may be prepared (see Attachment P). In static systems, the overlying water volume should be changed every 4-7 d by siphoning down to a level just above the substrate, and slowly adding freshly prepared water (Batac-Catalan and White 1982). When using a static culture system, extra care must be taken to ensure that proper water quality is maintained. For example, supplemental aeration will likely be required to maintain adequate concentrations of dissolved oxygen. The air supply should be determined to be free of impurities, such as oil, by inclusion of a filter in the air line, if necessary.

9.1.6. Temperature and Photoperiod

The temperature for culturing *C. tentans* should be maintained at 23°C (Denny et al. 1992). A photoperiod of 16 h light and 8 h dark is recommended, with an intensity of 540 to 1080 lux or 50-100 ft-C. A photoperiod of 24 h of light also can result in normal development, but either no light or short periods of light (e.g., 8 L:16 D) prevent completion of the *Chironomus* life cycle (Englemann and Shapiro 1965, Townsend et al. 1981).

9.1.7. Food and Feeding

Various food items have been used for culturing *C. tentans* by different laboratories (ASTM 1993e), although some type of flaked fish food is used by most laboratories. Denny et al. (1992) adopted the use of Tetrafin® goldfish food, and methods

for preparing this food are described here. Tetrafin® flake food contains a minimum of 32% crude protein, 3% fat, and a maximum crude fiber content of 2%. The maximum moisture content is 6.5%, and the L-ascorbyl-2-phosphate content is ≥200 mg per 454 g.

In culture systems utilizing daily water renewal or continuous flow conditions, food should be prepared to provide a final concentration of approximately 0.04 mg dry solids/mL of culture water in each aquarium (Denny et al. 1992). Prepare a stock suspension of the solids in culture water such that a total volume of 5.0 mL of food suspension is added daily to each aquarium. For example, if a culture aquarium volume is 7.5 L, 300 mg of food would be added once each day. This would be accomplished by adding 5 mL of a 60 g/L stock suspension. The stock suspension should be initially shaken and then stirred immediately prior to withdrawal of an aliquot for each culture tank to ensure homogeneity of the food. Once prepared, the Tetrafin® food may be used for approximately two weeks if it is refrigerated between use. Specific details for food preparation are provided in Attachment Q.

9.1.8. Initiating a Culture

Organisms for initiating a culture are commonly received as egg masses from a commercial supplier or another laboratory. Two or three egg masses generally provide a sufficient number of organisms to start a new culture. In some cases, the embryos may have hatched during shipment. If the embryos have not hatched, acclimate the egg masses to the culture water and the desired culture temperature of 23°C in a glass beaker or crystallizing dish containing about 100 mL of culture water. The temperature change should not exceed 2°C per d. Allow the embryos to start hatching before adding a small amount (e.g., ≈4 mg dry solids) of suspended food particles to the water. Do not add food until the embryos are hatching to reduce the risk of oxygen depletion. When the hatch is judged to be complete or near completion, transfer the first instar larvae and remaining eggs into a larger culture aquarium. Larvae that have formed cases can be transferred with a gentle stream of water from a squeeze bottle. If larvae are already evident in the shipment of new brood stock, adjust the temperature of the shipped water containing larvae to the desired culture temperature at a rate not exceeding 2°C per 24 h. Add food at the rate of ≈4 mg per 100 mL of water to the shipped water. After proper acclimation, place food (≈4 mg) into a container of culture water (100 mL), and transfer the larvae and remaining eggs into the beaker or crystallizing dish immediately. Allow the larvae to feed in the confined beaker or finger bowl for a day prior to transferring them into a larger culture aquarium containing substrate, overlying water and food.

9.1.9. Culture Maintenance

Beakers or crystallizing dishes containing two to three egg masses should be examined under a dissecting microscope to determine hatching success. When most of the larvae have hatched, transfer the hatched larvae plus the remainder of the egg masses into an aquarium that contains substrate, overlying culture water and an initial increment of food (e.g., in the 19 L aquaria set-up, 5 mL of concentrated food suspension per 7.5 L of culture water to yield a final concentration of 0.04 mg/mL of solids in the aquarium).

The temperature of the aquarium water should be maintained at 23°±1°C. Dissolved oxygen levels should be monitored, and maintained at concentrations of 3.4 mg/L or above (i.e., ≥40 percent of saturation). If dissolved oxygen levels approach or drop below 3.4 mg/L, place an airstone into the chamber, and gently aerate. If the overlying water is automatically renewed, the flow should be regularly monitored to provide a measure of the daily rate of total volume turnover.

If culturing is being performed in a static system, fresh water should be added every four to seven days by siphoning the old water to just above the substrate (Batac-Catalan and White 1982), and slowly adding in the new water. A screened siphon tube should be used to avoid the removal of larvae and substrate (Denny et al. 1992). Care should be taken to avoid disturbance of the substrate and larvae during siphoning and replacement of the water. On days when water is renewed, add food after the water has been changed. Laboratories utilizing static cultures should develop lower feeding rates specific to their systems.

At a culture temperature of 23°C, larvae should have readily attained the second instar by 8 d post-hatch (Denny et al. 1992). The second and third instars are the desired ages for initiation of a 10-d toxicity test. Adult emergence will begin approximately 24 d post-oviposit at this temperature.

Once adults begin to emerge, they should be gently siphoned into a dry aspirator flask on a daily basis. An aspirator can be readily made from a 250 or 500 mL Erlenmeyer flask, a two-hole stopper, some short sections of 0.25 inch glass tubing, and Tygon or rubber tubing for collecting and providing suction (Figure G-8).

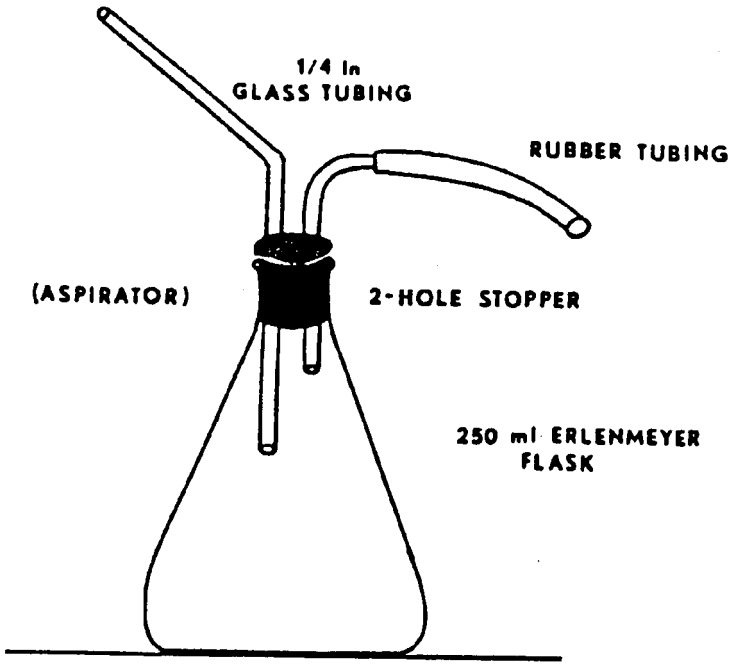


Figure G-8. Aspirating flask for collection of adults (from Batac-Catalan and White, 1982).

Aspirate with short, sharp inhalations to avoid injuring the adults. Check the sex ratio of collected adults to ensure that a sufficient number of males are available for mating and fertilization. Males are readily distinguished from females by their large plumose antennae (ASTM 1993e). One male may fertilize more than one female. However, a ratio of 50% or more males will ensure good fertilization.

A mating and oviposition chamber may be prepared in several different ways (Figure G-9). The flask in which the adults were collected may be used by simply adding a volume of water (e.g., 50-75 mL) to the flask and tipping the flask (Fig. G-9A, Batac-Catalan and White 1982). Denny et al. (1992) used a 500 mL collecting flask (Fig. G-9B), which included a length of Nitex® screen positioned vertically and extending into the water when water was subsequently added. The Nitex® screen is used by the females to position themselves just above the water during oviposition. The two-hole stopper and tubing of the aspirator should be replaced by screened material, a cotton plug, or perforated aluminum foil to allow for adequate air exchange in the oviposition chamber.

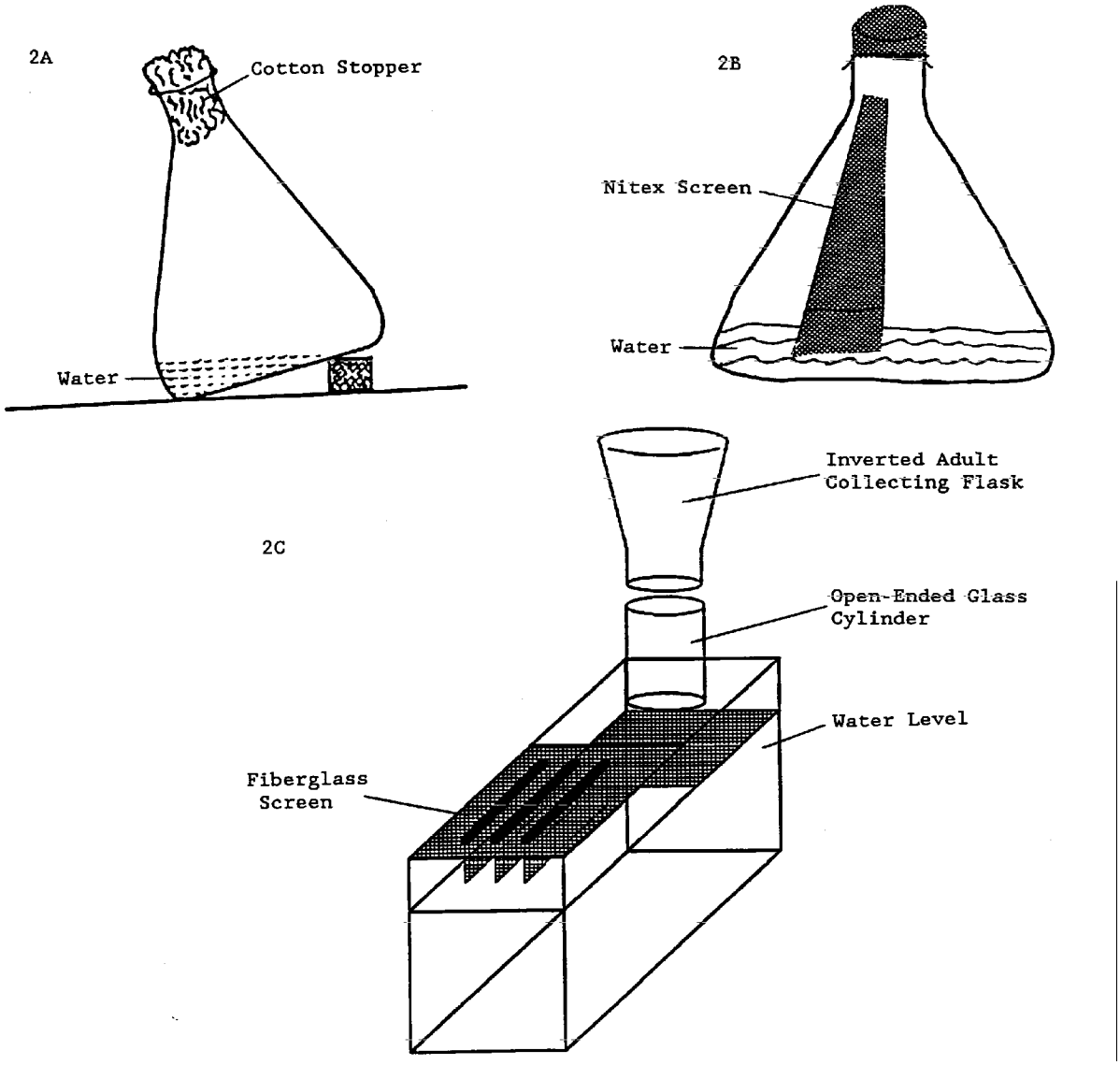


Figure G-9. Several styles of mating and oviposition chambers: 2A from Batac-Catalan and White, 1982; 2B from Denny et al., 1992; 2C from R. Venkataramani and S. McGovern, University of Wisconsin-Superior, unpublished.

A chamber designed by the University of Wisconsin-Superior (Fig. G-9C) provides for attachment of an inverted 250 mL Erlenmeyer adult collecting flask, sufficient room for mating, and a surface area for females to position themselves for egg deposition in the water. The overall dimensions are

approximately 20 cm x 10 cm x 14 cm (L x W x H). The rectangular chamber consists of two sections to allow for removal of the upper section and collection of the egg masses. The inverted adult collection flask is taped to the mouth of a 120 mL glass bottle with the bottom cut away and glued to a glass portion of the chamber cover. The lower portion of the chamber (~20 cm x 10 cm x 10.5 cm, L x W x H) should contain a volume of water of 8-9 cm depth and sufficient Nitex® or fiberglass window screen positioned such that it either extends into the water or is just above the water to allow for deposition of egg masses upon the water.

Egg masses should be collected daily, and depending upon the culture and testing needs of the laboratory, should regularly (e.g., weekly, twice weekly) be used to initiate a new larval culture tank. Egg masses should be collected with a wide bore pipet and placed into a crystallizing dish where they will begin to hatch within 48 h at 23°C. Upon hatching, they can be assigned to rearing tanks. Egg masses to be used for larval rearing should be documented as to their date of deposition, so that an accurate record will be available of the age of the larvae throughout their life cycle.

9.1.10. Culture Evaluation

Cultures should be observed daily at the time of feeding to ensure that a healthy culture is maintained. Larvae should be actively feeding within their cases in the substrate, as a requirement of health. Water temperature is measured and recorded daily in each aquarium used for larval rearing. Dissolved oxygen concentrations should be monitored and recorded weekly. Observations of the larvae should indicate good growth as the larvae progress through the four instars. Records should be kept on the time to first emergence and the success of emergence for each aquarium.

A culture evaluation chart (Attachment R, Form R1) should be maintained, and updated monthly. If the culture is not showing normal growth (i.e., mean dry weight of at least 0.6 mg for ten 22-day old 4th instar larvae), survival, emergence of adults, hatching success, or performance in reference toxicant tests, the culturing conditions should be scrutinized and adjustments made to restore culture health. Any adjustments made may be considered to have resulted in an acceptable state of health for the culture when the culture produces a regular supply of vigorous larvae that perform acceptably in reference toxicant tests (see Section 4.3.8.).

9.2. TOXICITY TEST METHODS

9.2.1. Solid-Phase Sediment Preparation

See Section 5.0.

All glassware used in the tests must be initially clean and should also be thoroughly cleaned after each test. Glassware should be washed with detergent, and rinsed 3 times each with tap water and distilled or deionized water. Glassware should then be rinsed with clean 10% HCl, followed by several rinses with distilled or deionized water. Cleaning is completed by an acetone rinse followed by several rinses with distilled or deionized water.

9.2.2. Test Design

The basic design and conditions for performing a 10-d toxicity test with solid-phase dredged material are given in Table G-15. In a typical test, one or more dredged material samples will be compared to a disposal site sediment sample. Ideally, disposal site sediment samples will have similar physical properties (e.g., grain size, organic matter) as the dredged material samples. In addition, a clean control sediment which serves as a basis for evaluating biological performance criteria for the test and determining test acceptability should be run simultaneously. The control material can be laboratory specific; however, previous testing should have demonstrated that the test organisms routinely survive and grow in an acceptable manner in the sediment. Exposures consist of a minimum of five replicates of each test sample plus a minimum of five replicates each of the disposal site material and control sediment in an exposure system designed to renew water overlying the sediment. The replicates for each test, disposal site, or control sediment should all be contained within their respective aquaria. Sediments from different sources should not be mixed within an aquarium.

Table G-15. Overview of Recommended Conditions for the 10-d Larval Survival and Growth Toxicity Test with *Chironomus tentans* and Solid-Phase Dredged Material.

1.	Test Type	Solid-phase sediment toxicity test with daily renewal of overlying water.
2.	Temperature	23±1°C
3.	Light quality	Fluorescent bulbs (wide spectrum)

Table G-15 (continued)

4.	Light intensity	10-20 uE/m^2/s, 540-1080 lux, or 50-100 ft-C (ambient laboratory levels).
5.	Photoperiod	16 h light, 8 h dark
6.	Test chamber	300 mL high-form beaker with two opposing holes (1.5 cm diameter centered 7.7 cm high above the bottom and covered with 60 mesh stainless steel screen).
7.	Sediment volume	100 mL
8.	Overlying water volume	150-175 mL; variable due to water renewal siphoning cycle.
9.	Renewal of overlying water	Two volume additions per day.
10.	Age of test organisms	Second or third instar larvae (all organisms must be at third instar or younger with at least 50 percent of the organisms at third instar).
11.	No. organisms per test chamber	10
12.	No. replicate test chambers per sample	5 minimum
13.	Total number of organisms per sample	50 minimum
14.	Feeding regime	Feed 1.5 mL daily to each beaker of blended Tetrafin® goldfish food containing 6.0 mg of dry solids.
15.	Aeration	Aerate if dissolved oxygen drops below 40% of saturation (i.e., 3.40 mg/L at 23°C).
16.	Overlying water	Similar to culture water or, if desired, site water should be measured twice during the test.
17.	Water quality monitoring	Daily measurements of water temperature and dissolved oxygen. Hardness, alkalinity, specific conductance, pH and total ammonia
18.	Test duration	10 d
19.	Test endpoints	Larval survival and growth (dry weight).
20.	Test acceptability	70% or greater survival in the control sediment. Maintenance of

		dissolved oxygen at ≥40% saturation and mean temperature of 23 ± 1°C. Test initiated with healthy, 8-12 day old (post-hatch) larvae. Satisfactory performance in reference toxicant test.
21.	Sample requirements	Storage of dredged material is at 4°C. Test should be initiated within 2 weeks of sample collection, and must be initiated with 8 weeks of collection.
22.	Dredged material volume required	A minimum of 500 mL from each test and disposal site.

The automated renewal of overlying water within each test chamber has a definite advantage in reducing labor hours as compared to manual renewal of water (Ankley et al. 1993). Several types of automated water renewal systems have been used, and are presented here as delivery system options. Any method of water renwal is acceptable, provided the recommended volumes of overlying water and their renewal rates are maintained, along with the recommended physical and chemical characteristics of the overlying water. Mount/Brungs (1967) diluters have been modified for use in sediment testing, and other automated water delivery systems have been used, as well (e.g., Maki 1977, Ingersoll and Nelson 1990, Benoit et al. 1993, Zumwalt et al. 1994).

Thoroughly homogenized sediment (100 mL) is added to each 300 mL high-form exposure beaker, and the sediment allowed to settle for 24 h in the test system before introduction of test organisms. The overlying water flows over the sediment during this 24 h period at approximately two volume additions per day.

A summary of daily activities prior to and during a test is presented in Attachment S. This schedule assumes that all materials are on hand, and that a healthy culture of animals is being maintained.

9.2.3. Test Chambers

Five 300 mL high-form beakers with side-walls drilled and screened (two opposing holes of 1.5 cm diameter, centered 7.7 cm up from the beaker floor, and covered with 60 mesh stainless steel screen) are required for each dredged material, control or disposal site sediment sample. The screened holes allow for renewal of overlying water, thereby allowing for a renewed supply of dissolved oxygen.

A single test site sediment and a disposal site sediment can be tested simultaneously in a portable mini-flow exposure system

of the size described in Benoit et al. (1993), using five replicates per sample. A maximum of 12 samples (60 total replicates), including reference and control samples, can be tested simultaneously in a modified mini-diluter system.

9.2.4. Water Renewal

Laboratory culture water or water with similar characteristics may serve as overlying water for the exposures. The overlying water should be of high quality whereby it does not contribute contaminants to the exposure system. In certain projects, it may be desired that disposal site water be used; however, this may prove formidable from a logistical standpoint. The exposure system is set to provide approximately two volume additions per day. This renewal rate will likely require supplemental aeration for many sediments. Aeration of the overlying water should be initiated if the dissolved oxygen concentration drops to 40 percent of saturation or below (i.e., 3.40 mg/L at 23°C). Overlying water within the tanks in which the exposure chambers are positioned should be aerated for all samples if the dissolved oxygen concentration drops to 40 percent or less in one or more exposure chambers.

9.2.5. Temperature and Photoperiod

Tests should be performed at 23±1°C. The daily photoperiod should be 16 L:8 D, using ambient laboratory lighting of 50-100 ft-C.

9.2.6. Organism Introduction

A sufficient number of second and third instar larvae (8-12 d post-hatch) are removed from the paper toweling or sand substrate in the culture rearing chamber to provide 10 organisms per replicate. They should be handled gently in freeing them of substrate, and placed directly into randomly chosen test beakers, after which each beaker is returned to its respective test holding tank. Larvae with their cases may first be withdrawn from the culture chamber with a fire-polished wide-bore pipet and transferred to an enamel pan containing culture water. The larvae may be gently forced out of their cases by touching the ends of their cases with a small, soft-bristled artist's paint brush. Larvae may also be transferred in their cases if they are not readily removed. Their presence inside of their cases can be confirmed by placing them into a transparent dish and inspecting them under a dissecting scope with strong backlight conditions.

9.2.7. Food and Feeding

Tetrafin® goldfish food should be prepared in distilled water to yield a concentrated suspension of 4.0 mg dry solids/mL (Ankley et al. 1993). Since stock culture food (56 mg dry solids/mL) is 14 times more concentrated than the desired concentration for feeding in a toxicity test, dilute 71.5 mL of

thoroughly mixed culture food concentrate to 1,000 mL with distilled water to yield the test food concentration of 4 mg dry solids/mL. Each replicate test beaker receives 1.5 mL of well-mixed food suspension daily. The food should be stirred between each replicate feeding. A total volume of 75 mL is required for the duration of the test (10 d) for each type of sediment sample (i.e., dredged site, disposal site and control) containing five replicates.

9.2.8. Test Organism Monitoring

Observe the beakers daily. The chironomids will form cases in the sediment, and most likely will not be visible if they are in good health. The openings of their tubes, however, may be visible. Organisms on the sediment surface that are not inside cases may be indicative of a stressful environment. Record the observations for each beaker.

9.2.9. Water Quality Monitoring

Water should be monitored daily for temperature and dissolved oxygen concentrations. The temperature should be maintained within ± 1°C of the desired temperature (23°C) at all times. Dissolved oxygen concentrations should be maintained at or above 40 percent of saturation. Hardness, alkalinity, specific conductance and pH should be measured at the beginning and end of the test from one of the replicates. Because ammonia may be elevated in some test sediments (e.g. Ankley et al. 1991a), measurement of total ammonia may aid in test interpretation. Total ammonia should be measured near the beginning and end of each test. It may be desirable to have an additional replicate for chemistry measurements only. Water quality parameters should be recorded on a data form (see Attachment R, Form R2).

9.2.10. Test Termination

After 10 d of exposure, sediment from each replicate is sieved through a fine-meshed screen sufficiently small to retain the fourth instar larvae (e.g., U.S. Standard No. 30, having a 0.59 mm mesh size). Larvae are placed into a crystallizing dish or beaker containing culture water and, if necessary, viewed under a dissecting microscope to determine if the larvae are alive. A small volume of carbonated water may be added to the volume of water in a beaker to immobilize the larvae, thereby facilitating their transfer to a weighing pan. Surviving larvae are freed of any remaining substrate and placed into a pre-weighed aluminum weighing pan. The larvae are then oven-dried for at least 4 h at 100°C (until a steady weight is obtained). The sample is allowed to come to room temperature in a desiccator, and weighed to the nearest 0.01 mg. The larvae from a given replicate are weighed together.

9.2.11. Data Reporting and Statistical Analysis

See Section 12.

10.0 *Hyalella azteca* **SOLID-PHASE TOXICITY TEST**

Hyalella azteca (Figure G-10) is a freshwater crustacean (Amphipoda:Talitridae) which is widely distributed in North America and South America (Pennak 1989). This species was chosen as a test organism for several reasons: (1) ease of culturing and testing, (2) widespread and common occurrence, (3) rapid growth and short generation time, (4) ecological importance, (5) close association with sediments and (6) sensitivity to a variety of environmental pollutants. *Hyalella azteca* is an omnivorous feeder. It prefers foods high in protein (de March 1981) and will browse on the film of bacteria and microscopic plants, animals and organic debris (aufwuchs) covering leaves, stems and other substrates (Pennak 1989). Bluegreen and green algae are less preferred as food and are not assimilated as efficiently (de March 1981). In most amphipods, and probably *H. azteca* as well, food is held by the gnathopods and anterior pereiopods and chewed directly (Pennak 1989).

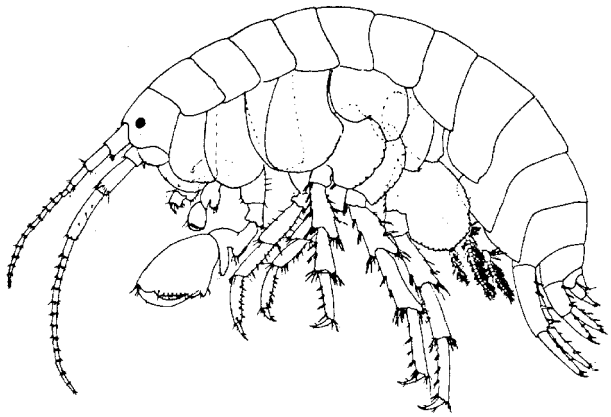


Figure G-10. *Hyalella azteca*, X14 (From Cole and Watkins 1977).

Reproduction by *H. azteca* is obligately sexual. Males pair with females by grasping the females (amplexus) with their enlarged second gnathopods while on the backs of the females. After feeding together for 1 to 7 days the female is ready to molt and the two animals separate for a short time while the female sheds her old exoskeleton. Once the exoskeleton is shed, the two animals reunite and copulation occurs. The male places sperm near the marsupium of the female and her pleopods sweep the

sperm into the marsupium. The animals separate and the female releases eggs from her oviducts into the marsupium where they are fertilized. *H. azteca* averages about 18 eggs per brood (Pennak 1989) with larger animals having the most eggs (Cooper 1965).

The developing embryos and newly hatched young are retained in the marsupium until the next molt. At 24 to 28°C, hatching has been reported to occur from 5 to 10 d after fertilization (Bovee 1950, Cooper 1965, Embody 1911). The time between molts for females is 7 to 8 d at the temperature range of 26 to 28°C (Bovee 1950); therefore, about the time embryos hatch, the female molts and releases the young. *H. azteca* averages 15 broods in 152 d (Pennak 1989). Pairing of the sexes is simultaneous with embryo incubation of the previous brood in the marsupium.

H. azteca has a minimum of nine instars in its life history (Geisler 1944). There are 5 to 8 pre-reproductive instars (Cooper 1965) and an indefinite number of post-reproductive instars. The first five instars form the juvenile stage of development, instar stages 6 and 7 form the adolescent stage when sexes can be differentiated, instar stage 8 is the nuptial stage and all subsequent instars are the adult stages of development (Pennak 1989).

Occurrence of *H. azteca* is most common in warm (20-30°C for much of the summer) mesotrophic or eutrophic lakes which support aquatic plants and periphyton. It is also found in ponds, sloughs, marshes, rivers, ditches, streams and springs, but in lower numbers. They have achieved densities of >10,000m^2 in preferred habitats (de March 1981).

Hyalella azteca avoids bright light, preferring to hide under litter and feed during the day. Activity levels increase at night; however, de March (1977) reported that during a laboratory study conducted with a 16-h light and 8-h dark photoperiod and 20 to 30°C, *H. azteca* reproduced well at 55 μE/m2/s but not at 12 μE/m^2/s light intensity. (Average room light intensity is 10 μE/m^2/s.) She also reported that a photoperiod duration of 16 h or more was conducive to reproductive success. However, some laboratories (e.g., U.S. FWS Columbia, MO; UW-Superior; USEPA, Duluth, MN) have reported successful reproduction at light intensities of 8 to 16 μE/m^2/s.

Temperatures tolerated by *H. azteca* range from 0 to 33°C (Bovee 1949, Embody 1911, Sprague 1963). At temperatures less than 10°C the organisms rest and are immobile (de March 1977, 1978). At temperatures of 10 to 18°C some reproduction occurs and juveniles grow slowly into large adults. Smaller adults result when organisms are grown at temperatures in the range of 18 to 28°C and reproductive output is high. The highest

reproduction occurs in the temperature range of 26 to 28°C (de March 1978) while lethality, due to temperature, occurs at 33 to 37°C (Bovee 1949, Sprague 1963).

Hyalella azteca is found in widely varying water conditions in which dissolved oxygen can range from saturation to very stagnant conditions. Sprague (1963) reported a 24-h LC50 at 20°C of 0.7 mg/L dissolved oxygen, and Pennak and Rosine (1976) reported a similar value. Little is known of *H. azteca's* preference for various ions or ionic concentrations in water. de March (1981) reported that *H. azteca* was not observed when calcium was less than 7 mg/L and that salinity to the concentration of sea water is tolerated if the organisms are acclimated slowly to increasing concentrations of seawater ions.

Hyalella azteca tolerate a wide range of substrate conditions. Ingersoll and Nelson (1990) reported that they tested *H. azteca* in long-term studies using sediments ranging from more than 90% silt- and clay-sized particles to 100% sand-sized particles without detrimental effects on either survival or growth of the organisms. Ankley et al. (1993) found that organisms tested on quartz sand with four water renewals/d without food had poor survival (30%) and that feeding a 0.8 mg/d ration of yeast, cereal leaves and trout chow yielded better survival (90%). Organisms tested in the same system with a sediment containing about 8% organic carbon did not benefit from feeding. Therefore, feeding of tests is necessary to eliminate the confounding effect of sediment organic carbon content.

A number of studies have used *H. azteca* to assess toxicity of sediments (e.g., Nebeker et al. 1984a, Borgmann and Munawar 1989, Ingersoll and Nelson 1990, Ankley et al. 1991a,b) with favorable results. The amphipods are often among the most sensitive species tested.

This report describes methods used to culture *H. azteca,* to perform a 10-d exposure of this organism to solid-phase sediments with either an intermittent- or continuous-flow overlying water renewal system; and methods for data analysis. The endpoint in the toxicity test is survival, although growth can also be monitored as an endpoint in this test (Ingersoll and Nelson 1990).

10.1. CULTURE METHODS

The culturing methods described in this report are based on the methods developed by USEPA (Denny and Collyard 1991, Denny et al. 1993). Other culture methods have been successfully used for *Hyalella azteca* (e.g., ASTM 1993e); any of these are acceptable provided that a stable stock of healthy, reproducing test animals results. Required materials are listed in Attachment T.

10.1.1. Organism Source

Organisms for the initiation of a laboratory culture should be obtained from a source which has a verified culture of *H. azteca*. Organisms that are adapted to laboratory conditions and free of disease must be used as the brood stock. Juveniles or adults are equally suited for the initial stock and are easy to transport. Stock from wild populations should be avoided unless cultured through at least one generation to ensure their identity is verified, they are disease-free and of adequate vigor (Denny et al. 1993). Starter cultures are available from several government and commercial suppliers (Attachment U).

10.1.2. Acclimation

Environmental stress on the organisms in the starter culture must be minimized to facilitate normal growth and embryo production. The temperature of the water containing the brood stock animals should be measured upon their arrival and gradually adjusted to the desired culture temperature of 23±1°C. Changes in water temperature >2°C in any 24-h period should be avoided and, in general, water temperature should not change more than 3°C in a 72-h period (ASTM 1993a). The dissolved oxygen concentration should be maintained between 60 and 100% of saturation. Gentle aeration (1 bubble/sec/L of water from an air line terminating with a disposable glass pipet with an approximate 1mm diameter opening) with oil-free compressed room air is desirable. Supersaturation by dissolved gases should be avoided to prevent entrapment of organisms at the water surface. If the culture medium differs in hardness, alkalinity or pH from that in which the organisms were received, animals from the starter culture should be transferred to the new culture medium gradually over a period of 1 to 2 d to avoid stress (ASTM 1993a).

Reproductively mature (≥30-d old when cultured at 23°C) individuals must be segregated into breeding groups to successfully produce embryos of known age.

10.1.3. Reference Organism

It is recommended that several organisms in the brood stock, especially when obtained from wild populations, be examined by a competent invertebrate taxonomist to ensure that the brood stock is a pure culture of *Hyalella azteca*. Several taxonomic references are available to distinguish members of the crustacean Order Amphipoda (e.g., Bousfield 1958, Pennak 1989, Covich and Thorp 1991). Verification should be documented in writing, including the name of the individual responsible for the taxonomy, the taxonomic key used, the date of identification and the source of the individuals used in the identification.

10.1.4. Culture Chambers

Chambers for mass culturing of *H. azteca* may be constructed

of glass, plastic, fiberglass, or stainless steel, although glass or plastic is preferred. An example of a single culturing system would be a 2-L glass battery jar or polycarbonate beaker, although larger aquaria work well, too. The unit must be acid washed with 1N HCl or HNO_3 and rinsed with deionized water to remove any manufacturing residues. One air line with a disposable glass pipet attached serves each chamber.

White translucent plastic dishwashing pans commonly available in stores (e.g., 53 cm x 40 cm x 12 cm, L x W x H) make good sorting containers for separation of adults from juveniles. The water temperature within this pan should be maintained at 23±1°C. Juveniles and adults can easily be seen in the pans if placed in bright light or on a light table. A supply of low pressure (≈3 psi) air (compressed, oil-free room air) is needed to mix the water in the culture chambers to keep the culture water from becoming supersaturated with dissolved oxygen due to the abundance of green algae in the cultures.

10.1.5. Culturing Substrates

Hyalella azteca hide beneath any available materials during the light portion of the photoperiod. For cultures, suitable culturing substrates have been provided with presoaked maple (*Acer* ap.), poplar (*Populus* sp.), alder (*Alnus* sp.) or birch (*Betula* sp.) leaves (Ingersoll and Nelson 1990; Nebeker et al. 1984a). Other more standardized choices are plastic mesh and presoaked cotton gauze. Plastic mesh of 10 to 15 meshes/cm and either 100% cotton cheesecloth or surgical gauze works well (Borgmann and Munawar 1989, Denny et al. 1993). Plastic mesh large enough to stand obliquely in the culture chamber or a single 10 x 15 cm piece of cotton gauze added to each culture chamber works well with about 50 adults present in the chamber.

10.1.6. Culture Water

An adequate supply of water, such as spring, well, reconstituted (ASTM 1993a; Attachment B) or controlled surface water, is necessary to culture *H. azteca*. Water quality parameters of hardness, alkalinity, conductivity and pH should fall within the following ranges: hardness, 60-300 mg/L as $CaCO_3$; alkalinity, 50-300 mg/L as $CaCO_3$; conductivity, 50-500 μmhos/cm; and pH, 6.5 to 9.0. Dechlorinated water can be used when dechlorinated with sodium bisulfite (sodium sulfite can be used but is less desirable), which also removes chloramines (ASTM 1993a), or by dechlorination with aeration in an open chamber of sufficient retention time (>1h) to completely remove the chlorine and chloramines. Chemical monitoring of the water for residual chlorine or chloramine concentration must be conducted to ensure that concentrations of these chemicals do not exceed 3 μg/L. Municipal drinking water may contain copper, lead, zinc and fluoride which can be removed, when excessive, by using

appropriate ion-exchange resins (ASTM 1993a). The national water quality criteria to prevent chronic effects on freshwater organisms exposed to copper, lead and zinc are 12, 3.2 and 110 μg/L, respectively, at a water hardness of 100 mg/L as $CaCO_3$ (USEPA 1987). Different chronic values must be calculated if the hardness of the culture water differs from 100 mg/L as $CaCO_3$. No criterion is available for fluoride.

10.1.7. Temperature and Photoperiod

Water temperature for culturing *H. azteca* should be maintained at 23±1°C. This temperature is suitable for reproduction, incubation and growth. Temperatures below 15°C and above 28°C reduce reproduction of *H. azteca* (de March 1977, 1978).

A photoperiod of 16 h light and 8 h dark during each 24-h period is recommended. Wide spectrum fluorescent lights with minimum luminescence of 10 to 20 $\mu E/m^2/s$ (540-1080 lux; 50-100 ft-c) at the water surface are preferred.

10.1.8. Food and Feeding

Adult and juvenile *H. azteca* are fed three times each week (MWF) 10 to 15 mL/L of yeast-Cerophyll®-trout chow (YCT) mixture, and a 60-mL inoculum of a green alga (a single cell alga such as *Ankistrodesmus* sp. works well but filamentous green algae will also work) at the time of culture renewal (Attachment V). This results in a large green algal population in the culture chamber in 48 to 72 h.

Other commercial diets (e.g., Tetrafin®, TetraMin®, rabbit chow) also are acceptable. However, they should result in a similar rate of growth in adults and a similar rate of young production as the recommended diet.

10.1.9. Chamber Cleaning

Chambers containing breeding *H. azteca* should be washed weekly when the culture chambers are renewed. Satisfactory cleaning is accomplished by washing the culture chambers with soap and rinsing with either distilled, deionized or culture water.

10.1.10. Handling

Care must be taken to avoid disturbance of the juvenile and adult *H. azteca* by unnecessary movement, noise, or extraneous lighting. Organism handling should be kept at a minimum. Juvenile and adult *H. azteca* can be carefully transferred using a glass or clear plastic pipet which has a polished end with a 6 mm diameter opening. Organisms must be quickly released below the water surface to avoid stress.

10.1.11. Water Quality Monitoring

Water used to culture *H. azteca* should be monitored for temperature (standardized alcohol or electronic thermometer), dissolved oxygen (titrimetric or ion selective electrode), hardness (titrimetric total hardness), alkalinity (titrimetric total alkalinity), conductivity (conductivity meter), pH (electrode method) and any other characteristics [e.g. chlorine and chloramines (residual chlorine ion specific electrode method, Rigdon et al. 1978), sulfides (iodometric method, APHA 1985)] useful to indicate consistent quality. Temperature should be measured daily and dissolved oxygen twice weekly in the culturing tanks. Hardness and alkalinity should be measured weekly at the water supply source to the chambers. Because ammonia may be elevated in some test systems, measurement of total ammonia to ensure a concentration of <0.1 mg/L may aid in culturing success. Total ammonia should be measured near the end of each culture solution renewal.

10.1.12. Juvenile Production

Visually inspect the contents of all culture chambers every seventh day for juvenile production by pouring the contents of each culture chamber into a translucent white plastic pan (use of a light box to see the organisms is recommended). After the adults are removed, the remaining organisms will be the juveniles ranging in age from <1 to 7 d. When juveniles are present, transfer them with a 6 mm i.d. pipet to a 1-L beaker for holding for one week in preparation for a toxicity test, or place them into a mass culture chamber for use as brood adults later. Count the number of adults and juveniles in each culture chamber and record the counts on the culture record sheet (Attachment W). These records are useful to determine if cultures are maintaining a vigorous reproductive rate indicative of culture health.

The brood-board method is an alternative method of culturing *H. azteca* to produce juveniles of known ages. Mated adults are placed in a small beaker or plastic cup (one pair per chamber) and fed an amount proportionate to the larger mass culture chambers. A substrate may be added to each chamber but is not needed. The chambers may be inspected daily for production of offspring (3 to 4 young per female per week for peak reproduction rate); therefore, ages of juveniles can be more precisely determined than those produced in mass culture chambers.

10.1.13. Culture Evaluation

Brood stock evaluation is based upon survival and reproductive rate of the adults. Counts of surviving adults, breeding pairs and young production should be made at the time of culture renewals and the information should be recorded (e.g., Attachment W, Form W1). Some adult *H. azteca* can be expected to die in the culture tanks between weekly renewals, but any

unusually high death rate for the week in one of the brood stock chambers when compared to previous weekly mortalities should be a cause for concern. The first symptom of problems in the culture chambers is a reduction in the reproductive rate. Typical reproductive rates in culture chambers containing 50 adults range from 75 to as high as 100 juveniles per week. Once a reproductive rate has been established, any decrease in this rate can be attributed to a change in water or food quality, or brood stock health. Adult females usually continue to reproduce for several months; however, their fertility will gradually decrease as senescence approaches (≈100 d).

The <1- to 7-d old amphipods are held in separate culture chambers with presoaked cotton gauze for an additional 7 d, at which time the 7- to 14-d old *H. azteca* are used in sediment toxicity tests. During the seven days, the juveniles are fed 10 to 15 mL of YCT daily. After separation from the adults, each batch of juveniles should be observed daily for survival until used for testing. If >20% of the juveniles die during this time interval, that batch of juveniles should be considered unsuitable for testing.

10.1.14. Culture Records

A separate set of records should be maintained for the culture unit. The records should show dates of renewal of culture chambers and the estimated number of surviving adults and production of juveniles per culture chamber. In addition, there should be daily records (Attachment W) of water temperature and feedings.

10.2. TOXICITY TEST METHODS

10.2.1. Solid-Phase Sediment Preparation

See Section 5.0.

10.2.2. Test Design

The basic design and conditions for performing a 10-d toxicity test with solid-phase dredged material are given in Table G-16. In a typical test, one or more dredged material samples will be evaluated by comparison with a disposal site sediment sample. In addition, a control sediment which serves as a basis for evaluating biological performance criteria for the test should be run simultaneously. The control material can be laboratory specific; however, previous testing should have demonstrated that test organism survival is routinely >80 percent, and that they grow and reproduce in the sediment. Exposures consist of a minimum of five replicates of each test sample and disposal site material in an exposure system designed to renew water overlying the sediment. The replicates for each test disposal site or control sediment should all be contained

within their respective aquaria. Sediments from different sources should not be mixed within an aquarium.

Table G-16. Overview of Recommended Test Conditions for the 10-d Solid-Phase Dredged Material *Hyalella azteca* Survival Toxicity Test.

1.	Test Type	Solid-phase sediment toxicity test with renewal of overlying water (2 volume additions/day).
2.	Temperature	23±1°C
3.	Light quality	Fluorescent bulbs (wide spectrum)
4.	Light intensity	10-20 µE/M^2/s, 540-1080 lux, or 50-100 ft-c
5.	Photoperiod	16 h light, 8 h dark
6.	Test chamber	300-mL high-form beaker
7.	Test sediment volume	100 mL
8.	Overlying water volume	150-175 mL; variable due to water renewal siphoning cycle
9.	Renewal of overlying water	2 volume additions/day done continuously or intermittently, such as one volume addition every 12 h
10.	Age of test organisms	7- to 14-days old
11.	No. of organisms per test chamber	10
12.	No. replicate test chambers per treatment	5 minimum
13.	No. organisms per treatment site	50 minimum
14.	Feeding regimen	YCT[a] food, fed 1.5 mL daily to each test chamber.
15.	Aeration	Add aeration to each test chamber if dissolved oxygen in overlying water falls below recommended minimum in any test chamber.
16.	Overlying water	Culture water, test site water, well water, surface water or reconstituted water.

Table G-16. (continued)

17.	Test chamber cleaning	If test chamber screens become clogged during the test, gently brush outside of screen only.
18.	Dissolved oxygen minimum	40% of saturation; if this saturation level cannot be maintained, add aeration to each large test chamber.
19.	Test duration	10 d
20.	Endpoint	Survival and growth.
21.	Test acceptability	80% or greater survival in the control sediments; dissolved oxygen $\geq$40% saturation; mean test temperature 23±1°C; and satisfactory results from a reference toxicant test.
22.	Sample requirements	Storage of dredged material at 4°C; sediment should be sieved and homogenized and tests initiated as soon as possible but must be within 8 weeks of collection.
23.	Sediment volume required	A minimum of 500 mL from each test and disposal site.

[a] YCT is a food mixture comprised of yeast, cereal leaves and trout chow (see Attachment V).

The automated renewal of overlying water within each test chamber has a definite advantage in reducing labor hours as compared to manual renewal of water (Ankley et al. 1993). Several types of automated water renewal systems are available (e.g., Mount/Brungs 1967, Maki 1977, Ingersoll and Nelson 1990, Benoit et al. 1993, Zumwalt et al. 1994), and are presented here as options. Any method of water renewal is acceptable, provided the recommended volumes of water and their renewal rates are maintained, along with the recommended physical and chemical characteristics of overlying water.

Homogenized [sieved through a coarse (5 mm) screen then blended for a few minutes in the storage container with a wooden or plastic rod] sediment (100 mL) is added to each 300-mL high-form (taller than standard 300-mL beakers) exposure beaker, and the sediment allowed to settle for 24 h in the test system before introduction of test organisms. The overlying water flows over the sediment during this 24-h period at approximately two volume additions/d.

A summary of daily activities prior to and during a test is presented in Attachment X. This schedule assumes that all materials are on hand, and that a healthy culture of animals is being maintained.

10.2.3. Test Chambers

Five 300-mL high-form (taller than standard 300-mL beaker) beakers with side-walls drilled and screened (two opposing holes of 1.5 cm diameter, centered 7.7 cm up from the beaker floor, and covered with 60 mesh stainless steel screen) are required for each sediment sample tested (Benoit et al. 1993). The screened openings facilitate the exchange of water over the sediments when the water renewal system operates. The five beakers containing replicate samples of a test sediment must be in the same larger test chambers containing the overlying water with no mixing of sediments from other sites.

A single test site sediment (5 total replicates) and a disposal site sediment can be tested simultaneously in a portable mini-flow exposure system of the size described in Benoit et al. (1993) using five replicates per sample. Other types of renewal systems can be used (see Section 10.2.2.). At the time of construction, the dimensions of the portable mini-flow exposure system can be enlarged to the proportions necessary to accommodate tests for a larger number of sediment samples. A maximum of twelve samples (60 total replicates) can be tested simultaneously in a modified mini-diluter system (Benoit et al. 1993).

10.2.4. Water Renewal

Laboratory culture water or water with similar characteristics may serve as overlying water for the exposures. In certain projects, it may be desirable to use disposal site water; however, this may prove formidable from a logistical and test organism acclimation standpoint. The exposure system should be set to provide approximately two volume additions/d. This renewal rate may not result in satisfactory dissolved oxygen levels for most sediments. If the recommended minimum dissolved oxygen concentration cannot be maintained in all test beakers, then each chamber containing the five replicate test beakers, including control and disposal site sediment containing chambers, must be aerated for the remainder of the test.

10.2.5. Temperature and Photoperiod

Tests should be performed at 23±1°C. The daily photoperiod should be 16 L:8 D with a light intensity of 50-100 ft-C provided by wide spectrum fluorescent lamps.

10.2.6. Organism Introduction

A sufficient number of 7- to 14-d old juveniles are removed

from the juvenile culturing beakers to provide 10 organisms per replicate. They should be handled gently (use a glass or plastic pipet with 6 mm diameter opening that has been fire polished) and placed beneath the water surface directly into randomly chosen test beakers, after which each beaker is returned to its respective test holding tank. Any organisms trapped (floaters) in the water surface tension must be submerged with a drop of water or a blunt-ended probe. If some test organisms persist in floating, they can be removed and replaced during the first 24 h of the test.

10.2.7. Food and Feeding

Previously prepared YCT (Attachment V), which has been kept refrigerated (it should not be more than 14-d old), is fed daily at the rate of 1.5 mL to each test chamber. Food should be added at the end or after an overlying water renewal cycle to prevent food from leaving the test systems. YCT is prepared (Attachment Y) at a concentration of 1800 mg/L dry solids; therefore, feeding 1.5 mL results in 2.7 mg dry solids/feeding/test beaker. The food should be stirred or shaken before each feeding. A total volume of 75 mL is required for the duration of the test (10 d) for each sample of five replicates.

10.2.8. Test Organism Monitoring

Observe the contents of the beakers daily. The amphipods will burrow in the sediment or graze upon the sediments and may not be visible if they are in good health. A stressful environment may be indicated when all or most organisms in a beaker are observed to be persistently darting about in the overlying water and are apparently not feeding on the sediments. Dead or severely affected organisms, when they occur, may be seen lying motionless on the surface of the sediment or floating at the water surface. Record the observations for each beaker.

10.2.9. Water Quality Monitoring

Water should be monitored daily for temperature, and dissolved oxygen concentrations must be measured on even numbered days plus d 1. The temperature should be maintained within ±1°C of 23°C. Dissolved oxygen concentrations must be maintained at or above 40 percent of saturation. Hardness, alkalinity, specific conductance and pH should be measured near the beginning (d 1) and near the end (d 9) of the test from one of the replicates (Attachment W). Because ammonia may be elevated in some test sediments (e.g., Ankley et al. 1990), measurement of total ammonia may aid in test interpretation. Total ammonia should be measured near the beginning (d 1) and end (d 9) of each test. Determination of the worst case conditions for dissolved oxygen and ammonia is made by measuring concentrations just above the test sediment prior to the next overlying water renewal cycle. Water quality parameters should be recorded on a data

form (see Attachment W, Form W2).

10.2.10. Test Termination

After ten d of exposure, easily captured organisms can be removed with a pipette to save time before sediment from each replicate is sieved through a fine-meshed screen sufficiently small to retain the juvenile amphipods (e.g., U.S. Standard No. 30, having a 0.59 mm mesh size). Other methods of test organism removal from the test beakers such as swirling the overlying water with the pipet to lift organisms from the sediment may save time. Amphipods are placed into a clear viewing pan or beaker containing culture water and, if necessary, viewed under a dissecting microscope to determine if any movement occurs to indicate viability. Once survival determinations have been made, a determination for growth differences may be made. The live organisms should first be quickly killed by over dosing with an anesthetic or by freezing. There they are placed in a pre-dried and weighed metal pan, and placed in an oven at 100°C for about 4 h or at 60°C for about 12 h. After cooling the pans in a desiccator, the organisms are weighed to 0.01 mg.

10.2.11. Data Reporting and Statistical Analysis

See Section 12.

11.0. *Lumbriculus variegatus* CHEMICAL ACCUMULATION

Aquatic sediments are well known to act as sinks or reservoirs for nonionic, hydrophobic organic chemicals (Larsson 1985, 1986, Bierman 1990) and heavy metals (Malueg et al. 1984, Fallon and Horvath 1985, Poulton et al. 1988, Ankley et al. 1991a, West et al. 1993). They are also sources of toxicants, releasing chemicals into aquatic ecosystems through processes such as diffusion, resuspension and bioaccumulation through benthic and pelagic food chains (Rice and White 1987, Chapman 1988, Schuytema et al. 1988). The extent to which sediment-associated chemicals may be available to benthic organisms is of serious concern (DiToro et al. 1991).

Several studies have shown that hydrophobic organic compounds are bioaccumulated from sediment by freshwater infaunal organisms including larval insects, such as *Chironomus tentans* (Adams et al. 1985, Adams 1987) and *Hexagenia limbata* (Gobas et al. 1989); oligochaete worms, such as *Tubifex tubifex* and *Limnodrilus hoffmeisteri* (Oliver 1984, 1987, Connell et al. 1988); and by marine organisms, such as polychaete worms, *Nephtys incisa,* and molluscs, *Mercenaria mercenaria* and *Yoldia limatula* (Lake et al. 1990). Since these and related organisms are components of food webs containing higher consumers from all of

the vertebrate classes, the possibility exists for chemical bioaccumulation and/or biomagnification at higher trophic levels. It is important, therefore, to examine the uptake of chemicals by the benthos from contaminated sediments, as well as the toxicity of contaminated sediments to benthos.

Various species of organisms have been suggested for use in studies of chemical bioaccumulation from aquatic sediments. Several criteria should be considered before a species is adopted for routine use. These criteria include: ready availability of healthy organisms throughout the year, known chemical exposure history, adequate tissue masses for trace chemical analysis, ease of handling and tolerance of a wide range of sediment physico-chemical conditions (e.g., particle size), amenability to long-term exposures, and ability to accurately reflect concentrations of contaminants in field organisms (i.e., exposure is realistic). With these criteria in mind, the advantages and disadvantages of several potential freshwater taxa are discussed briefly below.

Freshwater clams provide an adequate tissue mass, are quite easily handled, and can be used in long-term exposures. However, few freshwater species are appropriate for testing, and the exposure is uncertain due to valve closure. Chironomids can be readily cultured, are quite easily handled, and reflect appropriate routes of exposure. However, large numbers of individuals are required to provide an adequate tissue mass for low-level residue analysis, and their rapid life-cycle makes it difficult to perform long-term exposures with highly hydrophobic compounds which equilibrate very slowly between sediment, pore water and animal tissue. Larval mayflies (i.e., *Hexagenia limbata*) reflect appropriate routes of exposure, have adequate tissue mass for residue analysis and can be used in long-term tests. However, they cannot be continuously cultured in the laboratory and consequently are not always available. Furthermore, the exposure history and health of field-collected individuals may be uncertain. Amphipods (e.g., *Hyalella azteca*) can be cultured in the laboratory, are easily handled, and reflect appropriate routes of exposure. However, their collective tissue mass may be insufficient for convenient trace residue analysis, and they are relatively sensitive to chemical parameters in the sediment. Although fishes (e.g., fathead minnows) provide an adequate tissue mass, are readily available and easily handled, and can be used in long-term exposures, they do not have the same routes of exposure to sediment-associated contaminants as benthic invertebrates.

As a group, oligochaetes represent infaunal benthic organisms that meet many of the test criteria described above. Certain oligochaete species are easily handled and cultured, provide reasonable biomass for residue analyses, and are tolerant

of varying sediment physical/chemical characteristics. Oligochaetes are exposed to contaminants via all appropriate routes of exposure, including pore water and ingestion of sediment particles. Various oligochaete species have been used in toxicity and bioaccumulation evaluations (Chapman et al. 1982a,b, Wiederholm et al. 1987, Keilty et al. 1988a,b), and field populations have been used as indicators of pollution of aquatic sediments (Brinkhurst 1980, Spencer 1980, Lauritsen 1985, Robbins et al. 1989).

Lumbriculus variegatus (Figure G-11) is a freshwater oligochaete that has been successfully cultured in the laboratory, and used in both chemical toxicity and bioaccumulation studies. Toxicity studies have been performed in water-only exposures of toxicants (Bailey and Liu 1980, Hornig 1980, Ewell et al. 1986, Nebeker et al. 1989, Ankley et al. 1991a,b), in effluent tests (Hornig 1980), and in solid-phase sediment toxicity evaluations (Nebeker et al. 1989, Ankley et al. 1991a,b, 1992b,c, Call et al. 1991, Carlson et al. 1991, Phipps et al. 1993, West et al. 1993). Several studies have reported the use of *L. variegatus* to examine chemical bioaccumulation from the sediment (Schuytema et al. 1988, Nebeker et al. 1989, Ankley et al. 1991a, 1992a, Call et al. 1991, Carlson et al. 1991).

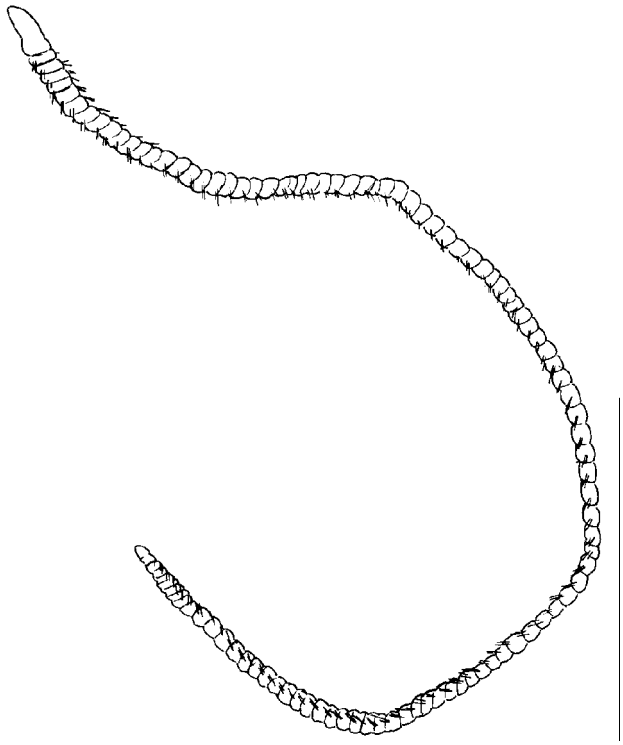


Figure G-11. *Lumbriculus variegatus* adult, X 10.

Lumbriculus variegatus inhabits a variety of sediment types throughout the United States and Europe (Chekanovskaya 1962, Cook 1969, Spencer 1980, Brinkhurst 1986). It typically inhabits the upper aerobic zone of sediments from reservoirs, rivers, lakes, ponds and marshes, in which it will tunnel through the sediment while actively feeding. When not tunneling, it will bury its anterior portion in the sediment and undulate its posterior portion in the overlying water for respiratory exchange.

Lumbriculus variegatus adults attain body lengths as great as 40 to 90 mm and a diameter from 1.0 to 1.5 mm (Phipps et al. 1993). They may vary in wet weight from about 5-12 mg (Call et al. 1991, Phipps et al. 1993). The lipid content of the animals is about 1.0 percent on a wet weight basis (Ankley et al. 1992b). They most commonly reproduce asexually by architomy or budding, although they can reproduce sexually (Chekanovskaya 1962). Sexual reproduction appears to occur infrequently, at least in culture, as newly hatched worms have never been observed in cultures at the University of Wisconsin-Superior or the Environmental Research Laboratory (ERL)-Duluth (Phipps et al. 1993). Rather, the cultures appear to consist of adults of various sizes. Under culturing conditions at ERL-Duluth, the population doubles every 10-14 d at 20°C (Phipps et al. 1993).

The use of *L. variegatus* in laboratory bioaccumulation studies has been field-validated with natural populations of oligochaetes. After a 30-d laboratory exposure of *L. variegatus* to sediments from the lower Fox River and Green Bay, Wisconsin, total PCB concentrations in laboratory-exposed *L. variegatus* compared well with concentrations measured in field-collected oligochaetes from the same sites (Ankley et al. 1992a). PCB homologue patterns also were similar between laboratory-exposed and field-collected oligochaetes, with a tendency for the more highly chlorinated PCBs to show slightly greater bioaccumulation in the field-collected organisms. In contrast, a comparison of total PCBs in laboratory-exposed fish (*Pimephales promelas*) and field-collected fish (*Ictalurus melas)* revealed poor agreement in bioaccumulation relative to sediments.

This report provides methods used in studies of chemical bioaccumulation from aquatic sediments using the oligochaete, *L. variegatus*. It describes methods for maintaining a continuous culture, collection and preparation of sediment, preparation of the exposure system, performance of the exposure, and treatment of the data.

11.1. CULTURE METHODS

The culturing methods described below are based on methods in use at ERL-Duluth (USEPA) and described in "Standard Operating

Procedure for the Culture of *Lumbriculus variegatus*" (Juenemann and Denny 1992) and a methods paper by Phipps et al. (1993). Required materials are listed in Attachment Y.

11.1.1. Organism Source

Organisms for the initiation of a laboratory culture may be obtained from a laboratory (Attachment Z) with a verified culture. Collection from the field should be avoided to eliminate the possibility of initiating a culture with a different species. Organisms received should be carefully examined by a qualified taxonomist using a key (e.g., Brinkhurst and Cook 1966, Brinkhurst 1986, Pennak 1989) to verify the species. One or more organisms should be cleared and mounted to serve as reference material. Methods for clearing and mounting aquatic oligochaetes are provided in Stimson et al. (1982) and Pennak (1989). Organisms should all be of a single species, *Lumbriculus variegatus*, and be disease-free. They should possess very low contaminant body burdens.

11.1.2. Acclimation of New Brood Stock

Environmental stress on the starter culture should be minimized to facilitate the rapid development of a healthy culture. Although *L. variegatus* is generally tolerant to changes in temperature, dissolved oxygen and pH (Phipps et al. 1993), it is prudent to habituate newly acquired organisms gradually to their new culture water. Measure the temperature of the water containing the stock animals upon their arrival and gradually adjust it to the desired culture temperature. A temperature of 20-23°C has been used for culturing (Juenemann and Denny 1992, Phipps et al. 1993). A temperature of 23°C is recommended for both culturing and testing, although many toxicity tests have been performed over a temperature range of 17-22°C. A gradual adjustment of the new brood stock water characteristics to those of the desired culture and test water may be accomplished by incremental dilution of the brood stock water with culture water over a period of two or more days. This is continued until the water meets the requirements for the desired culture water. Culture water should be maintained at the same temperature as the test water.

11.1.3. Culture Chambers

Standard 57-L glass aquaria are recommended for use as culture chambers. The water level should be maintained at a depth of about 25 cm, thereby providing a water volume of approximately 45 L.

11.1.4. Water Renewal

Due to the potential for a rapid increase in biomass, a relatively high culture water renewal rate (i.e., 20 volume exchanges per day) is recommended in a flow-through system. If a

lower renewal rate is used and oxygen concentrations are diminished, the animals will aggregate in clusters, necessitating aeration of the aquaria. The culture water can be from a variety of sources including untreated well water, dechlorinated tap water, natural surface (e.g., lake) water, or various reconstituted waters. Methods for preparing synthetic, reconstituted culture and dilution water are available (e.g., ASTM 1993h, USEPA 1989, 1993). When provided with adequate food, *Lumbriculus variegatus* appears quite tolerant of a wide variety of water quality characteristics (e.g., hardness, alkalinity, pH).

A static culture system can be used successfully if it is well aerated and carefully maintained. A regular schedule of water replacement is recommended. Static systems should be monitored frequently for dissolved oxygen concentrations.

11.1.5. Temperature and Photoperiod

The recommended temperature for culturing *L. variegatus* is 23±1°C. The recommended photoperiod is 16 h light and 8 h dark with a light intensity of approximately 50-100 ft-C (10-20 $\mu E/m^2/s$) at the water surface.

11.1.6. Substrate

Several substrates have been found to work well for culturing *L. variegatus*, including maple and/or poplar leaves, sand, clean sediments of high organic carbon content, and brown paper toweling (Bailey and Liu 1980, Phipps et al. 1993, Juenemann and Denny 1992). Toweling is recommended because it is readily available, uniform in composition, and allows for easy removal of animals.

Prepare the substrate by first unfolding ordinary brown paper towels and either cutting them into strips (about 2.5 cm wide) or passing them through a paper shredder. The strips are next placed into a conditioning tank. For conditioning, place a volume of dry towel strips (4,000 mL) into an aquarium equipped with two water lines, each having a flow capacity of 100 mL/min. One line is placed below and one above the towel mass. A glass weight, consisting of several 2.5 cm x 25.4 cm glass strips standing on edge and glued on both ends to glass strips approximately 50 cm in length, is placed on the mass to prevent floating. This method creates a uniform water flow throughout the mass of intertwined toweling strips, and minimizes fouling of the strips. The strips of paper are soaked in this manner for at least one week. Following substrate conditioning, the towel mass is removed and evenly distributed over the entire bottom of a culture chamber. A glass weight as described above is placed over the toweling to keep it in place.

Approximately 500-1,000 oligochaetes are transferred to the new culture chamber. The substrate is renewed with pre-conditioned towels when thin or bare areas appear (see Section 11.1.9., "General Culture Maintenance"). A fresh substrate will generally last for 2-3 months.

11.1.7. Food and Feeding

Aquatic oligochaetes ingest their substrate and are believed to obtain their nourishment from the organic matter in the substrate as it passes through their digestive tract (Pennak 1989). Food is provided to the cultures by distributing 10 mL (~5.5 g) of trout starter on the water surface three times weekly. The particles will temporarily disperse on the surface film, break through the surface tension, and settle out over the substrate.

11.1.8. Handling

Oligochaetes inhabiting substrate can be transferred from culture aquaria to a white or light-colored shallow pan with a fine-meshed brine shrimp dipnet (e.g., 7.6 cm, Penn Plax, Inc., Garden City, NY). Those organisms not associated with the towel substrate can be easily captured and moved with a glass pipette (20 cm long, 5 mm I.D. opening, fire polished on both ends) fitted with a pipette bulb (Phipps et al. 1993). When the annelids aggregate into a cluster, a gentle stream of culture water from the pipet or a squeeze bottle will serve to spread them out for capture either as individuals or small groups of individuals. Organisms should not be handled with forceps, as they may be injured and/or fragmented. Injured organisms should be removed from the culture and not used for testing.

11.1.9. General Culture Maintenance

The culture should be examined daily to assess general condition of health, and to ensure that disruptions in aeration or water flow have not occurred. The temperature of the culture water should be measured daily in each chamber either manually or by a continuous temperature monitor with a chart recorder. *L. variegatus* cultures have the potential to develop low concentrations of dissolved oxygen and/or high concentrations of ammonia; therefore, routine monitoring of dissolved oxygen and total ammonia concentrations is advised. The dissolved oxygen concentration should be maintained at $\geq$40 percent of saturation, while the total ammonia concentration should not exceed 0.1 mg/L.

New pre-conditioned paper toweling should be added when the substrate appears thin or when bare spots are observed in the substrate. Depending upon the required culture size, extra organisms may be used to increase the number in additional tanks. Place a mass of desired size (e.g., 5-15 g) into the new chamber. A doubling in population density occurs about every 10-14 d at

20° C.

Snails (*Helisoma* sp.) added to the culturing chambers at ERL-Duluth assist in keeping the chamber walls clean, thereby reducing or eliminating the necessity of cleaning the chambers frequently. If snails are used, their number will have to be thinned regularly, as their populations proliferate under these culturing conditions. *Helisoma* sp. are available from ERL-Duluth upon request (J. Denny, 218-720-5717).

11.1.10. Culture Evaluation

Reproduction should be at a normal level and organisms should be judged to be in good condition before they are used in a bioaccumulation study. The culture population should be doubling about every 10-14 d. Individual animals should appear to be of normal adult size and coloration, and should be highly responsive to a gentle touch with a probe. A culture evaluation chart (Attachment AA, Form AA1) should be maintained, and updated monthly. If the organisms do not meet the criteria above, the culturing conditions should be scrutinized and adjustments made to restore culture health and increase reproduction. Any adjustments made may be considered to have resulted in an acceptable state of health for the culture when the culture meets the above criteria of reproduction, appearance, and responsiveness.

11.2 ACUTE TOXICITY SCREENING TEST

Prior to or concurrent with the full 28-d bioaccumulation study, a 10-d toxicity screening test should be performed with each sediment. It is important to screen the sediment for toxicity, evidenced either by mortalities or behavioral effects (i.e., avoidance of sediment by not burrowing), to determine if the full 28-d test should be performed.

This screening test can be performed in 300 mL high-form beakers containing screened holes in the walls for exchange of overlying water, as are used in conducting sediment toxicity tests with *Chironomus tentans* or *Hyalella azteca*. Test details are provided in Phipps et al. (1993). Briefly, the test should be performed with 100 mL of sediment placed into each beaker, and the beakers then placed within aquaria which provide overlying water to the beakers. The test system in which the beakers are placed should receive two volume renewals daily of overlying water. Aeration should be available and monitored to ensure that dissolved oxygen levels are maintained at 40 percent of saturation or greater. Ten organisms per replicate beaker should be added after the sediment has been allowed to settle for 24 h. The animals should not be fed during the 10-d test period. After 10 d, sediment samples from the toxicity screening test should be sieved, and the animals counted to determine survival and

reproduction. They should be observed for any abnormal behavior, and then oven-dried and weighed to obtain a measure of growth. Results from each dredged material site are compared to results from control and disposal site sediments. Survival of controls must be ≥70 percent for the test to be considered acceptable. These observations should allow for a determination of the appropriateness of either starting or continuing with a 28-d bioaccumulation study.

11.3. BIOACCUMULATION TEST METHODS

11.3.1. Solid-Phase Sediment Preparation

See Section 5.0.

11.3.2. Test Design

The basic design and conditions for performing a bioaccumulation test with dredged material are given in Table G-17. The exposure consists of five or more replicates of each sample in an exposure system designed to renew overlying water at 1 h intervals at a rate to provide a total renewal of about two volume exchanges each day. Several types of automated water renewal systems have been developed (e.g., Mount/Brungs 1967, Maki 1977, Ingersoll and Nelson 1990, Benoit et al. 1993, Zumwalt et al. 1994), and are presented here as delivery system options. Variations of these systems are acceptable, provided the recommended volumes of overlying water and their renewal rates are maintained, along with the recommended physical and chemical characteristics of the overlying water. It should be noted that rectangular glass tanks are used for exposure chambers rather than beakers, as in sediment toxicity tests. Thoroughly homogenized sediment (1,600 mL) is added into each exposure chamber, and the sediment is allowed to settle for 24 h before introduction of test animals. The overlying water flows over the sediment during this 24 h period. The sediment:water volume ratio should be from 1:1.7 to 1:2.1 over the course of the overlying water renewal cycle.

11.3.3. Test Chambers

Five or more, if desired, replicate 5.5 L [15.8 x 29.3 x 11.7 cm, W x L x H (OD)] rectangular glass chambers are recommended for each sediment sample tested. A maximum of two sediment samples (10 total replicates), or one disposal site sediment and one test sediment, can be tested simultaneously in a standard mini-flow exposure system of the size described in Benoit et al. (1993), using five replicates per sample.

11.3.4. Water Renewal

Laboratory culture water may serve as overlying water for the exposures. The exposure system is set to provide about two volume exchanges per day.

Table G-17. Overview of Recommended Test Conditions for 28-d Bioaccumulation Tests with *Lumbriculus variegatus*.

1.	Test type	Chemical bioaccumulation from sediments with renewal of overlying water.
2.	Temperature	23±1°C
3.	Light quality	Fluorescent bulbs (wide spectrum)
4.	Light intensity	10-20 $\mu E/m^2/s$, 540-1080 lux or 50-100 ft-c (ambient laboratory levels)
5.	Photoperiod	16 h light, 8 h dark
6.	Test chamber	5.5 L glass tank (15.8 x 29.3 x 11.7 cm, W x L x H)
7.	Sediment volume	1,600 mL
8.	Overlying water depth	6 to 7.5 cm with top of standpipe siphon at 11 cm
9.	No. of volume renewals of overlying water	2 per day
10.	Age of test organisms	Adults
11.	No. of replicates per sample	Minimum of 5
12.	Initial mass of organisms per replicate	1.0 to 5.0 g, depending upon analytes of concern
13.	Initial No. of organisms per replicate	80-1,000
14.	Feeding regime	No feeding
15.	Aeration	Aerate if dissolved oxygen drops below 40% of saturation (i.e., 3.40 mg/L)
16.	Overlying water	Culture water, (e.g., untreated well water, dechlorinated tap water, reconstituted water)
17.	Test duration	28 d for all chemicals
18.	Measurements (possible-not all would be run on every test)	Animal tissue weight (wet and dry), chemical concentration in sediment and animal tissue, total organic carbon or acid-volatile sulfide content of sediment, organism lipid content.

Table G-17. (continued)

19.	Water Quality Monitoring	Daily measurements of water temperature and dissolved oxygen. Hardness, alkalinity, specific conductance, pH and total ammonia should be measured twice during the test.
20.	Sample storage	Store sediment at 4°C. Test should be initiated within 2 weeks of sample collection, and must be initiated within 8 weeks of collection.
21.	Sediment volume required	8.2 L from each test site (1.6 L for each of five replicates, remainder for analytical chemistry).
22.	Test acceptability criteria	Test initiated with animals from healthy culture; animals burrowed into sediment; 10-day toxicity test survival was not significantly different from controls; dissolved oxygen concentration should exceed 40% of saturation at all times; mean temperature was 23 ± 1°C and did not deviate ≥3°C at any time; total ammonia concentrations averaged 0.1 mg/L; satisfactory results in a reference toxicant test.

This renewal rate will likely require supplemental aeration for many sediments. Aeration of the overlying water should be initiated if the dissolved oxygen concentration drops to 40 percent of saturation or below (i.e., 3.40 mg/L at 23°C).

11.3.5.Temperature and Photoperiod

Tests should be performed at 23±1°C. The daily photoperiod should be 16L:8D, using ambient laboratory lighting of 50-100 ft-C.

11.3.6. Organism Introduction

A biomass of approximately 1.0 to 5.0 g of adult oligochaetes is weighed and added to each chamber on d 0. The initial mass will depend upon the analyte(s) of concern, and their respective lower limits of detection during chemical analysis. Tissue weights at the end of the exposure period required to achieve various analytical detection limits are presented in Table G-18. Assuming that no net weight change occurred during exposure, the initial weight required would be the same as the required final weight. However, either negative or positive weight changes are likely to occur, and the initial weight should be adjusted accordingly. Dependent upon their

size, this will be from 40 to 1,000 animals. An actual count of the organisms is optional but not necessary.

Table G-18. Grams of *Lumbriculus variegatus* Tissue (Wet Weight) Required for Various Analytes at Selected Lower Limits of Detection.

Analyte		Grams of Tissue 1.0	2.0	5.0
		Lower Limit of Detection (μg/g)		
PCBs				
PCB (total)[1]	level of chlorination	0.600	0.300	0.120
PCB (congeners)[2]	1-3	0.025	0.0125	0.005
	4-6	0.050	0.025	0.010
	7-8	0.075	0.375	0.015
	9-10	0.125	0.0625	0.025
Organochlorine Pesticides				
p,p'-DDE[1]		0.050	0.025	0.010
p,p'-DDD[1]		0.050	0.025	0.010
p,p'-DDT[1]		0.050	0.025	0.010
o,p'-DDE[1]		0.050	0.025	0.010
o,p'-DDD[1]		0.050	0.025	0.010
p,p'-DDT[1]		0.050	0.025	0.010
Alpha-Chlordane[1]		0.050	0.025	0.010
Gamma-Chlordane[1]		0.050	0.025	0.010
Dieldrin[1]		0.050	0.025	0.010
Endrin[1]		0.050	0.025	0.010
Heptachlorepoxide[1]		0.050	0.025	0.010
Oxychlordane[1]		0.050	0.025	0.010
Mirex[1]		0.050	0.025	0.010
Trans-Nonachlor[1]		0.050	0.025	0.010
Toxaphene[1]		0.600	0.300	0.120
PAHs				
PAHs (GC Method)[3]		0.012	0.006	0.002

Table G-18. (continued)

Inorganics			
Cadmium[4]	0.005	0.0025	0.001
Copper[4]	0.005	0.0025	0.001
Lead[4]	0.005	0.0025	0.001
Zinc[4]	0.005	0.0025	0.001
	Lower Limit of Detection (ng/g)		
Dioxins			
TCDD[5]	0.020	0.010	0.004
PAHs			
PAHs (HPLC-FD Method)[6]			
Benzo(a)pyrene[6]	0.01	0.005	0.002
Pyrene[6]	0.03	0.015	0.006
Benzo(k)fluoranthene[6]	0.03	0.015	0.006
Dibenzo(a,h)anthracene[6]	0.03	0.015	0.006
Anthracene[6]	0.10	0.050	0.020
Benz(a)anthracene[6]	0.10	0.050	0.020
Benzo(e)pyrene[6]	0.10	0.050	0.020
Benzo(b)fluoranthene[6]	0.10	0.050	0.020
Benzo(g,h,i)perylene[6]	0.10	0.050	0.020
3-Methylcholanthrene[6]	0.10	0.050	0.020

[1] Schmitt et al. (1990); [2] USEPA (1990b); [3] Vassilaros et al. (1982); [4] Schmitt and Finger (1987); [5] USEPA (1990); [6] Obana et al. (1981).

The annelids and substrate are removed from a culture tank with a 7.6 cm fine-meshed dipnet and placed directly into a glass bowl or stainless steel pan (approximately 20 cm or more in diameter) containing culture water. With a pipette (20 cm long, 5 mm I.D. opening, fire polished on both ends) fitted with a pipette bulb, gently pulse the animals with water to loosen the substrate. Allow the animals to reform in a cluster in the bottom of the container and decant or siphon off most of the water and substrate. Repeat this process until the animals are free of substrate. Refill the pan with stream of water from a squeeze bottle. Pick them up with a dissecting needle or dental pick, blot the mass on paper toweling, and place into a tared weighing pan. Experience will help approximate the size of the mass of annelids to equal the desired 1.0 to 5.0 g per replicate.

The weighing must be done rapidly, as the animals should not be allowed to desiccate or warm up in the weighing pan. When the desired weight is attained, water should be added to the weighing pan. Each aliquot of annelids should take 2-3 min. to dilution water and gently separate masses of annelids of desired size with a portion and weigh, and each aliquot should be randomly added to its respective exposure chamber immediately after weighing. The annelids should be observed to determine if they immediately burrow into the sediment.

Organism loading should result in an organic carbon ratio between animal tissue (dry weight) and sediment (dry weight) that preferably lies between 1:50 and 1:100. The ratio should not be less than 1:10.

11.3.7. Food and Feeding

No food is provided during the bioaccumulation exposure. The addition of food would alter the organic carbon content of the sediment, which could influence bioavailability of chemicals in the sediment (Phipps et al. 1993).

11.3.8. Test Organism Monitoring

The health of the animals should be observed at least twice daily. Animals which are not actively feeding and tunneling will be observed to be oriented with the anterior portion of their bodies in the sediment and the posterior portion in the overlying water. If no animals are evident with this orientation, it is possible that the sediment is toxic, and an insufficient biomass will be available for chemical analysis at the end of the exposure period. In such a case, it may be desirable to terminate the exposure early. Results from the 10-d acute toxicity screening test, if performed concurrently with the 28-d bioaccumulation study, will provide evidence for a decision regarding termination. See Section 11.2. on the 10-d acute toxicity screening test for specific criteria regarding test acceptability.

11.3.9. Water Quality Monitoring

Water should be monitored daily for temperature and dissolved oxygen concentrations. The temperature should be maintained within ± 1°C of 23°C at all times. Dissolved oxygen concentrations should be maintained at or above 40 percent of saturation. Hardness, alkalinity, specific conductance and pH should be measured at the beginning and end of the test from one of the replicates of each sediment. Because ammonia may be elevated in some test sediments (Ankley et al. 1990, Call et al. 1991), measurement of total ammonia should be performed regularly. Total ammonia concentrations should be monitored twice weekly in the overlying water from one of the replicates for each sample. Total ammonia concentrations should not exceed

0.1 mg/L. Water quality parameters should be recorded on a data form (see Attachment AA, Form AA4 as an example).

11.3.10. Test Duration

The duration of the test should be sufficient to allow time for the chemicals to equilibrate between sediment, pore water and oligochaete tissue. At present, a minimum exposure period of 28 d is recommended for all chemicals. In general, the larger the K_{ow} (octanol-water partition coefficient) of a nonionic organic chemical, the longer it will take to come into equilibrium or steady-state in animal tissue. For the purposes of environmental risk assessment, it is essential that decisions be made based on steady-state concentrations from the laboratory exposure. There are two ways to help ensure that steady-state concentrations are reached in laboratory tests with *L. variegatus*. The first is to run tests for longer than 28 d if it is suspected that chemicals of concern will not come to equilibrium tissue concentrations in this time period. This may be expected to be the case when the log K_{ow} of the analyte of concern is $\geq$5.5 to 6.0 (Ankley et al., unpublished manuscript). Alternatively, if it is impractical to run the bioaccumulation test for more than 28 d, but the target nonionic chemicals are in a log K_{ow} range where there may be concern about equilibrium (i.e., $\geq$5.5 - 6.0), it is possible to conduct multiple samplings during the 28 d test (e.g., d 1, 3, 4, 7, 14, and 28) and use a kinetic analysis to project or estimate steady-state tissue concentrations. This kinetic approach is described in detail elsewhere (USEPA and USACE 1993). Note that if this approach is used, it will be necessary to set up correspondingly greater numbers of replicate test chambers for the multiple sampling.

11.3.11. Test Termination

The sediment from each replicate is sieved through a fine-meshed screen sufficiently small to retain the oligochaetes (e.g., U.S. Standard No. 35 or 40, having 500 or 425 μm mesh sizes, respectively). The organisms may then be transferred for removal of associated substrate to a light-colored shallow pan by a gentle stream of water. Upon cleaning, they are transferred to a 1 L beaker containing test water with no sediment present for elimination of sediment from the alimentary canal. The beaker should be well aerated with an airstone to maintain a satisfactory level of dissolved oxygen. The animals should be held in the water for a 24-h period to allow for alimentary tract clearance of most of the sediment. Brooke et al. (unpublished manuscript) observed that *Lumbriculus variegatus* cleared most of its gut contents within 12 h in water without sediment. Following clearance of the alimentary canal, the annelid mass is collected, blotted to remove excess water, and weighed to determine wet weight (biomass). The animal tissue mass should not be dried prior to preparation for chemical analysis. If a

dry weight is desired, subsample 0.25 to 0.50 g of annelids, place the subsample into a pre-weighed pan, and obtain a total wet weight. Then oven-dry the annelids for at least 4 h at 100°C (until a steady weight is obtained). Allow to come to room temperature in a desiccator, and weigh to the nearest 0.01 mg.

The remaining mass of animals is then weighed and placed into a suitable clean container (e.g., 10 mL glass vial), sealed, and frozen for later analysis. The containers should be free of contaminants achieved by a thorough cleaning by approved methods (USEPA 1990a) prior to use. Vials should be placed inside of freezer containers to minimize "freezer burn" and dehydration during storage.

11.4. General Analyses

Certain chemical analyses may aid in the interpretation of test results. Measurement of tissue total lipid content in the test organism and total organic carbon (TOC) content of the sediment may help explain the partitioning of some organic chemicals between sediment and biota. Measurement of acid volatile sulfide (AVS) content of the sediment may help explain the bioavailability of divalent metals in the sediment. Their methods of analysis are referenced below.

11.4.1. Annelid Total Lipid Analysis

Take a subsample (≈1 g) of the total oligochaete mass of each thawed replicate for total lipid analysis. Various methods of lipid analysis can yield considerably different results. Consequently it has been suggested (Randall et al. 1991) that the analytical method used for lipid analysis should be calibrated against the chloroform/methanol extraction method described by Folch et al. (1957) and Bligh and Dyer (1959).

11.4.2. Sediment Total Organic Carbon (TOC) Analysis

Sediment TOC may be determined for sediments when bioaccumulation of nonionic organic chemicals is of concern. A subsample from each replicate is analyzed at the end of the exposure. The analysis should be a measure of TOC and not a measure of "loss on ignition" or "total volatile solids". The method used should be specific to the measurement of TOC, such as that described by Cowan and Riley (1987).

11.4.3. Sediment Acid Volatile Sulfide Analysis

AVS and simultaneously extracted metals (SEM) may be determined in wet sediment samples when metals are being assessed for bioaccumulation. SEM measurements apply for cadmium, copper, lead, nickel and zinc. A USEPA methods manual is available for the analysis of AVS and SEM (Allen et al. 1991).

11.4.4. Data Reporting and Statistical Analysis

At the conclusion of the exposure and gut clearance period, information is recorded on chemical residues in the annelids and the sediment. If the optional analyses were performed, data would also be recorded on such measurements as total lipids in the annelids, or the TOC or AVS content of the sediment. A sample data form is presented in Attachment AA (Form AA2). See Section 12.0 for statistical analysis.

12.0. DATA REPORTING AND STATISTICAL ANALYSIS

12.1. Data Reporting

Data are reported for the various tests using the proper reporting forms. Examples of suitable forms are shown in the various Attachments (A through AA). Great care should be taken on each day to ensure that observations from the randomized array of exposure chambers are accurately transferred to the data forms. Once the data have been organized and suitably summarized, analysis can be accomplished using an appropriate method. Toxicity assessment protocols for five species are offered in this Appendix (H). Those for *D. magna*, *C. dubia*, and *P. promelas* assess dredged material elutriates. Acute toxicity tests use organism survival as an endpoint. Chronic toxicity tests with *D. magna* and *C. dubia* use both organism survival and reproduction as endpoints, while the 10-d exposure with *P. promelas* examines survival and growth. Assessments of solid-phase dredged materials are made using *C. tentans, H. azteca* and *L. variegatus* as test organisms. The *C. tentans* test uses survival and growth as endpoints. The latter endpoint is an option in the toxicity test with *H. azteca*. *L. variegatus* is used to assess tissue contamination due to chemical bioaccumulation from solid-phase dredged materials by comparing tissue concentrations of specific chemicals in organisms exposed to dredged material to concentrations in organisms exposed to disposal site sediment, or to an action level.

12.2. Statistical Analysis

12.2.1. Toxicity Test Data Analysis

Methods described in this section are based upon and in agreement with statistical methods described in the Inland Testing Manual (USEPA/USACE 1998) for analysis of Tier 3 test results. Statistical analysis of Tier 4 tests may differ from these methods, as Tier 4 tests are case-specific. Program statements and sample data set analyses are provided for survival and bioaccumulation data. It is highly recommended that the reader refer to this manual. The statistical treatment of all test data follow either a parametric or nonparametric approach (Figures G-12, G-13 and G-14). If the data are found to be

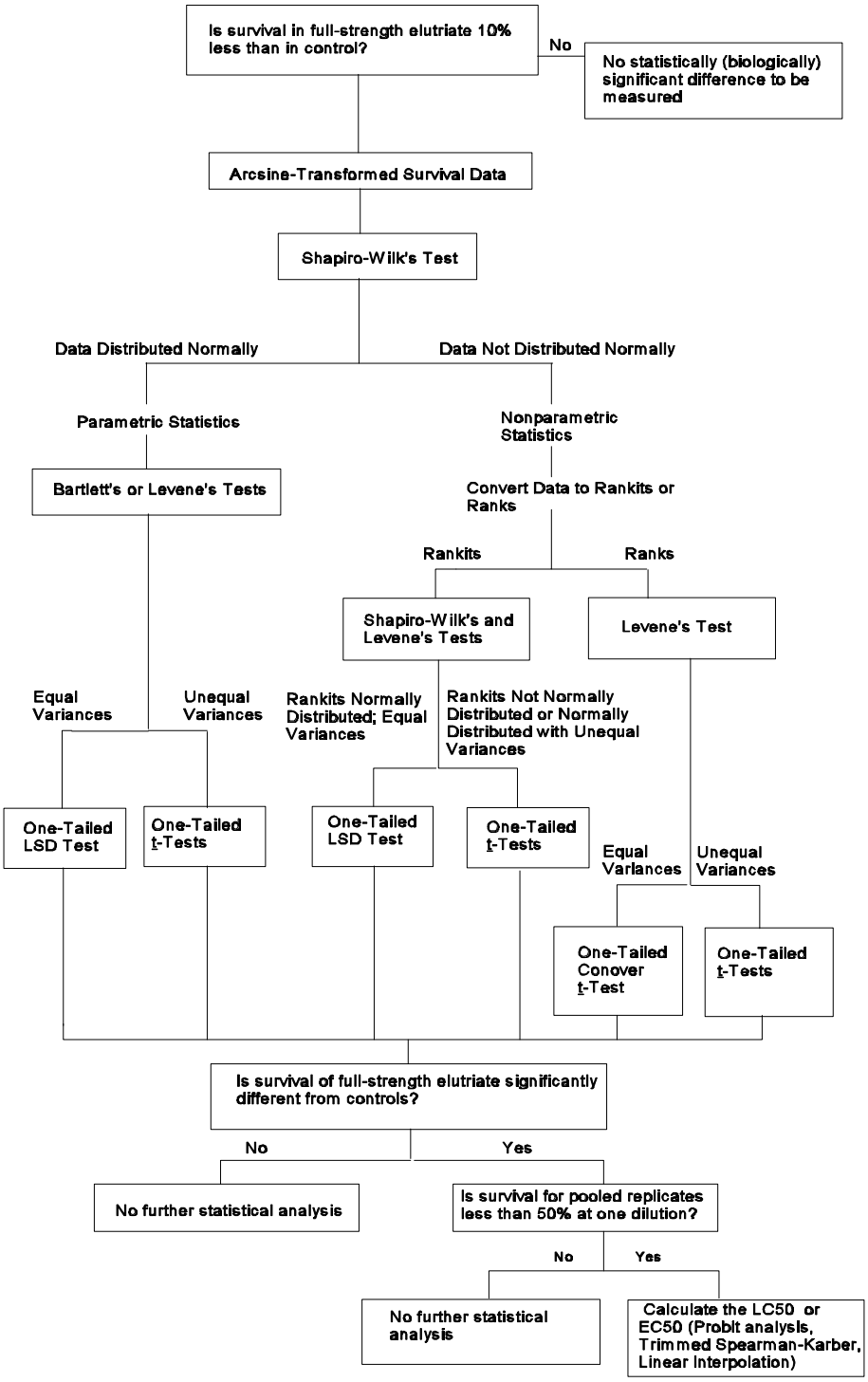


Figure G-12. Statistical treatment of survival data from toxicity tests with dredged material elutriates and *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (adapted from USEPA/U.S. ACE 1994).

normally distributed, a parametric approach is applied. If the data are not normally distributed, a nonparametric approach is used.

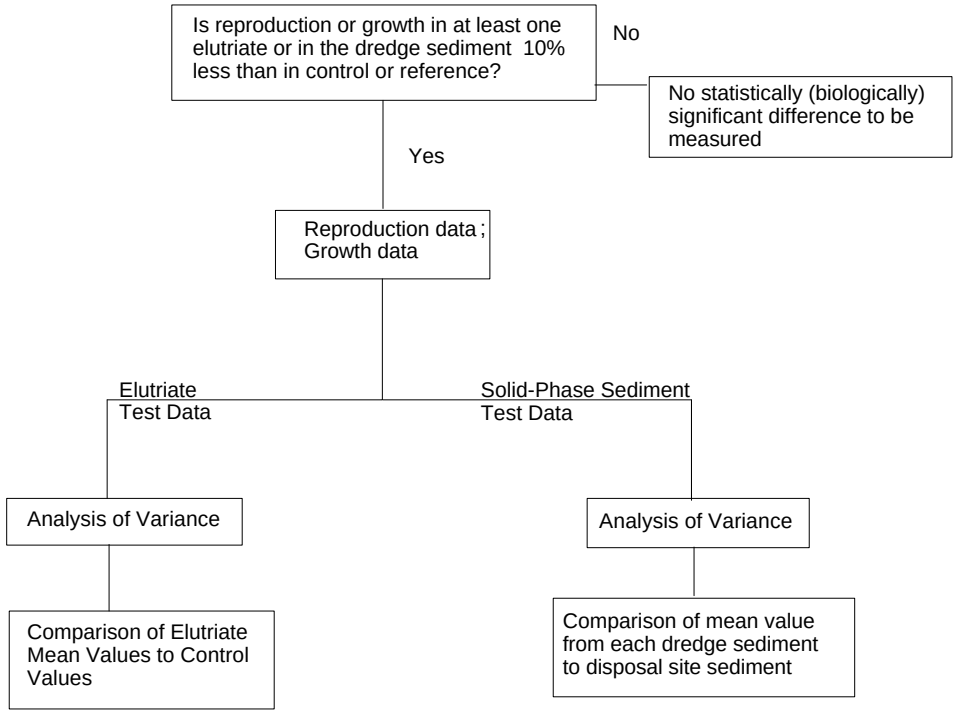


Figure G-13. Statistical treatment of reproduction or growth data from toxicity tests with dredged material elutriates and *Ceriodaphnia dubia, Daphnia magna* or *Pimephales promelas* or with solid-phase sediment and *Chironomus tentans* or *Hyalella azteca*.

Hypothesis testing is used initially to compare endpoints from either a full-strength elutriate to the control or from a dredged sediment to a disposal site sediment. The null hypothesis for toxicity tests is that there is no significant decrease between the specific endpoints (i.e., survival, reproduction or growth) of the test organism exposed to dredged sediments or elutriates when compared to either organisms exposed to disposal site sediment in the case of solid-phase tests or to dilution water (controls) in the case of elutriate tests. If survival in the full-strength elutriate is not reduced $\geq$10 percent relative to survival in the controls, no further statistical analysis is required. If survival is reduced $\geq$10 percent, the survival data are arcsine-transformed, tested for assumptions of normality of distribution and homogeneity of variances, and the survival means at each elutriate concentration compared to the mean survival of controls by a *t*-test. If *t*-test

results indicate that survival in the full-strength elutriate is not significantly different from survival in the controls, no further statistical analysis is required. If *t*-test results indicate that survival in the full-strength elutriate is significantly different from survival in the controls, then the data may be subjected to a point estimation of an effect level such as an LC50 or EC50. The point estimate can be used in a mixing model described in Appendix C of the Inland Testing Manual (USEPA/USACE 1998).

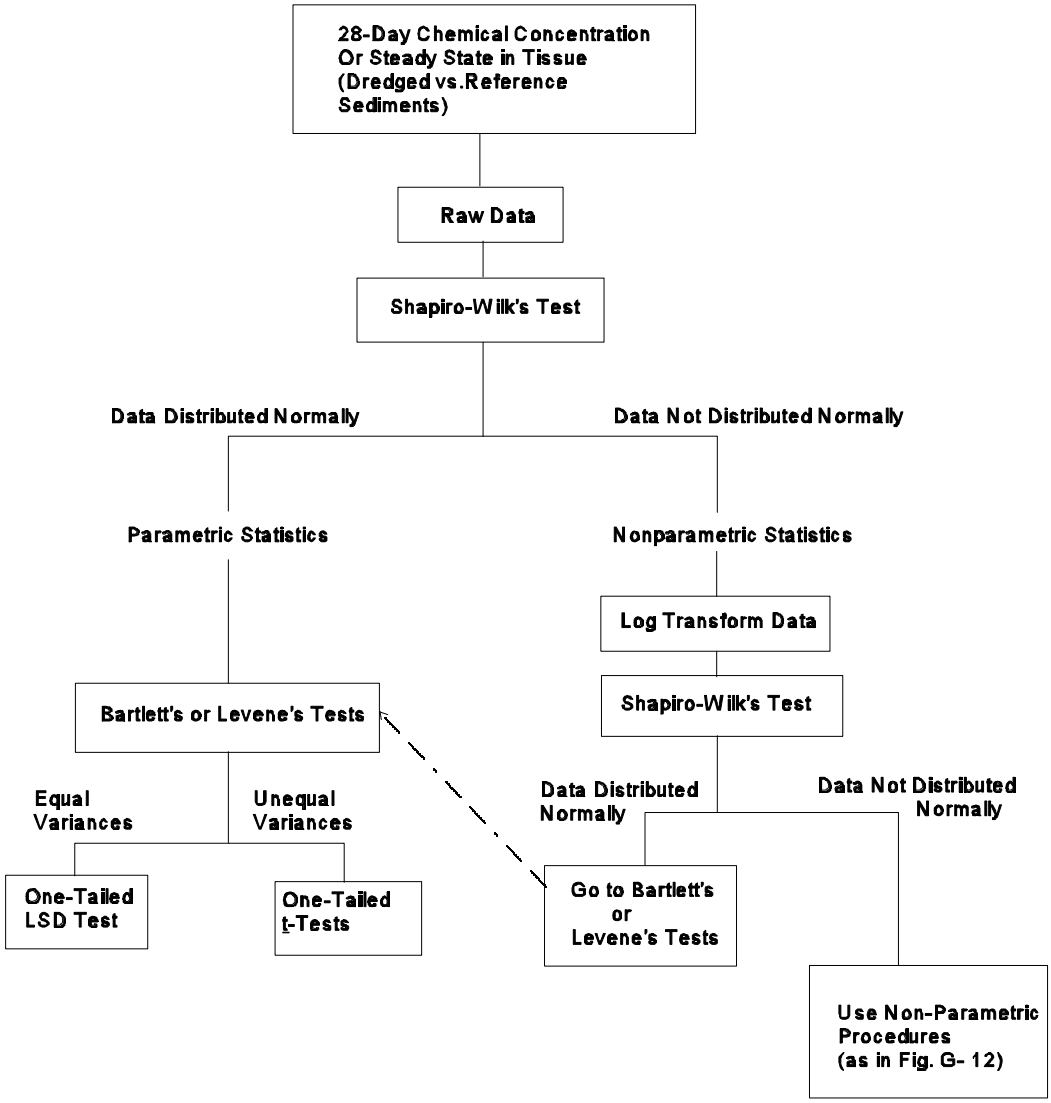


Figure G-14. Comparison of chemical residues in *Lumbriculus variegatus* tissues exposed to dredged site and disposal site sediments (based upon USEPA/USACE 1998).

The methods described for survival data are based upon methods described in the Inland Testing Manual (USEPA/USACE 1998). The methods provided in the Inland Testing Manual, complete with program statements and example data, use software products of the SAS Institute, Inc. (SAS 1985). These are IBM-compatible PC programs. Other acceptable hardware and software products are commercially available and may be used to perform the necessary analyses. While the specific statistical tests included in different software packages may vary in methods for determining data normality or equality of variances, it is important that these tests of assumptions are included in the software package used.

12.2.1.1. Two-Sample t-Test

The two-sample *t*-test (Snedecor and Cochran 1989) may be used in cases where an individual sediment or elutriate is being compared with a reference or control. Survival data should first be arcsine-transformed to reduce the heterogeneity of variance. A table for conversion of binomial percentage data is available in statistical tests (e.g., Steel and Torrie 1980, Snedecor and Cochran 1989). Data should then be tested for normality of distribution using a test such as the Shapiro-Wilk's Test. The normality test is run on the residuals (observations minus treatment mean) rather than on the raw data. Methods for determining the normality statistic, W, are found in USEPA (1993). Data that are normally distributed should then be tested for equality of variances to determine the proper equation for calculating the t-statistic. A calculated F-ratio [larger variance (S_1^2) over smaller variance (S_2^2)] is compared to a table value of F-ratios for the appropriate degrees of freedom for the two samples (see, e.g., Steel and Torrie 1980, Snedecor and Cochran 1989) to determine if the variances are significantly different. If variances are not significantly different, the equation is:

$$t = (\bar{x}_1 - \bar{x}_2) / \sqrt{S^2_{pooled}(1/n_1 + 1/n_2)}$$

where S^2_{pooled}, the pooled variance, is calculated as:

$$S^2_{pooled} = [S_1^2(n_1 - 1) + S_2^2(n_2 - 1)]/(n_1 + n_2 - 2),$$

and S_1^2 and S_2^2 are the sample variances of the two groups.

The calculated *t*-statistic is compared with the student *t* distribution (this is a one-tailed *t*-test and the table of *t* values must be used appropriately) in a statistics text (e.g., Steel and Torrie 1980, Snedecor and Cochran 1989) to determine if the null hypothesis should be rejected. If variances are unequal, the *t*-statistic is calculated by the equation:

$$t = (\bar{x}_1 - \bar{x}_2)/\sqrt{S_1^2/n_1 + S_2^2/n_2},$$

and the degrees of freedom calculated as follows (Satterthwaite 1946):

$$df = \frac{(S_1^2/n_1 + S_2^2/n_2)^2}{(S_1^2/n_1)^2/(n_1 - 1) + (S_2^2/n_2)^2/(n_2 - 1)}$$

Fractional degrees of freedom should be rounded down to the nearest integer (USEPA/USACE 1998). Suggested significance levels (") for normality and variance tests vary dependent upon number of replicates and evenness of statistical design and are given in Table G-19.

Table G-19. Suggested " Levels to Use for Tests of Assumptions.

Test	Number of Observations[a]	" When Design Is	
		Balanced	Unbalanced[b]
Normality	N = 3 to 9	0.10	0.25
	N = 10 to 19	0.05	0.10
	N = 20 or more	0.01	0.05
Equality of Variances	n = 2 to 9	0.10	0.25
	n = 10 or more	0.05	0.10

[a] N = total number of observations (replicates) in all treatments combined
n = number of observations (replicates) in an individual treatment.

[b] $n_{max} \geq 2n_{min}$.

(From USEPA/USACE 1998)

When testing the assumption that the dredged sediment is not significantly different from the disposal site sediment (null hypothesis), an error rate (") must be specified. Biological tests generally set "=0.05 which means that, on the average, the null hypothesis will be rejected in 5% of the tests when it is true. The recommended " is 0.05; however, there is nothing magical about the " level of 0.05 and the evaluator of a toxicity test may desire to use a larger ", such as 0.10. The larger " results in a more environmentally protective sediment evaluation

by rejecting a null hypothesis when it is true a higher percentage of the time (i.e., sediments may be considered contaminated when they are not).

12.2.1.2. Multiple Sample t-Test

A flow-diagram of statistical tests is presented (Figure G-12) with examples of specific statistical tests to test for assumptions, to compare means and to calculate point estimates. These will be discussed briefly. As in the case of the two-sample test, the data are first arcsine-transformed to reduce heterogeneity of variance. A table for conversion of binomial percentage data to arcsine data is available in statistical texts (e.g., see Steel and Torrie 1980, Snedecor and Cochran 1989). The arcsine-transformed survival data are tested for normality of distribution by a test such as Shapiro-Wilk's Test. Methods for determining the normality test statistic, W are found in USEPA (1993). Data that are normally distributed should then be tested for equality of variances to determine the proper equation for calculating the *t*-statistic. If the variances are equal, survival at each elutriate concentration can be compared to control survival by a one-tailed LSD test. If the variances are unequal, this survival comparison is accomplished by a one-tailed *t*-test (USEPA/USACE 1998).

Nonparametric statistics are used to compare data that are not distributed normally based upon Shapiro-Wilk's Test. The survival data are first converted to rankits or ranks. If converted to rankits, Shapiro-Wilk's and Levene`s Tests are performed to determine the assumptions of normality and equal variances. If the rankits are distributed normally and have equal variances, a one-tailed LSD test is recommended for comparing the mean survival at each elutriate concentration to mean survival of controls. If the rankits are not normally distributed, or if they are normally distributed but with unequal variances, a one-tailed *t*-test is used (Fig. G-12). If the data are converted to ranks, Levine's Test is applied to determine the equality of variances. If the variances are equal, a one-tailed Conover *t*-test is used. If the variances are unequal, a one-tailed *t*-test is used to compare mean survival in the various elutriate concentrations to mean survival of controls.

Following analysis by one of the *t*-tests listed in Fig. G-12, the statistical results are examined to determine if organism survival at the full-strength elutriate was significantly different ($p \leq 0.05$) than survival in the controls. If not, no further statistical analysis is required. If the difference is significant, further statistical analysis (i.e., a point estimation) may be performed for subsequent use in a mixing model. If survival for the pooled replicates of a given elutriate concentration is less then 50 percent, an LC50 may be

calculated. Probit analysis is recommended to provide a point estimate of the elutriate concentration that decreases survival to some level. Software with the capacity to calculate various LC values from LC1 to LC99 with 95 percent confidence limits is available through EPA. A compiled version of a program written in IBM PC Basic for IBM compatible PCs may be obtained by sending a double-sided (DS), high density (HD) diskette with a written request to: Environmental Monitoring Systems Laboratory-Cincinnati, Office of Modeling, Monitoring Systems and Quality Assurance, Office of Research and Development, U.S. Environmental Protection Agency, 3411 Church Street, Cincinnati, OH 45268. Other methods for determining a point estimate, such as the Trimmed Spearman-Karber method, or the Logistic Method, may also be used. Programs for these methods are available from EPA. If none of these three methods is available or the data do not meet the requirements of these methods, then the Linear Interpolation Method may be used. The Trimmed Spearman-Karber Method and the Linear Interpolation Method, with the 95 percent confidence intervals provided in addition to a point estimate, are both available on the same diskette as indicated above for Probit Analysis. The program for the Linear Interpolation Method accepts data that do not follow a pattern of monotonically decreasing survival with increasing elutriate concentration.

A SAS program called WATTOX.SAS performs the forementioned arcsine transformation, tests of assumptions, and *t*-tests for elutriate toxicity tests. Program statements from WATTOX.SAS and sample data sets are provided in the Inland Testing Manual (USEPA/USACE 1998). The only test endpoint that is used in WATTOX.SAS is survival, and the program compares survival in the control (dilution) water to survival in full-strength (100 percent) elutriate.

A SAS program called BENTOX.SAS compares benthic survival data from dredged sediments to survival data from a disposal site sediment. Program statements from BENTOX.SAS and the analytical results from sample data sets are provided in the Inland Testing Manual (USEPA/USACE 1998).

The software packages and statistical methods mentioned above (i.e., WATTOX.SAS, BENTOX.SAS, Probit Method, Trimmed Spearman-Karber Method, Logistic Method, and Linear Interpolation Method) are used to analyze survival data. These programs and test methods do not analyze growth or reproduction data. A general approach to analyzing growth or reproduction data from elutriate or solid-phase sediment toxicity tests is presented in Figure G-13. For elutriates, reproduction or growth data are subjected to an analysis of variance, and the treatment (elutriate) means are then compared to the control mean by an appropriate test, such as Dunnett's Test. The diskette that is

available from EPA (Cincinnati, OH) also contains a program for analyzing data by Dunnett's Procedure. This allows for a determination of whether growth or reproduction at the various elutriate concentrations is statistically different from that of the controls, and whether the null hypothesis can be rejected. Various software packages are available to accomplish this comparison. The data are first examined to determine normality of distribution and homogeneity of variance. If the data are not normally distributed and the variance is not homogeneous, data may be analyzed by the LSD test or t-test on the rankits.

Computer software called TOXSTAT® has been developed to analyze data in support of chronic test methods described in "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" (USEPA 1989). Originally developed for application with chronic toxicity test data for the fathead minnow (*p. promelas*) and the cladoceran, *C. dubia*, the program may also be applied to chronic data for *D. magna*. The IBM compatible program is menu-driven, and is sold by: WEST, Inc., 1402 S. Greeley Highway, Cheyenne, WY 82007-3031. Another commercial software package that includes the commonly used tests of assumptions, as well as specific methods for performing either hypothesis tests or point estimates is TOXCALC©. This package is sold by TidePool Scientific Software, P.O. Box 2203, McKinleyville, CA 95521.

Growth data from 10-d tests of solid-phase sediment with either *C. tentans* or *H. azteca* can likewise be analyzed by a number of software packages. There is not a graded series of concentrations for each dredged site sediment as in the case of elutriates. Therefore, the growth organisms exposed to the single dredged sediment is compared to the growth of organisms exposed to a disposal site sediment. The data are subjected to tests of normality of distribution and equality of variances prior to a comparison of growth means.

Commercial software packages that may be used to analyze growth data include SigmaStat™ Version 1.01 (Jandel Scientific, San Rafael, CA) or ToxCalc© (TidePool Scientific Software, McKinleyville, CA). Others may also be available. Only decreases in growth or reproduction at a dredged site relative to a disposal site are of concern relative to subsequent decision-making. Therefore, one-tailed tests are appropriate. Growth data may also be statistically analyzed using a SAS program called BIOACC.SAS, with the exception that one is interested in significantly decreased growth, rather than significantly increased bioaccumulation from test sediment exposures compared to disposal site sediment exposures. Program statements for BIOACC.SAS are available in USEPA/USACE (1994).

12.2.2. Bioaccumulation Test Data Analysis

Bioaccumulation data from the *L. variegatus* bioassay may be analyzed from either a single-time point study or from a time-sequenced study (USEPA/USACE 1998). In either case, analyses are performed on the data to provide for comparisons between each dredged sediment and the disposal site sediment, and for comparisons with an action level, when applicable. One-sided tests are appropriate, because the main concern is whether organisms exposed to dredged site material have accumulated significantly greater quantities of the chemical(s) of interest than organisms exposed to the disposal site sediment.

12.2.2.1. Comparison With a Disposal site Sediment

If only one dredged sediment is compared to a disposal site, then the procedure described in section 12.2.1. for comparing two samples is used. If more than one sediment is compared to a disposal site, then the procedures described in section 12.2.2. are used. However, an arcsine transformation of the raw data is not appropriate with residue data. Rather, the data are first analyzed in the raw form for assumptions of normality and homogeneity of variances. If they pass these tests of assumptions, the raw data are further analyzed as raw data. If the raw data fail these tests of assumptions, they should be log-transformed and reanalyzed for normality of distribution (USEPA/USACE 1998). If the transformed data fail the normality of distribution tests, they should be analyzed by nonparametric techniques. The scheme is presented in Figure G-14.

Two SAS programs that provide for statistical analysis of bioaccumulation data are BIOACC.SAS and BIOACCSS.SAS. Program statements and analysis of sample data sets are provided in USEPA/USACE (1994).

12.2.2.2. Comparison with an Action Level

In this comparison, the objective is to determine whether the mean bioaccumulation of contaminants in animals exposed to a dredged sediment is significantly less than a specified action level or standard (USEPA/USACE 1998). If the mean tissue concentration of one or more contaminants of concern is greater than or equal to the applicable action level, then no statistical testing is required. The conclusion would be that the dredged sediment does not meet the guidelines associated with the action level. If the mean tissue concentrations of a contaminant of concern are less than the applicable action level, then a confidence-interval approach is used to determine if these means are *significantly* less than the action level. One-sided tests are appropriate since there is concern only if bioaccumulation from the dredged sediment is not significantly less than the action level. There are two different approaches to conducting these tests, and both are acceptable.

The first is to calculate a value of *t*, much as in a *t*-test (this approach is often called a one-sample *t*-test):

$$t = \frac{\bar{x} - actionlevel}{\sqrt{s^2/n}}$$

where $\bar{X}$, s^2 and n refer to mean, variance, and number of replicates for contaminant bioaccumulation from the dredged sediment.

If tests of equality of variances in the comparison of dredged sediments with the disposal site indicate that variances are equal for all sediments, the MSE from the ANOVA is used as s^2, and calculated *t* is compared to $t_{0.95}$, with *N - k* degrees of freedom. If the variances are not equal, then s^2 for the individual sediment is used, and calculated *t* compared with $t_{0.95}$, with *n* - 1 degrees of freedom. If the data were log-transformed to normalize the distributions or equalize variances, then all calculations should be carried out on log-transformed values.

Another approach is to calculate the upper one-sided 95% confidence limit (UCL), and compare it to the action level:

$$UCL = \bar{x} + (t_{0.95,v})(\sqrt{s^2/n})$$

As in the first approach, the MSE is used in place of s^2 if variances are not significantly different, and the degrees of freedom (v) are N - k. If variances are significantly different, s^2 for the individual sediment is used, and v for each sediment i = n_1 - 1. There is a 0.95 probability that the true population mean tissue level is below the UCL. If the UCL is below the action level, there is a $\geq$0.95 probability that the population mean tissue level for the dredged sediment is below the action level, and we conclude that the action level is not exceeded. If the UCL is above the action level, it is uncertain whether the mean population tissue level is less than the action level.

Either of the above procedures may be used with the data that have failed the normality test, but the results should be considered approximate. The choice of which approach to use depends on the computer software and the presentation method to be used. In SAS, it is more convenient to calculate the UCL and compare with the action level, as in program BIOACC.SAS. In SYSTAT, it is simpler to conduct a one-sample *t*-test. Both approaches can easily be performed by hand.

12.3. Final Report

A final report for the biological test(s) performed should

be prepared which allows for an evaluation of the toxicity of the sediment to test animals and/or the accumulation of chemicals from the sediment by benthic organisms. This report should be of sufficient thoroughness that it provides readers with the necessary information to determine if the tests performed met the criteria for test acceptability.

Specific types of information in the final report should include the collection, handling and shipment of sediment samples, the date of receipt of sediment samples by the testing laboratory, storage conditions of sediment by the testing laboratory, and the time elapsed between receipt of the sediment and initiation of the specific biological tests performed. It should include a description of test methods used, and any deviations from the protocols described in this Appendix. The report should include raw data for the biological and/or chemical endpoints measured, as well as the accompanying water quality measurements performed during the test.

Statistical reduction methods should be specified, and the data, whether raw or summarized, used to determine statistical differences from controls or from a disposal site sediment should be included. A summary of the test results based upon statistical treatment of the data should be provided.

The final report should include information on quality control procedures implemented during each test. QA/QC audits performed during the tests should be provided in the final report, complete with requisite signatures by the performing laboratory's QA/QC officer and laboratory director.

13.0 REFERENCES

Adams, S.M., and D.L. DeAngelis. 1987. Indirect effects of early bass-shad interactions on predator population structure and food web dynamics. In: Predation in aquatic ecosystems (Kerfoot, W.C. and A. Sih Eds.). University of New England Press, Hanover, NH. pp. 103-117.

Adams, W.J. 1987. Bioavailability of neutral lipophilic organic chemicals contained in sediments: A review. In: Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems (Dickson, K.L., A.W. Maki and W.A. Brungs, Eds.). Pergamon Press, Elmsford, NY. pp. 219-244.

Adams, W.J. and B.B. Heidolph. 1985. Shortcut chronic toxicity estimates using *Daphnia magna*. In: Aquatic Toxicology and Hazard Assessment: Seventh Symposium, ASTM STP 854 (Cardwell, R.D., R. Purdy and R.C. Bahner, Eds.) Amer. Soc. for Testing and Materials, Philadelphia, PA. pp. 87-103

Adams, W.J., R.A. Kimerle and R.G. Mosher. 1985. Aquatic safety assessment of chemicals sorbed to sediments. In: Aquatic Toxicology and Hazard Assessment: Seventh Symposium, STP 854 (Cardwell, R.D., R. Purdy and R.C. Bahner, Eds.). Amer. Soc. Testing Materials, Philadelphia, PA. pp. 429-453.

Adams, W.J., P.S. Ziegenfuss, W.J. Renaudette and R.G. Mosher. 1986. Comparison of laboratory and field methods for testing the toxicity of chemicals sorbed to sediments. In: Aquatic Toxicology and Environmental Fate: Ninth Vol, ASTM STP 921 (Poston, T.M. and R. Purdy, Eds.). Amer. Soc. for Testing and Materials, Philadelphia, PA. pp. 494-513 .

Allen, H.E., F. Gongmin, W. Boothman, D. Di Toro and J.D. Mahony. 1991. Draft analytical method for determination of acid volatile sulfide in sediment. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.

Ankley, G.T., D.A. Benoit, R.A. Hoke, E.N. Leonard, C.W. West, G.L. Phipps, V.R. Mattson and L.A. Anderson. 1992a. Development and evaluation of test methods for benthic invertebrates and sediments: Effects of flow rate and feeding on water quality and exposure conditions. Arch. Environ. Contam. Toxicol. 25:12-19.

Ankley, G.T., P.M. Cook, A.R. Carlson, D.J. Call, J.A. Swenson, H.F. Corcoran and R.A. Hoke. 1992b. Bioaccumulation of PCBs from sediments by oligochaetes and fishes: Comparison of laboratory and field studies. Can. J. Fish. Aquat. Sci. 49:2080-2085.

Ankley, G.T., R.A. Hoke, D.A. Benoit, E.M. Leonard, C.W. West, G.L. Phipps, V.R. Mattson and L.A. Anderson. 1993. Development and evaluation of test methods for benthic invertebrates and sediments: Effects of flow rate and feeding on water quality and exposure conditions. Arch. Environ. Contam. Toxicol. 25:12-19.

Ankley, G.T., A. Katko and J.W. Arthur. 1990. Identification of ammonia as an important sediment associated toxicant in the lower Fox River and Green Bay, Wisconsin. Environ. Toxicol. Chem. 9:313-322.

Ankley, G.T., K. Lodge, D.J. Call, M.D. Balcer, L.T. Brooke, P.M. Cook, R.G. Kreiss, Jr., A.R. Carlson, R.D. Johnson, G.J. Niemi, R.A. Hoke, C.W. West, J.P. Geisy, P.D. Jones and Z.C. Fuying. 1992c. Integrated assessment of contaminated sediments in the Lower Fox River and Green Bay, Wisconsin. Ecotoxicol. Environ. Safety 23:46-63.

Ankley, G.T., G.L. Phipps, E.N. Leonard, D.A. Benoit, V.R. Mattson, P.A. Kosian, A.M. Cotter, J.R. Dierkes, D.J. Hansen and J.D. Mahoney. 1991a. Acid volatile sulfide as a factor mediating cadmium and nickel bioavailability in sediments. Environ. Toxicol. Chem. 10:1299-1307.

Ankley, G.T., M.K. Schubauer-Berigan and J.K. Dierkes. 1991b. Predicting the toxicity of bulk sediments with aqueous test fractions: Pore water versus elutriate. Environ. Toxicol. Chem. 10: 1359-1366.

APHA. 1985. Standard methods for the examination of water and wastewater. 16th ed. American Public Health Association, Washington, DC. 1268 pp.

ASTM. 1993a. Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E729-88a. Annual Book of ASTM Standards, Vol. 11.04. Amer. Soc. for Testing and Materials, Philadelphia, PA. pp. 456-475.

ASTM. 1993b. Standard guide for conducting renewal life-cycle toxicity tests with *Daphnia magna*. E 1193-87. Annual Book of ASTM Standards, Vol. 11.04. Amer. Soc. for Testing and Materials, Philadelphia, PA. pp. 866-882.

ASTM. 1993c. Standard guide for conducting three-brood, renewal toxicity tests with *Ceriodaphnia dubia*. E 1295-89. Annual Book of ASTM Standards, Vol. 11.04. Amer. Soc. for Testing and Materials, Philadelphia, PA. pp. 1028-1046.

ASTM. 1993d. Standard guide for collection, storage, characterization, and manipulation of sediments for toxicological

testing. E1391-90. Annual Book of ASTM Standards, Vol. 11. 04. Amer. Soc. for Testing and Materials, Philadelphia, PA. pp. 1200-1214.

ASTM. 1993e. Standard guide for conducting sediment toxicity tests with freshwater invertebrates. E1383-93. Annual Book of ASTM Standards, Vol. 11.04. Amer. Soc. for Testing and Materials, Philadelphia, PA. pp. 1173-1199.

ASTM. 1993f. Standard guide for conducting early life-stage toxicity tests with fishes. E 1241-92. Annual book of ASTM standards, Vol. 11.04. Am. Soc. Testing and Materials, Philadelphia, PA. pp. 941-968.

ASTM. 1993g. Standard practice for using brine shrimp nauplii as food for test animals in aquatic toxicology. E 1203-92. Annual book of ASTM standards, Vol. 11.04. Am. Soc. Testing and Materials, Philadelphia, PA. pp. 923-928.

Bailey, H.C. and D.H.W. Liu. 1980. *Lumbriculus variegatus*, a benthic oligochaete, as a bioassay organism. In: (Eaton, J.C., P.R. Parrish and A.C. Hendricks, Eds.). Aquatic Toxicology. ASTM STP 707. Amer. Soc. Testing Materials, Philadelphia, PA. pp. 205-215.

Bardach, J.E., J.J. Bernstein, J.S. Hart, and J.R. Brett, 1966. Tolerance to temperature extremes: Animals. Part IV. Fishes, pp. 37-80. In: Environmental Biology. P.L. Altman and D. Dittmer (Eds.). Fed. Am. Soc. Exp. Biol., Bethesda, MD.

Batac-Catalan, Z. and D.S. White. 1982. Creating and maintaining cultures of *Chironomus tentans* (Diptera: Chironomidae). Ent. News 93:54-58.

Beckett, D.C. and P.A. Lewis. 1982. An efficient procedure for slide mounting of larval chironomids. Trans. Amer. Fish. Soc. 101:96-99.

Benoit, D.A. 1982. User's guide for conducting life cycle chronic toxicity tests with fathead minnows (*Pimephales promelas*). U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN, EPA-600.8-81-011. 17 pp.

Benoit, D.A., V.R. Mattson and D.L. Olson. 1982. A continuous flow mini-diluter system for toxicity testing. Water Res. 16:457-464.

Benoit, D.A., G.L. Phipps and G.T. Ankley. 1993. A sediment testing intermittent renewal system for the automated renewal of overlying water in toxicity tests with contaminated sediments.

Water Res. 27:1403-1412.

Bentley, R.E., D.C. Surprenant and S.R. Petrocelli. 1986. Collaborative study of *Daphnia magna* static renewal assays. Final report, Contract no. DAMD17-80-C-0011, U.S. Army Medical Res. Dev. Command, Fort Detrick, Frederick, MD.

Berner, D.B. 1986. Taxonomy of *Ceriodaphnia* (Crustacea: Cladocera) in U.S. Environmental Protection Agency cultures. EPA/600/4-86/032. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

Bierman, V.J., Jr. 1990. Equilibrium partitioning and biomagnification of organic chemicals in benthic animals. Environ. Sci. Technol. 24:1407-1412.

Biesinger, K.E., L.R. Williams and W.H. Van der Schalie. 1987. Procedures for conducting *Daphnia magna* bioassays. EPA/600/8-87/011. Environmental
Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Las Vegas, NV.

Birge, W.J., J.A. Black, T.M. Short and A.G. Westerman. 1989. A comparative ecological and toxicological investigation of a secondary wastewater treatment plant effluent and its receiving stream. Environ. Toxicol. Chem. 8:437-456.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Borgmann, U. and M. Munawar. 1989. A new standardized sediment bioassay protocol using the amphipod *Hyalella azteca* (Saussure). Hydrobiologica 188/189:425-431.

Bousfield, E.L. 1958. Fresh-water amphipod crustaceans of glaciated North America. Can. Field Nat. 72:55-113.

Bovee, E.C. 1949. Studies on the thermal death of *Hyalella azteca* (Saussure). Biol. Bull. (Woods Hole) 96:123-128.

Bovee, E.C. 1950. Some effects of temperature on the rates of embryonic, postembryonic, and adult growth in *Hyalella azteca*. Proc. Iowa Acad. Sci. 57:439-444.

Brinkhurst, R.O. 1980. Pollution biology-the North American experience. In: (Brinkhurst, R.O. and G.C. Cook, Eds.). Proceedings of the First International Symposium on Aquatic Oligochaete Biology, Plenum Press, New York, NY. pp. 471-475.

Brinkhurst, R.O. 1986. Guide to the Freshwater Aquatic Microdrile Oligochaetes of North America. Can. Spec. Publ. Fish. Aquatic Sci. 84. Dept. Fisheries and Oceans, Ottawa, Can. 259 pp.

Brinkhurst, R.O. and D.G. Cook. 1966. Studies on the North American Oligochaeta III. Lumbriculidae and additional notes and records of other families. Proc. Acad. Nat. Sci. Phila. 118:1-33.

Brooke, L.T., D.J. Call, D.L. Geiger, and C.E. Northcott [Eds.]. 1984. Acute toxicities of organic chemicals to fathead minnows (*Pimphales promelas*). Vol. I. Center for Lake Superior Environmental Studies, Univ. of Wisconsin-Superior Press, Superior, WI. 414 pp.

Brooke, L.T., D.J. Call, S.H. Poirier, S.L. McGovern, G.T. Ankley and P.M. Cook. Gut content weight and content clearance rate for three species of freshwater invertebrates (manuscript in preparation).

Brooks, J. L. 1957. The Systematics of North American *Daphnia*. Memoirs Conn. Acad. Arts Sci. 13: 1-180.

Brungs, W.A. 1971. Chronic effects of constant elevated temperature on the fathead minnow (*Pimephales promelas* Rafinesque). Trans. Am. Fish Soc. 100:659-664.

Burton, G.A., Jr., A. Drotar, J.M. Lazorchak and L.L. Bahls. 1987. Relationship of microbial activity and *Ceriodaphnia* responses to mining impacts on the Clark Fork River, Montana. Arch. Environ. Contam. Toxicol. 16:523-530.

Burton, G.A., Jr., B.L. Stemmer, K.L. Winks, P.E. Ross and L.C. Burnett. 1989. A multitrophic level evaluation of sediment toxicity in Waukegan and Indiana harbors. Environ. Toxicol. Chem. 8:1057-1066.

Cairns, M.A., A.V. Nebeker, J.H. Gakstatter and W. Griffis. 1984. Toxicity of copper-spiked sediments to freshwater invertebrates. Environ. Toxicol. Chem. 3:435-446.

Call, D.J., M.D. Balcer, L.T. Brooke, S.J. Lozano and D.D. Vaishnav. 1991. Sediment quality evaluation in the Lower Fox River and southern Green Bay of Lake Michigan. Final report to Environmental Research Laboratory-Duluth, U.S. Environmental Protection Agency, Duluth, MN. University of Wisconsin-Superior, Superior, WI. 270 pp.

Carlson, A.R., G.L. Phipps, V.R. Mattson, P.A. Kosian and A.M.

Cotter. 1991. The role of acid-volatile sulfide in determining cadmium bioavailability and toxicity in freshwater sediments. Environ. Toxicol. Chem. 10:1309-1319.

Chapman, P.M. 1988. Marine sediment toxicity tests. In: Chemical and Biological Characterization of Sludges, Sediments, Dredge Spoils, and Drilling Muds. ASTM STP 976 (Lichtenberg, J.J., F.A. Winter, C.I. Weber and L. Fradkin, Eds.). Amer. Soc. Testing Materials, Philadelphia, PA. pp. 391-402

Chapman, P.M., M.A. Farrell and R.O. Brinkhurst. 1982a. Relative tolerances of selected aquatic oligochaetes to individual pollutants and environmental factors. Aquat. Toxicol. 2:47-67.

Chapman, P.M., M.A. Farrell and R.O. Brinkhurst. 1982b. Relative tolerances of selected aquatic oligochaetes to combinations of pollutants and environmental factors. Aquat. Toxicol. 2:69-78.

Chekanovskaya, O.V. 1962. Aquatic Oligochaeta of the U.S.S.R. Akademiya Nauk SSSR. Moscow, USSR.

Cole, G.A. and R.L. Watkins. 1977. *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. *Hydrobiologia* 52:175-184.

Connell, D.W., M. Bowman and D.W. Hawker. 1988. Bioconcentration of chlorinated hydrocarbons from sediment by oligochaetes. Ecotoxicol. Environ. Safety. 16:293-302.

Cook, D.G. 1969. Observations on the life history and ecology of some Lumbriculidae (Annelida, Oligochaeta). Hydrobiologia 34:561-574.

Cooper, W.E. 1965. Dynamics and production of a natural population of a fresh-water amphipod, *Hyalella azteca*. Ecol. Mong. 35:377-394.

Covich, A.P. and J.H. Thorp. 1991. Crustacea: Introduction and Peracarida. In: Ecology and Classification of North American Freshwater Invertebrates. Thorp, J.H. and A.P. Covich [Eds.]. Academic Press, Inc. San Diego, CA. pp. 775-689.

Cowan, C.E. and R.G. Riley. 1987. Guidance for Sampling of and Analyzing for Organic Contaminants in Sediments. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.

Cowgill, U.M. and D.P. Milazzo. 1991. The sensitivity of *Ceriodaphnia dubia* and *Daphnia magna* to seven chemicals using the

three-brood test. Arch. Environ. Contam. Toxicol. 20:211-217.

Cowgill, U.M., I.T. Takahashi and S.L. Applegath. 1985. A comparison of the effect of four benchmark chemicals on *Daphnia magna* and *Ceriodaphnia dubia-affinis* tested at two different temperatures. Environ. Toxicol. Chem. 4:415-422.

Curry, L.L. 1962. A survey of environmental requirements for the midge (Diptera: Tendipedidae). pp. 127-141 In: C.M. Tarzwell (ed.). Biological Problems in Water Pollution, 3rd Seminar. U.S. Public Health Serv. Publ. 999-WP-25.

Davenport, R. and A. Spacie. 1991. Acute phototoxicity of harbor and tributary sediments from Lower Lake Michigan. J. Great Lakes Res. 17:51-56.

de March, B.G.E. 1977. The effects of photoperiod and temperature on the induction and termination of reproductive resting stage in the freshwater amphipod *Hyalella azteca* (Saussure). Can. J. Zool. 55:1595-1600.

de March, B.G.E. 1978. The effects of constant and variable temperatures on the size, growth, and reproduction of *Hyalella azteca* (Saussure). Can. J. Zool. 56:1801-1806.

de March, B.G.E. 1981. *Hyalella azteca* (Saussure). In: Manual for the culture of selected freshwater invertebrates. Lawrence, S.G. (Ed.). Can. Spec. Pub. fish. Aquat. Sci. No. 54, Department of Fisheries and Oceans.

Denny, J.S. 1987. Guidelines for the culture of fathead minnows *Pimephales promelas* for use in toxicity tests. EPA-600/3-87/001. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN. 42 pp.

Denny, J. and S. Collyard. 1991. Standard Operating Procedure for the Culture of *Hyalella azteca*. Environ. Res. Lab.-Duluth, USEPA, Duluth, MN. Draft, 21 October 1991.

Denny, J. and K. Mead. 1991. Standard Operating Procedure for the Culture of *Chironomus tentans*. Environ. Res. Lab.-Duluth, USEPA, and AScI Corp., Duluth, MN. Draft, 10/21/91.

Denny, J.S., R.A. Hoke, K.E. Mead, S.A. Collyard, J.L. Juenemann and S.C. Yousuff. 1993. Standard operating procedure for culturing the invertebrates *Hyalella azteca, Chironomus tentans* and *Lumbriculus variegatus*. Environ. Res. Lab-Duluth, USEPA and AScI Corp., Duluth, MN. Lab Animal (Submitted manuscript).

Devine, G. 1968. A study of the smallmouth bass in ponds with

special consideration of minnows and decapods as forage. MA Thesis, Univ. of Missouri, Columbia, MO. 115 pp.

Dickson, K.L., W.T. Waller, J.H. Kennedy and L.P. Ammann. 1990. Assessing the relationships between ambient toxicity and instream biological response. Final Report for Cooperative Agreement No. CR-816284-01-0. U.S. Environmental Protection Agency, Duluth, MN.

Di Toro, D.M., C.S. Zarba, D.J. Hansen, W.J. Berry, R.C. Swartz, C.E. Cowan, S.P. Pavlou, H.E. Allen, N.A. Thomas and P.R. Paquin. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. Environ. Toxicol. Chem. 10:1541-1583.

Driver, E.A. 1977. Chironomid communities in small prairie ponds: some characteristics and controls. Freshwater Biol. 7:121-123.

Driver, E.A., L.G. Sugden and R.J. Kovach. 1974. Calorific, chemical and physical values of potential duck foods. Freshwater Biol. 4:281-292.

Dutka, B.J., T. Tuominen, L. Churchland and K.K. Kwan. 1989. Fraser River sediments and waters evaluated by the battery of screening tests technique. Hydrobiologia 188/189:301-315.

Eddy, S., and J.C. Underhill. 1974. Northern fishes. Univ. of Minnesota Press, Minneapolis, MN. 414 pp.

Embody, G.C. 1911. A preliminary study of the distribution, food and reproductive capacity of some freshwater amphipods. Int. Rev. gesamten Hydrobiol. Biol. Suppl. 3:1-33.

Englemann, W. and D.G. Shappiro. 1965. Photoperiodic control of the maintenance and termination of larval diapause in *Chironomus tentans*. Nature 207:548-549.

Ewell, W.S., J.W. Gorsuch, R.O. Kringle, K.A. Robillard and R.C. Spiegel. 1986. Simultaneous evaluation of the acute effects of chemicals on seven aquatic species. Environ. Contam. Toxicol. 5:831-840.

Fallon, M.E. and F.J. Horvath. 1985. Preliminary assessment of contaminants in soft sediments of the Detroit River. J. Great Lakes Res. 11:386-399.

Finney, D.J. 1948. The Fisher-Yates test of significance in 2 x 2 contingency tables. Biometrika 35:145-156.

Flannagan, J.F. 1971. Toxicity evaluation of trisodium

nitrilotriacetate to selected invertebrates and amphibians. Fish. Res. Board Can. Tech. Rep. 258. 21 p.

Folch, J., M. Lees and G.H.S. Stanley. 1957. A simple method for isolation and purification of total lipids from animal tissue. J. Biol. Chem. 226:497-509.

Gale, W.G., and G.L. Bunyak. 1982. Fecundity and spawning frequency of the fathead minnow. Trans. Am. Fish. Soc. 111:35-40.

Gauss, J.D., P.E. Woods, R.W. Winner and J.H,. Skillings. 1985. Acute toxicity of copper to three life stages of *Chironomus tentans* as affected by water hardness-alkalinity. Environ. Pollut. (Ser. A) 37:149-159.

Geiger, D.L., L.T. Brooke, and D.J. Call [Eds.]. 1990. Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*). Vol. V. Center for Lake Superior Environmental Studies, Univ. of Wisconsin-Superior Press, Superior, WI. 332 pp.

Geiger, D.L., D.J. Call, and L.T. Brooke [Eds.]. 1988. Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*). Vol. IV. Center for Lake Superior Environmental Studies, Univ. of Wisconsin-Superior Press, Superior, WI. 355 pp.

Geiger, D.L., C.E. Northcott, D.J. Call, and L.T. Brooke [Eds.]. 1985. Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*). Vol. II. Center for Lake Superior Environmental Studies, Univ. of Wisconsin-Superior Press, Superior, WI. 326 pp.

Geiger, D.L., S.H. Poirier, L.T. Brooke, and D.J. Call [Eds.]. 1986. Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*). Vol. III. Center for Lake Superior Environmental Studies, Univ. of Wisconsin-Superior Press, Superior, WI. 328 pp.

Geisler, F.S. 1944. Studies on the post-embryonic development of *Hyalella azteca* (Saussure). Biol. Bull. 86:6-22.

Gersich, F.M. and D.P. Milazzo. 1990. Evaluation of a 14-day static renewal toxicity test with *Daphnia magna* Straus. Arch. Environ. Contam. Toxicol. 19:72-76.

Giesy, J.P., R.L. Graney, J.L. Newsted, C.J. Rosiu, A. Benda, R.G. Kreis, Jr., and F.J. Horvath. 1988. Comparison of three sediment bioassay methods using Detroit River sediments. Environ. Toxicol. Chem. 7:483-498.

Giesy, J.P., C.J. Rosiu, R.L. Graney and M.G. Henry. 1990. Benthic invertebrate bioassays with toxic sediment and pore water. Environ. Toxicol. Chem. 9:233-248.

Gobas, F.A.P.C., D.C. Bedard, J.J.H. Ciborowski and G.D. Haffner. 1989. Bioaccumulation of chlorinated hydrocarbons by the mayfly (*Hexagenia limbata*) in Lake St. Clair. J. Great Lakes Res. 15:581-588.

Held, J.W., and J.J. Peterka. 1974. Age, growth and food habits of the fathead minnow, *Pimephales promelas*, in North Dakota saline lakes. Tran. Am. Fish. Soc. 103:743-756.

Hoke, R.A. 1989. Sediment toxicity assessments in the Lower Fox River and Green Bay using Microtox^{R} and 10-day *Daphnia magna* porewater assays. Data report submitted to Large Lakes Research Station, U.S. Environmental Protection Agency, Grosse Ile, MI, Sept. 25, 1989.

Hoke, R.A., J.P. Giesy, G.T. Ankley, J.L. Newsted and J.R. Adams. 1990. Toxicity of sediments from western Lake Erie and the Maumee River at Toledo,
Ohio, 1987: implications for current dredged material disposal practices. J. Great Lakes Res. 16:457-470.

Hoke, R.A. and B.L. Prater. 1980. Relationship of percent mortality of four species of aquatic biota from 96-hour sediment bioassays of five Lake Michigan harbors and elutriate chemistry of the sediments. Bull. Environ. Contam. Toxicol. 25:394-399.

Hornig, C.E. 1980. Use of the Aquatic Oligochaete, *Lumbriculus variegatus*, for Effluent Biomonitoring. EPA-600/D-80-005. National Technical Information Service, Springfield, VA.

Hughes, M.M., M.A. Heber, S.C. Schimmel, and W.J. Berry. 1987. Guidance manual for conducting complex effluent and receiving water larval fish growth-survival studies with the sheepshead minnow *Cyprinodon variegatus*). Contribution No. X104. In: Users guide to the conduct and interpretation of complex effluent toxicity tests at estuarine/marine sites (Schimmel, S. C. Ed.). Environmental Research Laboratory, U.S. Environmental Protection Agency, Narragansett, R. I. Contribution 796. 165 pp.

Ingersoll, C.G. and M.K. Nelson. 1990. Testing sediment toxicity with *Hyalella azteca* (Amphipoda) and *Chironomus riparius* Diptera. In: Aquatic Toxicology and Risk Assessment: Thirteenth Volume. ASTM STP 1096 (Landis, W.G. and W.H. van der Schalie Eds.). American Society for Testing and Materials. Philadelphia. pp. 93-109.

Johannsen, O.A. and L.C. Thomsen. 1937. Aquatic Diptera. Parts IV and V. Part IV, Chironomidae: subfamily Chironominae; Part V, Ceratopogonidae. Memoir 210, Cornell Univ. Agric. Exp. Station, Ithaca, N.Y.

Juenemann, J. and J. Denny. 1992. Standard Operating Procedure for the Culture of *Lumbriculus variegatus*. AScI Corp. and Environ. Res. Lab.-Duluth, USEPA, Duluth, MN. Draft, 3-30-92.

Keating, K.I. 1984. The influence of vitamin B_{12} deficiency on the reproduction of *Daphnia pulex* Leydig (Cladocera). J. Crustacean Biol. 5:130-136.

Keating, K.I. and B.C. Dagbusan. 1984. Effect of selenium deficiency on cuticle integrity in the Cladocera (Crustacea). Proc. Natl. Acad. Sci. U.S.A. 81:3433-3437.

Keilty, T.J., D.S. White and P.F. Landrum. 1988a. Short-term lethality and sediment avoidance assays with endrin-contaminated sediment and two oligochaetes from Lake Michigan. Arch. Environ. Contam. Toxicol. 17:95-101.

Keilty, T.J., D.S. White and P.F. Landrum. 1988b. Sublethal responses to endrin in sediment by *Limnodrilus hoffmeisteri* (Tubificidae), and in mixed-culture with *Stylodrilus heringianus* (Lumbriculidae). Aquat. Toxicol. 13:227-250.

Kemble, N.E., J.M. Berger, W.G. Brumbaugh, E.L. Brunson, T.J. Canfield, J.J. Coyle, F.J. Dwyer, J.F. Fairchild, C.G. Ingersoll, T.W. LaPoint, J.C. Meadows, D.P. Monda, B.C. Poulton, D.F. Woodward and J.L. Zajicek. 1993. ch. 2: Sediment toxicology. In: U.S. Fish and Wildlife Service and Univ. of Wyoming Final Report for the USEPA Milltown Endangerment Assessment Project. NTIS PB 93-215952, National Technical Information Service, Springfield, VA.

Knight, J.T. and W. T. Waller. 1987. Incorporating *Daphnia magna* into the seven-day *Ceriodaphnia* effluent toxicity test method. Environ. Toxicol. Chem. 6:635-645.

Lake, J.L., N.I. Rubinstein, H. Lee II, C.A. Lake, J. Heltshe and S. Pavignano. 1990. Equilibrium partitioning and bioaccumulation of sediment-associated contaminants by infaunal organisms. Environ. Toxicol. Chem. 9:1095-1106.

Larson, L.J. 1989. Method for the preliminary assessment of aquatic contamination sites using sediment extract toxicity tests. Bull. Environ. Contam. 42:218-225.

Larsson, P. 1985. Contaminated sediments of lakes and oceans

act as sources of release to water and atmosphere. Nature 317:347-349.

Larsson, P. 1986. Zooplankton and fish accumulate chlorinated hydrocarbons from contaminated sediments. Can. J. Fish. Aquat. Sci. 43:1463-1466.

Laskowski-Hoke, R.A. and B.L. Prater. 1981. Relationship of mortality of aquatic biota from 96-hour sediment bioassays and the change in chemical composition of the test water. Bull. Environ. Contam. Toxicol. 26:323-327.

Laurence, G.C. 1974. Growth and survival of haddock, *Melanogrammus aeglefinus*, larvae in relation to planktonic prey concentration. J. Fish. Res. Board Can. 31:1415-1419.

Lauritsen, D.D., S.C. Mozley and D.S. White. 1985. Distribution of oligochaetes in Lake Michigan and comments on their use as indices of pollution. J. Great Lakes Res. 11:67-76.

LeBlanc, G.A. and D.C. Surprenant. 1985. A method of assessing the toxicity of contaminated freshwater sediments. pp. 269-283 In: Aquatic toxicology and Hazard Assessment: Seventh Symposium, ASTM STP 854 (Cardwell, R.D., R. Purdy and R.C. Bahner, Eds.). Amer. Soc. for Testing and Materials, Philadelphia, PA.

Leiby, M.M. 1984. Life history and ecology of pelagic fish eggs and larvae. pp. 14-28 In K.A. Steidinger and L.M. Walker (eds.). Marine plankton life cycle strategies. CRC Press, Boca Raton, FL.

Lewis, P.A. and W.B. Horning, II. 1991. Differences in acute toxicity test results of three reference toxicants on *Daphnia* at two temperatures. Environ. Toxicol. Chem. 10:1351-1357.

Lewis, P.A. and W.B. Horning. 1988. A short-term chronic test using *Daphnia magna*. In: Aquatic Toxicology and Hazard Assessment: Tenth Volume, ASTM STP 971 (Adams, W.J., G.A. Chapman, and W.G. Landis, Eds.) Amer. Soc. for Testing and Materials, Philadelphia, PA. pp. 548-555.

Maki, A.W. 1977. Modifications of continuous flow methods for small aquatic organisms. Prog. Fish Cult. 39:172-174.

Malueg, K.W., G.S. Schuytema, J.H. Gakstatter and D.F. Krawczyk. 1984a. Toxicity of sediments from three metal-contaminated areas. Environ. Toxicol. Chem. 3:279-291.

Malueg, K.W., G.S. Schuytema, D.F. Krawczyk and J.H. Gakstatter. 1984b. Laboratory sediment toxicity tests, sediment chemistry and distribution of benthic macroinvertebrates in sediments from the

Keweenaw Waterway, Michigan. Environ. Toxicol. Chem. 3:233-242.

Marking, L.L., and V.K. Dawson. 1973. Toxicity of quinaldine sulfate to fish. Invest. Fish Contr. No. 48, U.S. Fish and Wildlife Service, Department of the Interior, Washington, DC. 8 pp.

May, R.C. 1971. Effects of delayed initial feeding on larvae of grunion, *Leuresthes tenius* (Ayres). Fish. Bull. 69:411-425.

Mayer, F.L., Jr., and M.R. Ellersieck. 1986. Manual of acute toxicity: Interpretation and data base for 410 chemicals and 66 species of freshwater animals. U.S. Fish and Wildl. Ser. Resource Publ. No. 160, Washington, DC. 506 pp.

McCarraher, D.B., and R. Thomas. 1968. Some ecological observations on the fathead minnow, *Pimephales promelas*, in the alkaline waters of Nebraska. Trans. Am. Fish. Soc. 97:52-55.

McKim, J.M. 1977. Evaluation of tests with early life stages of fish for predicting long-term toxicity. J. Fish. Res. Board Can. 34:1148-1154.

McLarney, W.O., S. Henderson and M.S. Sherman. 1974. A new method for culturing *Chironomus tentans* Fabricius larvae using burlap substrate in fertilized pools. Aquaculture 4:267-276.

Moore, D.W., T.M. Dillon, J.Q. Word and J.A. Ward. 1994. Quality assurance/quality control (QA/QC) guidance for laboratory dredged material bioassays. Miscellaneous Paper D-94-3, U.S. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS.

Mount, D.I. 1973. Chronic effect of low pH on fathead minnow survival, growth and reproduction. Water Res. 7:987-993.

Mount, D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1:21-30.

Mount, D.I. and T.J. Norberg. 1984. A seven-day life-cycle cladoceran toxicity test. Environ. Toxicol. Chem. 3:425-434.

Mount, D.I., T.J. Norberg-King, R. Keen and J.T. Taraldsen. 1987. A reference test water for cladocerans. Abstract, 11th Annual Symposium, Aquatic Toxicology and Hazard Assessment, Amer. Soc. for Testing and Materials, May 10-12, 1987, Cincinnati, OH.

Mount, D.I., A.E. Steen and T.J. Norberg-King [Eds.]. 1985. Validity of effluent and ambient toxicity testing for predicting biological impact, Five Mile Creek, Birmingham, Alabama. EPA-

600/8-85-015. U.S. Environmental Protection Agency, Duluth, MN.

Mount, D.I., N.A. Thomas, T.J. Norberg, M.T. Barbour, T.H. Roush and W.F. Brandes. 1984. Effluent and ambient toxicity testing and instream community response on the Ottawa River, Lima, Ohio. EPA-600/3-84-080. U.S. Environmental Protection Agency, Duluth, MN.

Nebeker, A.V., M.A. Cairns, J.H. Gakstatter, K.W. Malueg, G.S. Schuytema and D.F. Krawczyk. 1984a. Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates. Environ. Toxicol. Chem. 3:617-630.

Nebeker, A.V., M.A. Cairns, S.T. Onjukka and R.H. Titus. 1986a. Effect of age on sensitivity of *Daphnia magna* to cadmium, copper and cyanazine. Environ. Toxicol. Chem. 5:527-530.

Nebeker, A.V., M.A. Cairns and C.M. Wise. 1984b. Relative sensitivity of *Chironomus tentans* life stages to copper. Environ. Toxicol. Chem. 3:151-158.

Nebeker, A.V., W.L. Griffis, C.M. Wise, E. Hopkins and J.A. Barbitta. 1989. Survival, reproduction and bioconcentration in invertebrates and fish exposed to hexachlorobenzene. Environ. Toxicol. Chem. 8:601-611.

Nebeker, A.V., S.T. Onjukka and M.A. Cairns. 1988. Chronic effects of contaminated sediment on *Daphnia magna* and *Chironomus tentans*. Bull. Environ. Contam. Toxicol. 41:574-581.

Nebeker, A.V., S.T. Onjukka, M.A. Cairns and D.F. Krawczyk. 1986b. Survival of *Daphnia magna* and *Hyalella azteca* in cadmium-spiked water and sediment. Environ. Toxicol. Chem. 5:933-938.

Nimmo, D.W.R., D. Link, L.P. Parrish, G.J. Rodriguez and W. Wuerthle. 1989. Comparisons of on-site and laboratory toxicity tests: derivation of site-specific criteria for un-ionized ammonia in a Colorado transitional stream. Environ. Toxicol. Chem. 8:1177-1189.

Norberg, T.J., and D.I. Mount. 1985. A new fathead minnow (*Pimephales promelas*) subchronic toxicity test. Environ. Toxicol. Chem 4:711-718.

Norberg-King, T.J. and D.I. Mount [Eds.]. 1986. Validity of effluent and ambient toxicity tests for predicting biological impact, Skeleton Creek, Enid, Oklahoma. EPA-600/8-86/002. U.S. Environmental Protection Agency, Duluth, MN.

Obana, H., S. Hori and T. Kushimoto. 1981. Determination of polycyclic aromatic hydrocarbons in marine samples by high-

performance liquid-chromatography. Bull. Environ. Conatam. Toxicol. 26:613-620.

Oliver, B.G. 1984. Uptake of chlorinated organics from anthropogenically contaminated sediments by oligochaete worms. Can. J. Fish. Aquat. Sci. 41:878-883.

Oliver, B.G. 1987. Biouptake of chlorinated hydrocarbons from laboratory-spiked and field sediments by oligochaete worms. Environ. Sci. Technol. 21:785-790.

Oliver, D.R. 1971. Life history of the chironomidae. Ann. Rev. Entomol. 16:211-230.

Oris, J.T., R.W. Winner and M.V. Moore. 1991. A four-day survival and reproduction toxicity test for *Ceriodaphnia dubia*. Environ. Toxicol. Chem. 10:217-224.

Pearson, E.S. and T.O. Hartley. 1962. Biometrika tables for statisticians. Vol. 1. Cambridge Univ. Press, England. pp. 65-70.

Pennak, R.W. 1989. Freshwater Invertebrates of the United States. John Wiley and Sons, Inc. New York, NY. 628 pp.

Pennak, R.W. and W.A. Rosine. 1976. Distribution and ecology of Amphipoda (Crustacea) in Colorado. Am. Midl. Nat. 96:325-331.

Phipps, G.L., G.T. Ankley, D.A. Benoit and V.R. Mattson. 1993. Use of the aquatic oligochaete *Lumbriculus variegatus* for assessing the toxicity and bioaccumulation of sediment-associated contaminants. Environ. Toxicol. Chem. 12:269-279.

Post, J.R., and A.B. Prankevicius. 1987. Size-selective mortality in young-of-the-year yellow perch (*Perca flavescens*): Evidence from otolith microstructure. Can. J. Fish. Aquat. Sci. 44:1840-1847.

Poulton, D.J., K.J. Simpson, D.R. Barton and K.R. Lum. 1988. Trace metals and benthic invertebrates in sediments of nearshore Lake Ontario at Hamilton Harbour. J. Great Lakes Res. 14:52-65.

Randall, R.C., H. Lee II, R.J. Ozretich, J.L. Lake and R.J. Pruell. 1991. Evaluation of selected lipid methods for normalizing pollutant bioaccumulation. Environ. Toxicol. Chem. 10:1431-1436.

Rawson, D.S., and J.E. Moore. 1944. The saline lakes of Saskatchewan. Can.Res. 22:141-201.

Rice, C.P. and D.S. White. 1987. PCB availability assessment of

river dredging using caged clams and fish. Environ. Toxicol. Chem. 6:259-274.

Rigdon, L.P., G.J. Moody and J.W. Frazer. 1978. Determination of residual chlorine in water with computer automation and a residual-chlorine electrode. Anal. Chem. 50:465-468.

Robbins, J.A., T.J. Keilty, D.S. White and D.N. Edgington. 1989. Relationships among tubificid abundances, sediment composition and accumulation rates in Lake Erie. Can. J. Fish. Aquat. Sci. 46:223-231.

Sadler, W.O. 1935. Biology of the midge *Chironomus tentans* Fabricius, and methods for its propagation. Memoir 173, Cornell Univ. Agric. Exp. Station, Ithaca, N.Y.

SAS Institute, Inc. 1985. SAS User's Guide: Statistics. Version 5 Edition. SAS Institute, Inc. Cary, NC. 956 pp.

Satterthwaite, F.W. 1946. An approximate distribution of estimates of variance components. Biom. Bull. 2:110-114.

Schmitt, C.J. and S.E. Finger. 1987. The effects of sample preparation on measured concentrations of eight elements in edible tissues of fish from streams contaminated by lead mining. Arch. Environ. Contam. Toxicol. 16:185-207.

Schmitt, C.J., J.L. Zajicek and P.H. Peterman. 1990. National contaminant biomonitoring program: Residues of organochlorine chemicals in U.S. freshwater fish, 1976-1984. Arch. Environ. Contam. Toxicol. 19:748-781.

Schuytema, G.S., D.F. Krawczyk, W.L. Griffis, A.V. Nebeker, M.L. Robideaux, B.J. Brownawell and J.C. Westall. 1988. Comparative uptake of hexachlorobenzene by fathead minnows, amphipods and oligochaete worms from water and sediment. Environ. Toxicol. Chem. 7:1035-1044.

Scott, W.B., and E.J. Crossman. 1973. Freshwater fishes of Canada. Fish. Res. Bd. Canada. Bulletin No. 184. Ottawa, Canada. 966 pp.

Siegfried, W.R. 1973. Summer food and feeding of the ruddy duck in Manitoba. Can. J. Zool. 51:1293-1297.

Sims, J.G., E. Gamble and D.W. Moore. 1993. Protocol for conducting sediment bioassays with materials potentially containing zebra mussels, (*Dreissena polymorpha*). Miscellaneous paper EL-93, U.S. Army Waterways Experiment Station, Vicksburg, MS.

Slooff, W. and J.H. Canton. 1983. Comparison of the susceptibility of 11 freshwater species to 8 chemical compounds. II. (Semi)chronic toxicity tests. Aquat. Toxicol. 4:271-282.

Smith, L.L., Jr., D.M. Oseid, I.R. Adleman and S.J. Broderius. 1976. Effect of hydrogen sulfide on fish and invertebrates: Part I - Acute and chronic toxicity studies. EPA-600/3-76-062a. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN. 286 pp.

Snedecor G.W. and G.C. Cochran. 1989. Statistical Methods. 8th Ed. The Iowa State University Press. Ames, IA. 507 pp.

Spencer, D.R. 1980. The aquatic oligochaetes of the St. Lawrence Great Lakes region. In: (Brinkhurst, R.O. and D.G. Cook, Eds.). Proceedings of the First International Symposium on Aquatic Oligochaete Biology, Plenum Press, New York, NY. pp. 115-164.

Sprague, J.B. 1963. Resistance of four freshwater crustaceans to lethal high temperature and low oxygen. J. Fish. Res. Board Can. 20:387-415.

Sprague, J.B. 1971. Measurement of pollutant toxicity to fish. III. Sublethal effects. Water Res. 5:245-266.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York, NY. 633 pp.

Stemmer, B.L., G.A. Burton, Jr. and G. Sasson-Brickson. 1990. Effect of spatial variance and collection method on cladoceran toxicity and indigenous microbial activity determinations. Environ. Toxicol. Chem. 9:1035-1044.

Stimson, K.S., D.J. Klemm, and J.K. Hiltunen. 1982. A Guide to Freshwater Tubificidae (Annelida:Clitellata:Oligochaeta) of North America. EPA-600/3-82-033. National Technical Information Services, Springfield, VA.

Topping, M.S. 1971. Ecology of larvae of *Chironomus tentans* (Diptera: Chironomidae) in saline lakes in central British Columbia. Can. Entomol. 193:328-338.

Townsend, B.E., S.G. Lawrence and J.F. Flannagan. 1981. *Chironomus tentans* Fabricius. pp. 109-126 In: S.G. Lawrence (ed.). Manual for the Culture of Selected Freshwater Invertebrates. Can. Spec. Publ. Fish Aquatic Sci., no. 54. Dept. of Fisheries and Oceans, Winnipeg, Canada.

Tucker, M.W. and W.J. Adams. 1986. Method for conducting acute

toxicity tests with the midge *Chironomus tentans*. Monsanto Environmental Sciences Center Report No. ESC-EAG-86-113, St. Louis, MO.

USEPA. 1985. Characterization of Hazardous Waste Sites - A Methods Manual (Vols. 1-3). EPA 600/4/84-075. Environmental Monitoring Support Lab, Las Vegas, NV. NTIS PB 85-215960.

USEPA. 1987. Quality criteria for water. 1986. EPA 440/5-86-001. U.S. Government Printing Office, Washington, DC. Order No. 955-002-00000-8.

USEPA. 1990a. Specifications and guidance for obtaining contaminant-free sampling containers. Directive No. 9240.0-05, Office of Solid Waste and Emergency Response, USEPA, April, 1990.

USEPA. 1990b. Analytical procedures and quality assurance plan for the determination of xenobiotic chemical contaminants in fish. EPA/600/3-90/023, USEPA, Environmental Research Laboratory, Duluth, MN. 23 p.

USEPA. 1990c. Analytical procedures and quality assurance plan for the determination of PCDD/PCDF in fish. EPA/600/3-90/022, USEPA, Environmental Research Laboratory, Duluth, MN. 32 p.

USEPA. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fourth edition. C.I. Weber [Ed.]. EPA-600/4-90-027F. Environmental Monitoring Systems Laboratory-Cincinnati, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH. 293 pp.

USEPA. 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates (Draft). EPA 600/R-94/924. Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN.

USEPA and USACE. 1990. Draft ecological evaluation of proposed discharge of dredged material into ocean waters. EPA-503-8-90/002. Office of Marine and Estuarine Protection, U.S. Environmental Protection Agency, Washington, D.C.

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

USEPA and USACE. 1998. Evaluation of dredged material proposed for discharge in waters of the U.S. - Testing Manual (Inland Testing Manual), EPA-823-B-98-004, Office of Water, U.S.

Environmental Protection Agency, Washington, D.C.

USEPA NETAC. 1990. Standard operating procedures for *Ceriodaphnia* culturing, reference toxicant testing and young production monitoring-effluent testing center. National Effluent Toxicity Assessment Center, Environ. Res. Lab.-Duluth, USEPA.

Vassilaros, D.C., P.W. Stoker, G.M. Booth and M.L. Lee. 1982. Capillary gas chromatographic determination of polycyclic aromatic compounds in vertebrate fish tissue. Anal. Chem. 54:106-112.

Wentsel, R., A. McIntosh and V. Anderson. 1977a. Sediment contamination and benthic macroinvertebrate distribution in a metal-impacted lake. Environ. Pollut. 14:187-193.

Wentsel, R., A. McIntosh and G. Atchison. 1977b. Sublethal effects of heavy metal contaminated sediment on midge larvae (*Chironomus tentans*). Hydrobiologia 56:153-156.

Wentsel, R. A., A. McIntosh and P.C. McCafferty. 1978. Emergence of the midge *Chironomus tentans* when exposed to heavy metal contaminated sediments. Hydrobiologia 57:195-196.

Werner, R.G., and J.H.S. Blaxter. 1980. Growth and survival of larval herring (*Clupea harengus*) in relation to prey density. Can. J. Fish. Aquat. Sci. 37:1063-1069.

Werner, E.E., and D.J. Hall. 1974. Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). Ecology 55:1042-1052.

Werner, E.E. and J.F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. Annu. Rev. Ecol. Syst. 15:393-425.

West, C.W., V.R. Mattson, G.L. Phipps, E.N. Leonard and G.T. Ankley. 1993. Comparison of the relative sensitivity of three benthic invertebrates to copper contaminated sediments from the Keweenaw Waterway. Hydrobiologia 262:57-63.

Wiederholm, T. and G. Dave. 1989. Toxicity of metal polluted sediments to *Daphnia magna* and *Tubifex tubifex.* Hydrobiologia 176/177:411-417.

Wiederholm, T., A.M. Wiederholm and G. Milbrink. 1987. Bulk sediment bioassays with five species of fresh-water oligochaetes. Water Air Soil Pollut. 36:131-154.

Winner, R.W. 1988. Evaluation of the relative sensitivities of 7-

d *Daphnia magna* and *Ceriodaphnia dubia* toxicity tests for cadmium and sodium pentachlorophenate. Environ. Toxicol. Chem. 7:153-159.

Zumwalt, D.C., F.J. Dwyer, I.E. Green and C.G. Ingersoll. 1994. A water-renewal system that accurately delivers small volumes of water to exposure chambers. Environ. Toxicol. Chem. 13:1311-1314.

Attachment A. Materials for Culturing and Conducting Toxicity Tests with *Daphnia magna*.

Biological Supplies

- *Daphnia magna* starter culture
- *Selenastrum capricornutum* starter culture (see Attachment C for sources)
- Trout chow

Glassware

- Culture beakers (2L)
- Test beakers (100 mL)
- Erlenmeyer flasks (1 and 2 L)
- Volumetric flasks and graduated cylinders (10-1,000 mL, Class A borosilicate glass or non-toxic plastic)
- Volumetric pipets (1-100 mL, Class A)
- Pipets (disposable, plugged, sterile/mL x 1/100 polystyrene)
- Microscope slides
- Counting chamber (Sedgwick-Rafter, Palmer-Maloney or hemacytometer)
- Burettes
- Fire-polished glass tubes (5 and 8 mm inside diameter)
- Plate glass sheets (double strength) for covering culture and test beakers
- Thermometers (National Bureau of Standards Certified, $\pm 0.1^{o}$ C)

Instruments and Equipment

- pH meter ($\pm$ 0.1 pH unit)
- Dissolved oxygen meter ($\pm$ 0.1 mg/L)
- Specific conductivity meter ($\pm$ 5 umhos/cm or equivalent)
- Continuous recording thermometer ($\pm$ 0.1^{o} C)
- Constant temperature environmental chambers for culturing *Daphnia magna* and *Selenastrum capricornutum*
- Light meter
- Deionized water system (MILLIPORE MILLI-Q^{R} or equivalent)
- Analytical balance (capable of accurately weighing to 0.0001 g)
- Reference weights (class S)
- Magnetic stir plates (for preparing elutriate water and algal cultures)
- Teflon®-coated stir bar magnets
- Microscope (compound scope with 10x, 45x and 100x objective lenses, 10x ocular lens, mechanical stage, substage condensor, and light source)
- Microscope (dissecting scope with substage lighting)
- Light box
- Centrifuge (plankton, or with swing-out buckets having a capacity of 15-100 mL)
- Centrifuge tubes (15-100mL, screw cap)

Autoclave
Refrigerator with freezer
Blender
Carboys (5 gal plastic with spigot)
Fluorescent lights ("Cool-white" for algae; "Grow-Lux" and "Vita-Life" for daphnids)
Drying oven

Reagents

Reagent-grade dry chemicals
$MgCl_2 \cdot 6\ H_20$
$MgSO_4$
$CaCl_2 \cdot 2\ H_2O$
$CaSO_4 \cdot 2H_2O$
$H3BO_3$
$MnCl_2 \cdot 4\ H_2O$
$ZnCl_2$
$FeCl_3 \cdot 6\ H_2O$
$CoCl_2 \cdot 6\ H_2O$
$Na_2Mo0_4 \cdot 2\ H_2O$
$Na_2EDTA \cdot 2\ H_2O$
$NaNO_3$
$MgSO_4 \cdot 7\ H2O$
K_2HPO_4
$NaHCO_3$
Na_2SeO_4
KCl
Hardness and alkalinity test reagents

Reagent-grade liquids
Water - MILLIPORE MILLI-Q^R (or equivalent)
pH buffers - 4, 7 and 10
Specific conductivity standards

Miscellaneous
Acid (1N HCl or H_2SO_4, 10% HNO_3)
Pipet bulbs and fillers
Wash bottles
Nitex^R screen (110 mesh)
Tape
Marking pens
0.45 um filters
Foam plugs (non-toxic, 35-45 mm diameter)

Attachment B. Preparation of Water for Culturing and Testing *Daphnia magna*.

Biesinger et al. (1987) recommend using reconstituted hard water to culture *Daphnia magna* and for use in toxicity tests. A volume of 4.8 L is required initially and at each renewal in a chronic test. Biesinger and co-workers recommend preparation of 19 L at a time according to the following methods:

1. Thoroughly rinse the 5 gallon carboy with a 10 percent solution of nitric acid. Slowly pour out acid solution into cold running water. Rinse carboy thoroughly with deionized distilled water at least five times. Accurately mark the 19 liter level in the carboy to facilitate preparation of water each time.

2. Weigh out stock chemicals one at a time in the following amounts:

   3.65 g $NaHCO_3$

   2.28 g $CaSO_4 \cdot 2H_2O$

   2.28 g MgSO4

   0.15 g KC1

   Extra stock mixtures can be weighed out in advance for use the next week if stored in tightly covered jars.

3. Add approximately 15 liters of deionized distilled water to the carboy. Add the chemicals in the order given, and mix thoroughly after each addition. Rinse storage jar with deionized distilled water and add rinse water to solution in carboy. Mix solution thoroughly. Add deionized distilled water to a total solution volume of 19 liters.

4. To assure complete mixing of chemicals and saturation with dissolved oxygen, stir with the lid removed (but covered with a foam plug or glass wool) for 24 hours using a magnetic stirrer.

5. Measure hardness, alkalinity, dissolved oxygen, and pH. The hardness must be from 160-180 mg/l $CaCO_3$; the alkalinity from 110-120 mg/l $CaCO_3$; and the pH from 7.6-8.5. This will verify proper measurement and mixing of salts in preparing the reconstituted water. If the hardness, alkalinity, and pH requirements are not met, the reconstituted water must be prepared again.

6. Reconstituted water may be stored and used for one month. Lesser volumes of hard reconstituted water of the same characteristics can be prepared when desired by adding 192.0, 120.1, 120.0 and 8.0 mg each of $NaHCO_3$, $CaSO_4$, $2H_2O$, $MgSO_4$ and KC1, respectively to 1 L of deionized water. This will produce water with characteristics corresponding to the "hard" category of Table B1.

7. This water may be deficient in some trace nutrients, and it may be desirable to add 2 ug/L of selenium (IV) and 1 ug/L of vitamin B_{12} (Keating 1984; Keating and Dagbusan 1984; ASTM 1991).

TABLE B1: PREPARATION OF SYNTHETIC FRESH WATER USING REAGENT GRADE CHEMICALS[a]

	Reagent Added (mg/L)[b]				Final Water Quality		
Water Type	$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KC1	pH[c]	Hardness[d]	Alka-linity[d]
Very soft	12.0	7.5	7.5	0.5	6.4-6.8	10-13	10-13
Soft	48.0	30.0	30.0	2.0	7.2-7.6	40-48	30-35
Moderately Hard	96.0	60.0	60.0	4.0	7.4-7.8	80-100	60-70
Hard	192.0	120.0	120.0	8.0	7.6-8.0	160-180	110-120
Very hard	384.0	240.0	240.0	16.0	8.0-8.4	280-320	225-245

[a]Taken in part from Marking and Dawson (1973).
[b]Add reagent grade chemicals to deionized water.
[c]Approximate equilibrium pH after 24 h of aeration.
[d]Expressed as mg $CaCO_3$/L.

Attachment C. Preparation of Trout Food and *Selenastrum capricornutum* Diets.

Preparation of Trout Food Diet (from National Effluent Toxicity Assessment Center, ERL-Duluth, USEPA)

1. Add 7.5 gm of No. 1 granule trout food to 400 ml of hard reconstituted water and blend for 15 min to liquify.

2. Let stand for 15 min; decant the upper 300 ml and discard the rest.

3. Pour into a graduated cylinder and record the volume.

4. Thoroughly mix the suspension and withdraw one 10 ml aliquot.

5. Dry the aliquot to a constant weight ($\pm$ 0.1 mg) in a pre-weighed pan (e.g. 50^{o} C for 24 hr).

6. At the end of the drying period, remove the sample from the oven, allow to cool in a desiccator, and weigh to the nearest 0.1 mg.

7. Calculate dry solids weight for 1 ml of suspension. The final concentration must be 5 mg dry solids per ml of food, so the volume must be adjusted by adding reconstituted water. The total volume of water (x) to add equals the number of ml in the sample after removal of the aliquot (290 ml) times the mg/ml of dry food weighed (y) divided by the mg/ml of dry food desired (5 mg/ml) minus the number of ml in the sample after removal of the aliquot.

   For example, if the dry food weighed 6.32 mg/ml (Y), the following equation will give (x):

$$x = \frac{(290)(6.32)}{5} - 290$$

   x = 76.6 ml of reconstituted water to add to 290 ml to give a concentration of 5 mg/ml of dry food.

Preparation of Selenastrum capricornutum Diet

A concentration of 10^{8} cells/L of *Selenastrum capricornutum* in addition to the trout food has been found to be satisfactory for a sustained culture of *Daphnia magna* (Biesinger et al. 1987). The following *Selenastrum* culturing methods are adapted from Weber et al. (1989) for preparation of a *Ceriodaphnia dubia* diet.

A "starter" culture of *Selenastrum capricornutum* is used to develop "stock" and "food" cultures maintained in a specific culture medium, described below. "Starter" cultures may be obtained in pure form from the following sources (Biesinger et al. 1987):

Selenastrum capricornutum ATC #22662	American Type Culture Collection 12301 Parklawn Drive Rockville, MD 20852
Selenastrum capricornutum UTEX 1648	The Starr Collection Department of Biology University of Texas at Austin Austin, TX 78712

Algal Culture Medium

1. Prepare (five) stock nutrient solutions using reagent grade chemicals as described in Table C1.

2. Add 1 mL of each stock solution, in the order listed in Table C1, to approximately 900 mL of MILLI-Q^{R} water. Mix well after the addition of each solution. Dilute to 1 L, mix well, and adjust the pH to 7.5 $\pm$ 0.1, using 0.1N NaOH or HC1, as appropriate. The final concentration of macronutrients and micronutrients in the culture medium is given in Table C2.

3. Immediately filter the pH-adjusted medium through a 0.45um pore diameter membrane at a vacuum of not more than 380 mm (15 in.) mercury, or at a pressure of not more than one-half atmosphere (8 psi). Wash the filter with 500 mL deionized water prior to use.

4. If the filtration is carried out with sterile apparatus, filtered medium can be used immediately, and no further sterilization steps are required before the inoculation of the medium. The medium can also be sterilized by autoclaving after it is place in the culture vessels.

5. Unused sterile medium should not be stored more than one week prior to use, because there may be substantial loss of water by evaporation.

Establishing and Maintaining "Stock" Cultures of Algae

1. Upon receipt of the "starter" culture (usually about 10 mL), a stock culture is initiated by aseptically transferring one

milliliter to each of several 250-mL culture flasks containing 100 mL algal culture medium (prepared as described above). The remainder of the starter culture can be held in reserve for 6-12 months in a refrigerator (in the dark) at 4°C.

2. The stock cultures are used as a source of algae to initiate "food" cultures for *Daphnia* toxicity tests. The volume of stock culture maintained at any one time will depend on the amount of algal food required for the *Daphnia* cultures and tests. Stock culture volume may be rapidly "scaled up" to several liters, if necessary, using 4-L serum bottles or similar vessels, each containing 3 L of growth medium.

3. Culture temperature is not critical. Stock cultures may be maintained at 25°C in environmental chambers with cultures of other organisms if the illumination is adequate (continuous "cool-white" fluorescent lighting of approximately 86 $\pm$ 8.6 uE/m^2-s, or 400 ft-c).

4. Cultures are mixed twice daily by hand.

5. Stock cultures can be held in the refrigerator until used to start "food" cultures, or can be transferred to new medium weekly. One-to-three milliliters of 7-day old algal stock culture, containing approximately 1.5 X 10^6 cells/mL, are transferred to each 100 mL of fresh culture medium. The inoculum should provide an initial cell density of approximately 10,000-30,000 cells/mL in the new stock cultures. Aseptic techniques should be used in maintaining the stock algal cultures, and care should be exercised to avoid contamination by other microorganisms.

6. Stock cultures should be examined microscopically weekly, at transfer, for microbial contamination. Reserve quantities of culture organisms can be maintained for 6-12 months if stored in the dark at 4°C. It is advisable to prepare new stock cultures from "starter" cultures obtained from established outside sources of organisms every four to six months.

Establishing and Maintaining "Food" Cultures of Algae

1. "Food" cultures are started seven days prior to use for *Daphnia* cultures and tests. Approximately 20 mL of 7-day-old algal stock culture (described in the previous paragraph), containing 1.5 X 10^6 cells/mL, are added to each liter of fresh algal culture medium (i.e., 3 L of medium in a 4-L bottle, or 18 L in a 20-L bottle). The inoculum should provide an initial cell density of approximately 30,000 cells/mL. Aseptic techniques should be used in preparing and

maintaining the cultures, and care should be exercised to avoid contamination by other microorganisms. However, sterility of food cultures is not as critical as in stock cultures because the food cultures are terminated in 7-10 days. A one-month supply of algal food can be grown at one time, and the excess stored in the refrigerator.

TABLE C1. NUTRIENT STOCK SOLUTIONS FOR MAINTAINING ALGAL STOCK CULTURES AND TEST CONTROL CULTURES

Nutrient Stock Solution	Compound	Amount dissolved in 500 mL MILLI-Q^R Water
1	$MgCl_2 \cdot 6H_2O$	6.08 g
	$CaCl_2 \cdot 2H_2O$	2.20 g
	H_3BO_3	92.8 mg
	$MnCl_2 \cdot 4H_2O$	208.0 mg
	$ZnCl_2$	1.64 mg[a]
	$FeCl_3 \cdot 6H_2O$	79.9 mg
	$CoCl_2 \cdot 6H_2O$	0.714 mg[b]
	$Na_2MoO_4 \cdot 2H_2O$	3.63 mg[c]
	$CuCl_2 \cdot 2H_2O$	0.006 mg[d]
	$Na_2EDTA \cdot 2H_2O$	150.0 mg
2	$NaNO_3$	12.75 g
3	$MgSO_4 \cdot 7H_2O$	7.35 g
4	K_2HPO_4	0.522 g
5	$NaHCO_3$	7.50 g

[a] $ZnCl_2$ - Weigh out 164 mg and dilute to 100 mL. Add 1 mL of this solution to Stock #1.
[b] $CoCl_2 \cdot 6H_2O$ - Weigh out 71.4 mg and dilute to 100 mL. Add 1 mL of this solution to Stock #1.
[c] $Na_2MoO_4 \cdot 2H_2O$ - Weigh out 36.6 mg and dilute to 10 mL. Add 1 mL of this solution to Stock #1.
[d] $CuCl_2 \cdot 2H_2O$ - Weigh out 60.0 mg and dilute to 1000 mL. Take 1 mL of this solution and dilute to 10 mL. Take 1 mL of the second dilution and add to Stock #1.

TABLE C2. FINAL CONCENTRATION OF MACRONUTRIENTS AND MICRONUTRIENTS IN THE CULTURE MEDIUM

Macronutrient	Concentration (mg/L)	Element	Concentration (mg/L)
$NaNO_3$	25.5	N	4.20
$MgCl_2 \cdot 6H_2O$	12.2	Mg	2.90
$CaCl_2 \cdot 2H_2O$	4.41	Ca	1.20
$MgSO_4 \cdot 7H_2O$	14.7	S	1.91
K_2HPO_4	1.04	P	0.186
$NaHCO_3$	15.0	Na	11.0
		K	0.469
		C	2.14

Micronutrient	Concentration (ug/L)	Element	Concentration (ug/L)
H_3BO_3	185	B	32.5
$MnCl_2 \cdot 4H_2O$	416	Mn	115
$ZnCl_2$	3.27	Zn	1.57
$CoCl_2 \cdot 6H_2O$	1.43	Co	0.354
$CuCl_2 \cdot 2H_2O$	0.012	Cu	0.004
$Na_2MoO_4 \cdot 2H_2O$	7.26	Mo	2.88
$FeCl_3 \cdot 6H_2O$	160	Fe	33.1
$Na_2EDTA \cdot 2H_2O$	300	--	----

2. Food cultures may be maintained at 25°C in environmental chambers with the algal stock cultures or cultures of other organisms if the illumination is adequate (continuous "cool-white" fluorescent lighting of approximately 86 $\pm$ 8.6 uE/m^2-s, or 400 ft-c).

3. Cultures are mixed continuously on a magnetic stir plate (with a medium size stir bar) or in a moderately aerated separatory funnel, or are mixed twice daily by hand. If the

cultures are placed on a magnetic stir plate, heat generated by the stirrer might elevate the culture temperature several degrees. Caution should be exercised to prevent the culture temperature from rising more than 2-3^{0}C.

Preparing Algal Concentrate for Use as Daphnia Food

1. An algal concentrate containing 1 x 10^{8} cells/mL is prepared from food cultures by centrifuging the algae with a plankton or bucket-type centrifuge, or by allowing the cultures to settle in a refrigerator for approximately two-to-three days and siphoning off the supernatant.

2. The cell density (cells/mL) in the concentrate is measured with an electronic particle counter, microscope and hemacytometer, fluorometer, or spectrophotometer, and used to determine the dilution (or further concentration) required to achieve a final cell count of 1 x 10^{8}/mL.

3. Assuming a cell density of approximately 1.5 X 10^{6} cells/mL in the algal food cultures at 7 days, and 100% recovery in the concentration process, a 3-L, 7-10 day culture will provide 4.5 X 10^{9} algal cells. This numberof cells would provide approximately 45 mL of algal cell concentrate (450 feedings at 0.1 mL/feeding) for use as food. This would be enough algal food for approximately seven feedings. Food must be administered nine times during the test.

4. Algal concentrate may be stored in the refrigerator for one month.

Attachment D. Sample Record Forms for Culturing *Daphnia magna* and Performing a Dredged Material Elutriate Chronic Toxicity Test.

Form D1. Sample Record Form of Survival and Young Production Data for *Daphnia magna* in a Pre-Test Culture.

Date Started: _____ Culturist: _____
Culture Water Medium Renewal
Batch No.: _____ Days: _____
Trout Chow Selenastrum
Batch No.: _____ Cell Density: _____

Repl.	Day	10-1	10-2	10-3	10-4	10-5	10-6	Remarks
10	0							
	1							
	2							
	4							
	6							
	8							
	10							
	12							
	14							
Total								

Repl.	Day	9-1	9-2	9-3	9-4	9-5	9-6	Remarks
9	0							
	1							
	2							
	4							
	6							
	8							
	10							
	12							
	14							
Total								

Repl.	Day	8-1	8-2	8-3	8-4	8-5	8-6	Remarks
8	0							
	1							
	2							
	4							
	6							
	8							
	10							
	12							
	14							
Total								

+ = OK 0 = No Young D = Dead M = Male E = Eggs Present

Form D1 (Cont.)

Repl.	Day	7-1	7-2	7-3	7-4	7-5	7-6	Remarks
7	0							
	1							
	2							
	4							
	6							
	8							
	10							
	12							
	14							
Total								

Repl.	Day	6-1	6-2	6-3	6-4	6-5	6-6	Remarks
6	0							
	1							
	2							
	4							
	6							
	8							
	10							
	12							
	14							
Total								

Repl.	Day	5-1	5-2	5-3	5-4	5-5	5-6	Remarks
5	0							
	1							
	2							
	4							
	6							
	8							
	10							
	12							
	14							
Total								

Repl.	Day	4-1	4-2	4-3	4-4	4-5	4-6	Remarks
4	0							
	1							
	2							
	4							
	6							
	8							
	10							
	12							
	14							
Total								

+ = OK 0 = No Young D = Dead M = Male E = Eggs Present

Form D1 (Cont.)

Repl.	Day	3-1	3-2	3-3	3-4	3-5	3-6	Remarks
3	0							
	1							
	2							
	4							
	6							
	8							
	10							
	12							
	14							
Total								

Repl.	Day	2-1	2-2	2-3	2-4	2-5	2-6	Remarks
2	0							
	1							
	2							
	4							
	6							
	8							
	10							
	12							
	14							
Total								

Repl.	Day	1-1	1-2	1-3	1-4	1-5	1-6	Remarks
1	0							
	1							
	2							
	4							
	6							
	8							
	10							
	12							
	14							
Total								

+ = OK 0 = No Young D = Dead M = Male E = Eggs Present

Form D2. Sample Data Form for Temperature and Water Chemistry in a Dredged Material Elutriate Chronic Toxicity Test with *Daphnia magna.*

Day	Temp. (°C)	D.O. (mg/L) C	L	M	H	pH C	L	M	H	Hardness (mg/L as $CaCO_3$) L	M	H	C	Alkalinity (mg/L as $CaCo_3$) C	L	M	H	Specific Conductance (μmhos/cm) C	L	M	H
0	____ ____ ____ ____																				
1	____ ____ ____ ____																				
2	____ ____ ____ ____																				
3	____ ____ ____ ____																				
4	____ ____ ____ ____																				
5	____ ____ ____ ____																				
6	____ ____ ____ ____																				
7	____ ____ ____ ____																				
8	____ ____ ____ ____																				

Form D2 (Cont.)

Day	Temp. (°C)	D.O. (mg/L) C	L	M	H	pH C	L	M	H	Hardness (mg/L as $CaCO_3$) L	M	H	C	Alkalinity (mg/L as $CaCO_3$) C	L	M	H	Specific Conductance (μmhos/cm) C	L	M	H
9	____ ____ ____ ____ ____																				
10	____ ____ ____ ____ ____																				
11	____ ____ ____ ____ ____																				
12	____ ____ ____ ____ ____																				
13	____ ____ ____ ____ ____																				
14	____ ____ ____ ____ ____																				
15	____ ____ ____ ____ ____																				
16	____ ____ ____ ____ ____																				
17	____ ____ ____ ____ ____																				

Form D2 (Cont.)

Day	Temp. (°C)	D.O. (mg/L) C	L	M	H	pH C	L	M	H	Hardness (mg/L as $CaCO_3$) C	L	M	H	Alkalinity (mg/L as $CaCo_3$) C	L	M	H	Specific Conductance (μmhos/cm) C	L	M	H
18	____ ____ ____ ____ ____																				
19	____ ____ ____ ____ ____																				
20	____ ____ ____ ____ ____																				
21	____ ____ ____ ____ ____																				

Form D3. Sample Record Form for Survival and Young Production Data From a Block-Randomized *Daphnia magna* Dredged Material Elutriate Chronic Toxicity Test.

Sample I.D.: _____
Sample Collection Date: _____
Test Start-Date/Time: _____
Test Organisms from
Pre-Test Culture No.: _____
Test Organism Age: _____

Selenastrum Cell Density: _____
Dilution Water
Batch No.: _____
Template No.: _____
Test Chamber Vol. _____
Vol. of Test Solution: _____
Investigator: _____

Repl.	Day	10-5	10-2	10-6	10-3	10-4	10-1	Remarks
10	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	11							
	12							
	13							
	14							
	15							
	16							
	17							
	18							
	19							
	20							
	21							
Total								

+ = OK 0 = No Young D = Dead M = Male E = Eggs Present

Form D3 (Cont.)

Repl.	Day	9-3	9-2	9-5	9-4	9-6	9-1	Remarks
9	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	11							
	12							
	13							
	14							
	15							
	16							
	17							
	18							
	19							
	20							
	21							
Total								

Repl.	Day	8-1	8-5	8-6	8-4	8-3	8-2	Remarks
8	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	11							
	12							
	13							
	14							
	15							
	16							
	17							
	18							
	19							
	20							
	21							
Total								

+ = OK 0 = No Young D = Dead M = Male E = Eggs Present

Form D3 (Cont.)

Repl.	Day	7-2	7-6	7-3	7-4	7-1	7-5	Remarks
7	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	11							
	12							
	13							
	14							
	15							
	16							
	17							
	18							
	19							
	20							
	21							
Total								

Repl.	Day	6-1	6-4	6-6	6-2	6-5	6-3	Remarks
6	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	11							
	12							
	13							
	14							
	15							
	16							
	17							
	18							
	19							
	20							
	21							
Total								

+ = OK 0 = No Young D = Dead M = Male E = Eggs Present

Form D3 (Cont.)

Repl.	Day	5-2	5-3	5-1	5-5	5-4	5-6	Remarks
5	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	11							
	12							
	13							
	14							
	15							
	16							
	17							
	18							
	19							
	20							
	21							
Total								

Repl.	Day	4-2	4-3	4-5	4-4	4-6	4-1	Remarks
4	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	11							
	12							
	13							
	14							
	15							
	16							
	17							
	18							
	19							
	20							
	21							
Total								

+ = OK 0 = No Young D = Dead M = Male E = Eggs Present

Form D3 (Cont.)

Repl.	Day	3-3	3-5	3-2	3-1	3-4	3-6	Remarks
3	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	11							
	12							
	13							
	14							
	15							
	16							
	17							
	18							
	19							
	20							
	21							
Total								

Repl.	Day	2-1	2-6	2-5	2-2	2-3	2-4	Remarks
2	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	11							
	12							
	13							
	14							
	15							
	16							
	17							
	18							
	19							
	20							
	21							
Total								

+ = OK 0 = No Young D = Dead M = Male E = Eggs Present

Form D3 (Cont.)

Repl.	Day	1-6	1-1	1-3	1-5	1-2	1-4	Remarks
1	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	11							
	12							
	13							
	14							
	15							
	16							
	17							
	18							
	19							
	20							
	21							
Total								

+ = OK 0 = No Young D = Dead M = Male E = Eggs Present

Form D4. Sample Summary Form for Suvival and Young Production in a *Daphnia magna* Dredged Material Elutriate Chronic Toxicity Test. (Summary of data from Form D3)

Sample I.D.: _____ Investigator: _____
Sample Collection Date: _____ Test Start-Date/Time: _____
Test End-Date/Time: _____

Elutriate Concentration	Total No. of Young Per Surviving Adult Replicate										No. of Live Adults
	1	2	3	4	5	6	7	8	9	10	
Control											
6.25%											
12.5%											
25.0%											
50.0%											
100%											

Attachment E. General Activity Schedule for Performing a Dredged Material Elutriate Chronic Toxicity Test with *Daphnia magna*[a].

Day	Activity
-14	Set up a pre-test culture of 60 beakers and add one neonate (≤24 hr old) to each of the beakers in 80 mL of culture medium containing food.
-12	Renew culture medium, add food and transfer individual daphnids.
-10	Renew culture medium, add food and transfer individual daphnids.
- 8	Renew culture medium, add food and transfer individual daphnids.
- 6	Renew culture medium, add food and transfer adult daphnids. Observe for young production, and record those adults which produced their first brood. Discard any young.
- 4	Renew culture medium, add food and transfer adult daphnids. Observe for young production, and record those adults which produced young, along with the brood number (i.e. first or second) and brood size. Discard any adults which have not produced young.
- 2	Renew culture medium, add food and transfer adult daphnids. Observe for young production, and record those adults which produced young, along with the brood number and size. Discard any young.
- 1	Observe the pre-test culture within 24 hr from the start of the test, and mark those beakers containing adults which may produce young that (1) will be <24 hr old, (2) will be producing their third or more brood, and (3) had at least nine young in the previous brood. Prepare the dredged material elutriate water and performance control water.
0	Add trout chow (final concentration of 5 mg/L) and *Selenastrum capricornutum* (final concentration of 10^8 cells/L) to the dredged material elutriate and performance control waters. Select 10 beakers of neonates to be placed into test solution, one beaker per replicate set of treatments. Add one neonate to each test beaker. Monitor all water quality parameters.
1	Observe test beakers for mortalities and monitor water temperature.
2	Renew test solutions and feed daphnids. Monitor water quality parameters in samples of "old" and "new" solutions.
3	Observe test beakers for mortalities and monitor water temperature.
4	Renew test solutions and feed daphnids. Monitor water quality parameters in samples of "old" and "new" solutions.

Day	Activity
5	Observe test beakers for mortalities and monitor water temperature.
6	Observe test beakers for mortalities and monitor water temperature.
7	Record mortalities and brood number and brood size for any daphnids that have young. Renew test solutions and feed adults. Discard the young. Monitor water quality parameters in samples of "old" and "new" solutions.
8	Observe test beakers for mortalities and brood production. Record occurrence of brood. Monitor water temperature.
9	Record mortalities and brood number and brood size for any daphnids that have young. Renew test solutions containing food and transfer adults. Discard the young. Monitor quality parameters in "old" and "new" solutions.
10	(Same as day 8)
11	(Same as day 9)
12	(Same as day 8)
13	(Same as day 8)
14	(Same as day 9)
15	(Same as day 8)
16	(Same as day 9)
17	(Same as day 8)
18	(Same as day 9)
19	(Same as day 8)
20	(Same as day 8)
21	Record mortalities, and brood number and brood size for any daphnids that have young. Monitor all water quality parameters. Discard all daphnids. Terminate test.

a Activity Schedule assumes that cultures of *D. magna* and *S. capricornutum* are already in existence at the laboratory, and that the culture water and diets have been prepared in advance.

Attachment F. Materials for Culturing of and Conducting Toxicity Tests with *Ceriodaphnia dubia*.

Biological Supplies

Ceriodaphnia dubia starter culture
Selenastrum capricornutum starter culture Trout Chow
Yeast
Cereal Leaves (see Attachment H for sources)

Glassware

Mass culturing chambers (1-2 L volume)
30 mL disposable polystyrene salad cups (1 oz) or glass beakers
Erlenmeyer flasks (250 mL - 3 L)
Separatory funnel (2 L)
Beakers (1-2 L)
Volumetric flasks and graduated cylinders (10-1,000 mL, class A borosilicate glass or non-toxic plastic)
Volumetric pipets (1-100 mL, class A)
Pipettor, adjustable volume repeating dispenser
Microscope slide
Counting chamber (Sedgwick-Rafter, Palmer-Maloney or hemocytometer)
Burettes
2-mm ID fire-polished glass tubes
Disposable pipets and droppers
Plate glass (double-strength) for covering brood board
Thermometer (National Bureau of Standards certified)

Instruments and Equipment

pH meter
Dissolved oxygen and specific conductivity meter
Constant temperature environmental chambers for culturing *Ceriodaphnia* and *Selenastrum* and for testing *Ceriodaphnia*.
Deionized water system (MILLIPORE MILLI-Q^{R} or equivalent)
Analytical balance (capable of weighing accurately to 0.0001g)
Reference weights (class S)
Magnetic stir plates (for algal cultures)
Teflon®-coated stir bar magnets
Light meter
Microscope (compound scope with 10X, 45X and 100X objective lenses, 10X ocular lens, mechanical stage, substage condensor and light source)
Microscope (dissecting scope with substage lighting)
Light box
Centrifuge - plankton, or with swing-out buckets having a capacity of 15-100 mL.
Centrifuge tubes - 15-100 mL, screw-cap
Continuous recording thermometer

Autoclave
Refrigerator with freezer
Blender

Reagents

Reagent-grade dry chemicals
- $MgCl_2 \cdot 6\ H_20$
- $MgSO_4$
- $CaCl_2 \cdot 2\ H_2O$
- $CaSO_4 \cdot 2H_2O$
- $H3BO_3$
- $MnCl_2 \cdot 4\ H_2O$
- $ZnCl_2$
- $FeCl_3 \cdot 6\ H_2O$
- $CoCl_2 \cdot 6\ H_2O$
- $Na_2Mo0_4 \cdot 2\ H_2O$
- $Na_2EDTA \cdot 2\ H_2O$
- $NaNO_3$
- $MgSO_4 \cdot 7\ H2O$
- K_2HPO_4
- $NaHCO_3$
- Na_2SeO_4
- KCl
- Hardness and alkalinity test reagents

Reagent-grade liquids
- Water - MILLIPORE MILLI-Q^R (or equivalent)
- pH buffers - 4, 7 and 10
- Specific conductivity standards

Miscellaneous
- Acid (1N HCl or H_2SO_4)
- Pipet bulbs and fillers
- Wash bottles
- Nitex^R screen (110 mesh)
- Brood board material (e.g. styrofoam insulation board, 50 cm x 30 cm x 2.5 cm)
- Tape
- Marking pens
- 0.45 um filters

Attachment G. Preparation of Water for Culturing and Testing *Ceriodaphnia dubia*.

Waters of different chemical characteristics may be used successfully in culturing and testing *Ceriodaphnia dubia*. Prepared water with a hardness (as CaC03) range between 40 mg/L ("soft" water) and 100 mg/L ("moderately hard" water) may be used for culturing. However, moderately hard water is recommended by EPA (Weber et al. 1989). The synthetic medium that is selected as the culture medium and diluent must result in survival and reproduction results that meet the criteria for acceptability.

Tables G1 and G2 below (from Weber et al. 1989) list the ingredients required to prepare either reconstituted synthetic fresh water or diluted mineral water, each at several hardnesses. The pH, hardness, and alkalinity of the prepared water should be measured and fall within the ranges indicated in the tables. The water must be aerated for 24 hr prior to determination of pH. Aeration should be performed with air that is known to be clean and not contain any oil or chemical residues from the compressor. In-line filters should be used if the potential for such contamination exists. It is recommended by the staff of the USEPA National Effluent Toxicity Assessment Center in Duluth, MN that 2 ug/L of selenium (Se^{+6}) be added to the water in the form of sodium selenite (Na_2SeO_3).

TABLE G1: PREPARATION OF SYNTHETIC FRESH WATER USING REAGENT GRADE CHEMICALS[a]

	Reagent Added (mg/L)[b]				Final Water Quality		
Water Type	$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KC1	pH[c]	Hardness[d]	Alka-linity[d]
Very soft	12.0	7.5	7.5	0.5	6.4-6.8	10-13	10-13
Soft	48.0	30.0	30.0	2.0	7.2-7.6	40-48	30-35
Moderately Hard	96.0	60.0	60.0	4.0	7.4-7.8	80-100	60-70
Hard	192.0	120.0	120.0	8.0	7.6-8.0	160-180	110-120
Very hard	384.0	240.0	240.0	16.0	8.0-8.4	280-320	225-245

[a]Taken in part from Marking and Dawson (1973).
[b]Add reagent grade chemicals to deionized water.
[c]Approximate equilibrium pH after 24 h of aeration.
[d]Expressed as mg $CaCO_3$/L.

TABLE G2. PREPARATION OF SYNTHETIC FRESH WATER USING MINERAL WATER[a]

Water Type	Volume of Mineral Water Added (mL/L)[b],	Proportion of Mineral Water (%)	Final Water Quality		
			pH[c]	Hardness	Alka-linity[d]
Very soft	50	2.5	7.2-8.1	10-13	10-13
Soft	100	10.0	7.9-8.3	40-48	30-35
Moderately Hard	200	20.0	7.9-8.3	80-100	60-70
Hard	400	40.0	7.9-8.3	160-180	110-120
Very hard[e]	---	---	---	---	---

[a]From Mount et al., 1987, and data provided by Philip Lewis, EMSL-Cincinnati.
[b]Add mineral water to Milli-Q^R water or equivalent to prepare DMW (Diluted Mineral Water).
[c]Approximate equilibrium pH after 24 h of aeration.
[d]Expressed as mg $CaCO_3$/L.
[e]Dilutions of PERRIER^R Water form a precipitate when concentrations equivalent to "very hard water" are aerated.

Attachment H. Preparation of YCT and *Selenastrum capricornutum* Diets.

Preparation of YCT Diet (from Weber et al. 1989)

The YCT diet is a mixture of yeast, cereal leaves, and trout chow. This produces an organic-rich microbial culture as the food source for *Ceriodaphnia dubia.* Preparation of the diet requires one week. Equal volumes of the yeast, cereal leaves, and trout chow preparations are combined, and divided into smaller aliquots (e.g. 50 mL) to be frozen for later use. The three ingredients are prepared as follows:

1. Trout Chow[a] - Add 5.0g of trout chow pellets to 1 L of deionized water, and thoroughly mix in a blender (~ 15 min). Transfer to a 2-L separatory funnel and, in a fume hood, continuously aerate from the bottom of the funnel for 7 days at room temperature. Replace any water lost due to evaporation. Place the vessel in a refrigerator and allow to settle for 1 hr. Filter through a fine mesh screen and combine with the other ingredients.

2. Yeast - Add 5.0 g of baker's yeast, (e.g. Fleischmann's[R]) to 1 L of deionized water and thoroughly blend with a mixer at a slow speed. Use immediately.

3. Cereal leaves[b] - Add 5.0 g of dried, powdered, cereal leaves to 1 L of deionized water, and mix in a blender at high speed for 5 min. Allow to settle overnight in a refrigerator before using.

---

[a] Trout chow (starter or No. 1 pellets) may be purchased from a commercial supplier of animal and pet foods.

[b] Dried, powdered cereal leaves are available as "CEREAL LEAVES" from Sigma Chemical Company, P.O. Box 14508, St. Louis, MO 63178, (800-325-3010); or as CEROPHYLL[R] from Ward's Natural Science Establishment, Inc., P.O. Box 92912, Rochester, NY 14692-9012 (716-359-2502). Dried, powdered alfalfa leaves from health food stores have served as satisfactory substitutes (Weber et al. 1989).

Preparation of Selenastrum Capricornutum Culture and Diet for Ceriodaphnia dubia (from Weber et al. 1989)

A "starter" culture[a] of *Selenastrum capricornutum* is used to develop "stock" and "food" cultures maintained in a specific culture medium, described below.

Algal Culture Medium

1. Prepare (five) stock nutrient solutions using reagent grade chemicals as described in Table H1.

2. Add 1 mL of each stock solution, in the order listed in Table D1, to approximately 900 mL of MILLI-Q^{R} water. Mix well after the addition of each solution. Dilute to 1 L, mix well, and adjust the pH to 7.5 $\pm$ 0.1, using 0.1N NaOH or HC1, as appropriate. The final concentration of macronutrients and micronutrients in the culture medium is given in Table H2.

3. Immediately filter the pH-adjusted medium through a 0.45um pore diameter membrane at a vacuum of not more than 380 mm (15 in.) mercury, or at a pressure of not more than one-half atmosphere (8 psi). Wash the filter with 500 mL deionized water prior to use.

4. If the filtration is carried out with sterile apparatus, filtered medium can be used immediately, and no further sterilization steps are required before the inoculation of the medium. The medium can also be sterilized by autoclaving after it is placed in the culture vessels.

5. Unused sterile medium should not be stored more than one week prior to use, because there may be substantial loss of water by evaporation.

Establishing and maintaining "Stock" Cultures of Algae

1. Upon receipt of the "starter" culture (usually about 10 mL), a stock culture is initiated by aseptically transferring one milliliter to each of several 250-mL culture flasks containing 100 mL algal culture medium (prepared as described above). The remainder of the starter culture can be held in reserve for up to six months in a refrigerator (in the dark) at 4^{0}C.

2. The stock cultures are used as a source of algae to initiate "food" cultures for *Ceriodaphnia* toxicity tests. The volume of stock culture maintained at any one time will depend on the amount of algal food required for the *Ceriodaphnia*

cultures and tests. Stock culture volume may be rapidly "scaled up" to several liters, if necessary, using 4-L serum bottles or similar vessels, each containing 3 L of growth medium.

3. Culture temperature is not critical. Stock cultures may be maintained at 25^{0}C in environmental chambers with cultures of other organisms if the illumination is adequate (continuous "cool-white" fluorescent lighting of approximately 86 $\pm$ 8.6 uE/m^2/s, or 400 ft-c).

4. Cultures are mixed twice daily by hand.

5. Stock cultures can be held in the refrigerator until used to start "food" cultures, or can be transferred to new medium weekly. One-to-three milliliters of 7-day old algal stock culture, containing approximately 1.5 X 10^6 cells/mL, are transferred to each 100 mL of fresh culture medium. The inoculum should provide an initial cell density of approximately 10,000-30,000 cells/mL in the new stock cultures. Aseptic techniques should be used in maintaining the stock algal cultures, and care should be exercised to avoid contamination by other microorganisms.

6. Stock cultures should be examined microscopically weekly, at transfer, or microbial contamination. Reserve quantities of culture organisms can be maintained for 6-12 months if stored in the dark at 4^{0}C. It is advisable to prepare new stock cultures form "starter" cultures obtained from established outside sources of organisms every four to six months.

Establishing and Maintaining "Food" Cultures of Algae

1. "Food" cultures are started seven days prior to use for Ceriodaphnia cultures and tests. Approximately 20 mL of 7-day-old algal stock culture (described in the previous paragraph), containing 1.5 X 10^6 cells/mL, are added to each liter of fresh algal culture medium (i.e., 3 L of medium in a 4-L bottle, or 18 L in a 20-L bottle). The inoculum should provide an initial cell density of approximately 30,000 cells/mL. Aseptic techniques should be used in preparing and maintaining the cultures, and care should be exercised to avoid contamination by other microorganisms. However, sterility of food cultures is not as critical as in stock cultures because the food cultures are terminated in 7-10 days. A one-month supply of algal food can be grown at one time, and the excess stored in the refrigerator.

2. Food cultures may be maintained at 25^{0}C in environmental

chambers with the algal stock cultures or cultures of other organisms if the illumination is adequate (continuous "cool-white" fluorescent lighting of approximately 86 $\pm$ 8.6 $uE/m^2/s$, or 400 ft-c).

TABLE H1. NUTRIENT STOCK SOLUTIONS FOR MAINTAINING ALGAL STOCK CULTURES AND TEST CONTROL CULTURES

Nutrient Stock Solution	Compound	Amount dissolved in 500 mL MILLI-Q^R Water
1	$MgCl_2 \cdot 6H_2O$	6.08 g
	$CaCl_2 \cdot 2H_2O$	2.20 g
	H_3BO_3	92.8 mg
	$MnCl_2 \cdot 4H_2O$	208.0 mg
	$ZnCl_2$	1.64 mg[a]
	$FeCl_3 \cdot 6H_2O$	79.9 mg
	$CoCl_2 \cdot 6H_2O$	0.714 mg[b]
	$Na_2MoO_4 \cdot 2H_2O$	3.63 mg[c]
	$CuCl_2 \cdot 2H_2O$	0.006 mg[d]
	$Na_2EDTA \cdot 2H_2O$	150.0 mg
2	$NaNO_3$	12.75 g
3	$MgSO_4 \cdot 7H_2O$	7.35 g
4	K_2HPO_4	0.522 g
5	$NaHCO_3$	7.50 g

[a] $ZnCl_2$ - Weigh out 164 mg and dilute to 100 mL. Add 1 mL of this solution to Stock #1.

[b] $CoCl_2 \cdot 6H_2O$ - Weigh out 71.4 mg and dilute to 100 mL. Add 1 mL of this solution to Stock #1.

[c] $Na_2MoO_4 \cdot 2H_2O$ - Weigh out 36.6 mg and dilute to 10 mL. Add 1 mL of this solution to Stock #1.

[d] $CuCl_2 \cdot 2H_2O$ - Weigh out 60.0 mg and dilute to 1000 mL. Take 1 mL of this solution and dilute to 10 mL. Take 1 mL of the second dilution and add to Stock #1.

TABLE H2. FINAL CONCENTRATION OF MACRONUTRIENTS AND MICRONUTRIENTS IN THE CULTURE MEDIUM

Macronutrient	Concentration (mg/L)	Element	Concentration (mg/L)
$NaNO_3$	25.5	N	4.20
$MgCl_2 \cdot 6H_2O$	12.2	Mg	2.90
$CaCl_2 \cdot 2H_2O$	4.41	Ca	1.20
$MgSO_4 \cdot 7H_2O$	14.7	S	1.91
K_2HPO_4	1.04	P	0.186
$NaHCO_3$	15.0	Na	11.0
		K	0.469
		C	2.14

Macronutrient	Concentration (ug/L)	Element	Concentration (ug/L)
H_3BO_3	185	B	32.5
$MnCl_2 \cdot 4H_2O$	416	Mn	115
$ZnCl_2$	3.27	Zn	1.57
$CoCl_2 \cdot 6H_2O$	1.43	Co	0.354
$CuCl_2 \cdot 2H_2O$	0.012	Cu	0.004
$Na_2MoO_4 \cdot 2H_2O$	7.26	Mo	2.88
$FeCl_3 \cdot 6H_2O$	160	Fe	33.1
$Na_2EDTA \cdot 2H_2O$	300	--	----

3. Cultures are mixed continuously on a magnetic stir plate (with a medium size stir bar) or in a moderately aerated separatory funnel, or are mixed twice daily by h and. If the cultures are placed on a magnetic stir plate, heat generated by the stirrer might elevate the culture temperature several degrees. Caution should be exercised to prevent the culture temperature from rising more than 2-3^{0}C.

Preparing Algal Concentrate for use as Ceriodaphnia Food

1. An algal concentrate containing 3.0 to 3.5 X 10^7 cells/mL is prepared from food cultures by centrifuging the algae with a plankton or bucket-type centrifuge, or by allowing the cultures to settle in a refrigerator for approximately two-to-three weeks and siphoning off the supernatant.

2. The cell density (cells/mL) in the concentrate is measured with an electronic particle counter, microscope and hemocytometer, fluorometer, or spectrophotometer. and used to determine the dilution (or further concentration) required to achieve a final cell count of 3.0 to 3.5 X 10^7/mL.

3. Assuming a cell density of approximately 1.5 X 10^6 cells/mL in the algal food cultures at 7 days, and 100% recovery in the concentration process, a 3-L, 7-10 day culture will provide 4.5 X 10^9 algal cells. This number of cells would provide approximately 150 mL of algal cell concentrate (1500 feedings at 0.1 mL/feeding) for use as food. This would be enough algal food for four Ceriodaphnia tests.

4. Algal concentrate may be stored in the refrigerator for one month.

Attachment I. Sample Record and Data Forms for Culturing *Ceriodaphnia dubia* and Performing Elutriate Chronic Toxicity Tests.

Form I1. Sample Record Form of Survival and Young Production Data for *Ceriodaphnia dubia* in a Culture Brood Board.

Date Started: _____ Culturist: _____
Culture Water Medium Renewal Days: _____
Batch No.: _____ Brood Board No: _____
YCT Batch No.: _____ *Selenastrum* Cell Density: _____

Repl.	Day	10-1	10-2	10-3	10-4	10-5	10-6	Remarks
10	0							
	2							
	4							
	7							
	9							
	11							
	14							
Total								

Repl.	Day	9-1	9-2	9-3	9-4	9-5	9-6	Remarks
9	0							
	2							
	4							
	7							
	9							
	11							
	14							
Total								

Repl.	Day	8-1	8-2	8-3	8-4	8-5	8-6	Remarks
8	0							
	2							
	4							
	7							
	9							
	11							
	14							
Total								

Repl.	Day	7-1	7-2	7-3	7-4	7-5	7-6	Remarks
7	0							
	2							
	4							
	7							
	9							
	11							
	14							
Total								

+ = OK 0 = No Young D = Dead M = Male E = Eggs Present

Form I1 (Cont.)

Repl.	Day	6-1	6-2	6-3	6-4	6-5	6-6	Remarks
6	0							
	2							
	4							
	7							
	9							
	11							
	14							
Total								

Repl.	Day	5-1	5-2	5-3	5-4	5-5	5-6	Remarks
5	0							
	2							
	4							
	7							
	9							
	11							
	14							
Total								

Repl.	Day	4-1	4-2	4-3	4-4	4-5	4-6	Remarks
4	0							
	2							
	4							
	7							
	9							
	11							
	14							
Total								

Repl.	Day	3-1	3-2	3-3	3-4	3-5	3-6	Remarks
3	0							
	2							
	4							
	7							
	9							
	11							
	14							
Total								

+ = OK 0 = No Young D = Dead M = Male E = Eggs Present

Form I1 (Cont.)

Repl.	Day	2-1	2-2	2-3	2-4	2-5	2-6	Remarks
2	0							
	2							
	4							
	7							
	9							
	11							
	14							
Total								
Repl.	Day	1-1	1-2	1-3	1-4	1-5	1-6	Remarks
1	0							
	2							
	4							
	7							
	9							
	11							
	14							
Total								

+ = OK 0 = No Young D = Dead M = Male E = Eggs Present

Form I2. Sample Data Form for Temperature and Water Chemistry in a Dredged Material Elutriate Chronic Toxicity Test with *Ceriodaphnia dubia*.

Day	Temp. (°C)	D.O. (mg/L)				pH				Hardness (mg/L as CaCO3)				Alkalinity (mg/L as CaCo3)				Specific Conductance (umhos/cm)			
		C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H
0	____ ____ ____ ____																				
1	____ ____ ____ ____																				
2	____ ____ ____ ____																				
3	____ ____ ____ ____																				
4	____ ____ ____ ____																				
5	____ ____ ____ ____																				
6	____ ____ ____ ____																				
7	____ ____ ____ ____																				
8	____ ____ ____ ____																				

Form I3. Sample Record Form for Survival and Young Production Data from a Block-Randomized *Ceriodaphnia dubia* Dredged Material Elutriate Chronic Toxicity Test.

Sample I.D.: _____
Sample Collection Date: _____
Test Start-Date/Time: _____
Test Organisms from
Brood Board No.: _____
Test Organism Age: _____
YCT Batch No.: _____

Selenastrum Cell Density: _____
Dilution Water
Batch No.: _____
Template No.: _____
Test Chambers
(glass or plastic/vol.): _____
Investigator: _____

Repl.	Day	10-5	10-2	10-6	10-3	10-4	10-1	Remarks
10	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
Total								

Repl.	Day	9-3	9-2	9-5	9-4	9-6	9-1	Remarks
9	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
Total								

Repl.	Day	8-1	8-5	8-6	8-4	8-3	8-2	Remarks
8	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
Total								

Repl.	Day	7-2	7-6	7-3	7-4	7-1	7-5	Remarks
7	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
Total								

Repl.	Day	6-1	6-4	6-6	6-2	6-5	6-3	Remarks
6	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
Total								

Repl.	Day	5-2	5-3	5-1	5-5	5-4	5-6	Remarks
5	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
Total								

Repl.	Day	4-2	4-3	4-5	4-4	4-6	4-1	Remarks
4	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
Total								

Form I3 (Cont.)

Repl.	Day	3-3	3-5	3-2	3-1	3-4	3-6	Remarks
3	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
Total								
Repl.	Day	2-1	2-6	2-5	2-2	2-3	2-4	Remarks
2	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
Total								
Repl.	Day	1-6	1-1	1-3	1-5	1-2	1-4	Remarks
1	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
Total								

Form I4. Sample Summary Form for Survival and Young Production in a *Ceriodaphnia dubia* Dredged Material Elutriate Chronic Toxicity Test. (Summary of data from Form I3)

Sample I.D.: _____ Investigator: _____
Sample Collection Date: _____ Test Start-Date/Time: _____
Test End-Date/Time: _____

Elutriate Concentration	Total No. of Young Per Surviving Adult Replicate										No. of Live Adults
	1	2	3	4	5	6	7	8	9	10	
Control											
6.25%											
12.5%											
25.0%											
50.0%											
100%											

Attachment J. General Activity Schedule for Performing a Dredged Material Elutriate Chronic Toxicity Test with *Ceriodaphnia dubia*.[a]

Day	Activity
-7	Set up a brood board of 60 chambers and add one neonate (≤24 hr old) to each of the chambers. Feed brood animals.
-6	Feed brood animals.
-5	Transfer brood animals to new medium. Feed brood animals.
-4	Check chambers for production young. Count, record, and discard young produced. Feed brood animals.
-3	Check chambers for production of young. Transfer adults to new medium. Count, record, and discard young produced. Feed brood animals.
-2	Check chambers for production of young. Count, record and discard young produced. Feed brood animals.
-1	Check chambers for production of young. Mark all chambers with no young production. Transfer adults to new medium. Count, record, and discard young produced. Feed brood animals.
0	In morning, check marked chambers from preceding day for young production. Place a new mark on those with no young. Prepare sediment elutriate and performance control waters, and monitor all water parameters. Place 15 mL in each chamber in randomized test board. Introduce one neonate (≤24 hr old and all within 8 hr of one another in age) to each chamber. Feed test animals.
+1	Monitor water temperature. Observe test animals and record mortalities. Feed test animals.
+2	(Same as Day 1)
+3	Observe test animals and record mortalities and young production. Renew the test media (i.e. sediment elutriate and performance control waters), and transfer animals to new media. Monitor all water parameters of "old" and "new" solutions. Feed test animals.
+4	Observe test animals and record mortalities and young production. Monitor water temperature. Feed test animals.
+5	(Same as day 3)
+6	(Same as day 4)
+7	Observe test animals and record mortalities and young production. If 60 percent or more of the control animals have had their third brood, the test is terminated. If not, the test is continued. Monitor water parameters of temperature, dissolved oxygen, and pH. If test is continued, feed test animals.

+8	Observe test animals and record mortalities and young production. More than 60 percent of the control animals should have had their third brood, and the test is terminated.

---

[a] Activity schedule assumes that mass and individual cultures have already been established, and that the culture water and diets have been prepared in advance.

Attachment K. Materials for Culturing of and Conducting Toxicity Tests with Fathead Minnows.

Biological Supplies

- *Pimephales promelas* starter culture
- Brine shrimp
  - Frozen adult
  - Cysts

Glassware

- Mass culturing chambers (40 or 57 L volume)
- Erlenmeyer flasks (250 mL and 3 L) for exposure chambers and mixing elutriate stocks
- Separatory funnel (2 L)
- Volumetric flasks and graduated cylinders (10-1,000 mL, class A of borosilicate glass or non-toxic plastic)
- Volumetric pipets (1-100 mL, class A)
- Burettes ($\pm$ 0.05 mL)
- 5-mm ID fire-polished glass tubes
- Disposable pipets and droppers
- Plate glass (double-strength) or 1/4 inch plastic sheets for covering exposure chambers
- Beakers (1000 mL)

Instruments and Equipment

- Thermometer ($\pm$ 0.1^{o} C, National Bureau of Standards certified)
- pH meter ($\pm$ 0.1 pH units)
- Dissolved oxygen ($\pm$ 0.1 mg/L) and specific conductivity meter ($\pm$ 5 umhos/cm or equivalent)
- Constant temperature environmental room for culturing fathead minnows and brine shrimp and for testing fathead minnows
- Deionized water system (MILLIPORE MILLI-Q^{R} or equivalent)
- Analytical balance (capable of weighing accurately to 0.00001g)
- Reference weights (class S)
- Magnetic stir plates (for elutriate solution production)
- Light meter ($\pm$ 5 lux)
- Microscope (40 x magnification dissecting scope with substage lighting)
- Light box
- Centrifuge with swing-out buckets having a capacity of 500-1000 mL ($\geq$ 10,000 x g)
- Centrifuge tubes - 100-200 mL, Teflon®-lined screw-cap
- Continuous recording thermometer ($\pm$ 1.0^{o} C)
- Refrigerator with freezer
- Compressed air supply pump
- Plastic dish washing pan (white)

Automatic light control timer
pH/ion meter (± 0.1 mV)
Residual chlorine ion specific electrode
Ice bath

Reagents

(Reagent grade or better)
Hardness and alkalinity test reagents
Na_2CO_3
H_2SO_4 (concentrated)
Bromcresol green sodium salt
Methyl red sodium salt
NH_4C1
NH_4OH
EDTA (magnesium salt)
Eriochrome Black T
Dissolved oxygen meter calibration reagents
$MnSO_4 \cdot 4H_2O$
NaN_3
NaOH
NaI
KI
H_2SO_4
Starch
$Na_2S_2O_3 \cdot 5H_2O$
$KH(I0_3)_2$
Chlorine analysis reagents
Chloramine-T trihydrate
Phenyl arsine oxide (PAO)
Sodium acetate trihydrate
KI

Reagent-grade liquids

Water - MILLIPORE MILLI-Q^R, or equivalent
pH buffers - 4, 7 and 10
Specific conductivity standards

Miscellaneous

Acid (1N HCl, HNO_3 or H_2SO_4)
NaC1
Pipet bulbs and fillers
Wash bottles
Tape (labeling)
Marking pens
Stainless Steel mesh (5 mm openings)
Silicone glue

Attachment L. Sample Record Forms for Culturing Fathead Minnows and Performing Dredged Material Elutriate Toxicity Tests.

Form L1. Record of Fathead Minnow Embryo Production in the Brood Culture and Checklist for Daily Maintenance of Brood Culture and Incubation Chambers.

Date	Chamber 1				Chamber 2				Chamber 3				Chamber 4				Chamber 5				Chamber 6				Chamber 7			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	C	A	B	C	D

Date	Brood Cultures							Incubating Pan					
	Temp. (C)	Feed Fish (2X) √	Water Flow √	Aeration √	Cleaned Chambers (Which)	Check Sub-strates	Mortali-ties (Where)	Temp. (C)	Aeration √	Remove Dead Embryos	No. of Embryos Started	No. of Embryos Hatched	Percent Hatched

Form L2. Data Form for the Fathead Minnow Larval Survival and Growth Test. Routine Chemical and Physical Determinations.[1]

Dredged Material Source: _____ Test Dates: _____
Collection Date: _____ Analyst: _____

	Day								
Control:	0	1	2	3	4	5	6	7	Remarks
Temp.									
D.O. Initial									
Final									
pH Initial									
Final									
Alkalinity									
Hardness									
Conductivity									

	Day								
Conc:	0	1	2	3	4	5	6	7	Remarks
Temp.									
D.O. Initial									
Final									
pH Initial									
Final									
Alkalinity									
Hardness									
Conductivity									

	Day								
Conc:	0	1	2	3	4	5	6	7	Remarks
Temp.									
D.O. Initial									
Final									
pH Initial									
Final									
Alkalinity									
Hardness									
Conductivity									

Form L2. (Cont.)

Dredged Material Source: _____ Test Dates: _____
Collection Date: _____ Analyst: _____

	Day								
Control:	0	1	2	3	4	5	6	7	Remarks
Temp.									
D.O. Initial									
Final									
pH Initial									
Final									
Alkalinity									
Hardness									
Conductivity									

	Day								
Conc:	0	1	2	3	4	5	6	7	Remarks
Temp.									
D.O. Initial									
Final									
pH Initial									
Final									
Alkalinity									
Hardness									
Conductivity									

	Day								
Conc:	0	1	2	3	4	5	6	7	Remarks
Temp.									
D.O. Initial									
Final									
pH Initial									
Final									
Alkalinity									
Hardness									
Conductivity									

[1] Adapted from Weber et al. 1989

Form L3. Survival Data for Fathead Minnow Larval Survival and Growth Test.[1]

Dredged Material Source: _____ Test Dates: _____
Collection Date: _____ Analyst: _____

Conc:	Rep. No.	No. Survivors								Remarks
		0	1	2	3	4	5	6	7	
Control										
Conc:										
Conc:										
Conc:										
Conc:										
Conc:										

Comments:

[1]Adapted from Weber et al. 1989.

Form L4. Weight Data for Fathead Minnow Larval Survival and Growth Test.[1]

Test Date(s):_____ Dredged Materia Source:_____
Weighing Date:_____ Collection Date: _____
Drying Temperature (°C):_____ Analyst:_____

Drying Time(h):_____

Conc:	Rep. No.	A Wgt. of boat (mg)	B Dry wgt. of boat and larvae (mg)	B-A Total dry wgt. of larvae (mg)	C No. of larvae	(B-A)/C Mean dry wgt. of larvae (mg)	Remarks
Control							
Conc:							
Conc:							
Conc:							
Conc:							
Conc:							

[1]Adapted from Hughes et al. 1987.

Form L5. Summary Data for Fathead Minnow Larval Survival and Growth Test.[1]

Sediment Source: _____ Test Dates: _____
Collection Date: _____ Analyst: _____

Treatment	Control					
No. of live larvae						
Survival (%)						
Mean dry wgt. of larvae (mg) ± SD						
Temperature range (°C)						
Dissolved oxygen range (mg/L)						
Hardness						
Conductivity						

Comments:

[1]Adapted from Hughes et al. 1987.

Attachment M. General Activity Schedule for Performing a Dredged Material Elutriate 7-d Toxicity Test With Fathead Minnow Larvae.[1]

Day	Activity
-14	Call supplier of fathead embryos, if purchasing them from a supplier, to establish test start date.
-4	Pull spawning substrates with enough embryos (~300 per sediment sample) to conduct dredged material elutriate toxicity test.
-3	Check spawning substrates for fungal infected embryos; remove if present.
-2	Check spawning substrates for unfertilized and fungal infected embryos, remove if present. Begin brine shrimp embryo incubation.
-1	Check spawning substrates for unfertilized and fungal infected embryos; remove if present. Prepare elutriate solution from dredged material and refrigerate.
0	Prepare dredged material elutriate dilutions and performance control waters. Place 100 mL in each chamber in randomized arrangement in temperature controlled room or water bath. Place $\leq$24-hr old ($\leq$ 48-hr if shipped from a remote site) larvae into chamber in order until 10 larvae are in each. Feed test animals. Measure water temperature, dissolved oxygen, pH, hardness, alkalinity and conductance in a control chamber and a low, medium and high concentration of elutriate for each dredged material tested.
+1	Observe test animals and record mortalities. Prepare renewal test solutions. Siphon exposure chambers and renew test solutions. Feed test animals. Measure water temperature, dissolved oxygen and pH in a control chamber and a low, medium and high elutriate concentration for each dredged material tested of "old" and "new" solutions.
+2	(Same as day 1)
+3	(Same as day 1)
+4	(Same as day 1)
+5	(Same as day 1)
+6	(Same as day 1)
+7	Do not feed test animals. Observe test animals and record mortalities. Measure water temperature, dissolved oxygen, pH, hardness, alkalinity and conductance in a control chamber and a low, medium and high concentration of elutriate for each dredged material tested. Place all surviving larvae from each test chamber in a preweighed boat and oven dry at 100 C for at least 2 hr. Cool in a dessicator and weigh to 0.00001 gm.

[1] Activity schedule assumes that brood cultures are already producing embryos.

Attachment N. Materials for Culturing of and Conducting Toxicity Tests with *Chironomus tentans*.

Biological Supplies

Chironomus tentans brood stock (egg masses or larvae)
Tetrafin® goldfish food

Glassware

Crystallizing dishes or beakers (200-300 mL volume)
Erlenmeyer flasks (250 and 500 mL)
Larval rearing chambers (e.g., 19 L capacity)
Exposure beakers (300 mL high-form)
Wide bore pipets (5 to 6 mm ID)
¼" glass tubing (for aspirating flask)
Burettes (for hardness and alkalinity determinations)
Graduated cylinders (assorted sizes, 10 mL to 2 L)

Instruments and Equipment

Dissecting microscope
Sieve (e.g., U.S. Standard No. 30 mesh)
Delivery system for overlying water
Photoperiod timers
Temperature controllers
Thermometer
Dissolved oxygen meter
pH meter
Specific ion meter
Ammonia electrode (or ammonia test kit)
Specific conductance meter
Drying oven
Dessicator
Balance (to 0.01 mg)
Blender
Paper shredder, cutter or scissors
Refrigerator
Freezer
Hot plate
Light box

Miscellaneous

White paper toweling (for substrate)
Acetone (for substrate preparation)
Air Supply
Airstones
Screening material (e.g., Nitex, window screen or panty hose)

Stainless steel screen (no. 60 mesh, for test beakers)
Glass hole-cutting bit
Glass glue
Coarse-mesh sieve ($\geq$ 5 mm mesh)
Aluminum weighing pans
Fluorescent light bulbs (for culture and toxicity test)
Tygon® tubing (0.25 inch diameter for aspirating flask)
Nalgene® bottles (500 and 1,000 mL, for food and substrate preparation and storage)
Deionized water
Aspirator top (for collecting adults)
Water squirt bottle
White dishpan

Attachment O. Some Laboratory Sources of *Chironomus tentans* Cultures.

Laboratory	Contact Person	Phone/Fax/email
Environmental Consulting & Testing 1423 North 8th St., Suite 118 Superior, WI 54880	Steven Poirier	T 800-377-3657 F 715-394-7414
Mid-Continent Ecology Division U.S. Environmental Protection Agency 6201 Congdon Blvd. Duluth, MN 55804	Michael Kahl	T 218-529-5179 F 218-529-5003 E epamdj@du4500.dul.epa.gov
Lake Superior Research Institute University of Wisconsin-Superior Superior, WI 54880	Larry Brooke	T 715-394-8318 F 715-394-8454 E lbrooke@staff.uwsuper.edu
Zoology Department Natural Sciences Building Michigan State University East Lansing, MI 48824	John Giesy	T 517-353-2000 F 517-432-1984 E JGIESY@aol.com
Institute for Environmental Quality Wright State University Dayton, OH 45435	G. Allen Burton	T 937-873-2201 F 937-775-4997 E aburton@wright.edu

Attachment P. Methods of Preparing Synthetic Fresh Water.

TABLE P1: PREPARATION OF SYNTHETIC FRESH WATER USING REAGENT GRADE CHEMICALS[a]

	Reagent Added (mg/L)[b]				Final Water Quality		
Water Type	$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KC1	pH[c]	Hardness[d]	Alka-linity[d]
Very soft	12.0	7.5	7.5	0.5	6.4-6.8	10-13	10-13
Soft	48.0	30.0	30.0	2.0	7.2-7.6	40-48	30-35
Moderately Hard	96.0	60.0	60.0	4.0	7.4-7.8	80-100	60-70
Hard	192.0	120.0	120.0	8.0	7.6-8.0	160-180	110-120
Very hard	384.0	240.0	240.0	16.0	8.0-8.4	280-320	225-245

[a]Taken in part from Marking and Dawson (1973).
[b]Add reagent grade chemicals to deionized water.
[c]Approximate equilibrium pH after 24 h of aeration.
[d]Expressed as mg $CaCO_3$/L.

TABLE P2. PREPARATION OF SYNTHETIC FRESH WATER USING MINERAL WATER[a]

			Final Water Quality		
Water Type	Volume of Mineral Water Added (mL/L)[b],	Proportion of Mineral Water (%)	pH[c]	Hardness	Alka-linity[d]
Very soft	50	2.5	7.2-8.1	10-13	10-13
Soft	100	10.0	7.9-8.3	40-48	30-35
Moderately Hard	200	20.0	7.9-8.3	80-100	60-70
Hard	400	40.0	7.9-8.3	160-180	110-120
Very hard[e]	---	---	---	---	---

[a]From Mount et al., 1987, and data provided by Philip Lewis, EMSL-Cincinnati.
[b]Add mineral water to Milli-Q[R] water or equivalent to prepare DMW (Diluted Mineral Water).
[c]Approximate equilibrium pH after 24 h of aeration.
[d]Expressed as mg $CaCO_3$/L.
[e]Dilutions of PERRIER[R] Water form a precipitate when concentrations equivalent to "very hard water" are aerated.

Attachment Q. Preparation of Food for *Chironomus tentans*.

The following is based upon a procedure presented by Denny and Mead (1991), and is designed for an aquarium containing 7 L of water.

1. One day in advance, place three marked aluminum weighing pans in an oven at 100°C, and heat overnight. Handle pans with forceps only.

2. Remove pans from the oven, allow to cool in a dessicator, and determine the tared weight of each pan.

3. Blend the Tetrafin® flake food for goldfish in distilled water for 30 seconds or until very finely ground. Use 100 mL of water for every 10 g of Tetrafin® food. The food can be frozen, so larger batches (e.g., 1 L) of food are recommended to reduce the effort in preparing food.

4. Filter the slurry through a #202 Nitex screen to remove large particles.

5. Shake well to ensure homogeneity, and pipet 5.0 mL of the slurry into each of the three tared pans. Dry at 100° C for at least 4 hr and reweigh.

6. Subtract the weight of each pan from the total weight (solids plus pan) to obtain the weight of the solids. This should be approximately 70,000-80,000 mg. Divide by a target value of 56,000 (56 g/L) to obtain a dilution factor.

7. Multiply the volume of the food suspension by the dilution factor to obtain the desired final volume. Dilute the food suspension to the final volume with distilled water.

8. Record all weights and calculations in a record book.

9. Pour food into 500 mL Nalgene® bottles. Keep one bottle for current use in a refrigerator. Freeze the remaining bottles for future use.

10. Shake the bottles vigorously prior to feeding and stir well between feeding of each culture aquarium to ensure a uniform distribution of solids.

Attachment R. Culture and Test Data Forms for *Chironomus tentans*.

Form R1. Sample Evaluation Form for the Health and Reproduction of a *Chironomus tentans* Culture.

Culture Aquarium	Date of Egg Mass Deposition	Date 4th Instar Larvae were Weighed	Age of Weighed 4th Instar Larvae	Mean Dry wt. of 4th Instar Larvae (n=10)	Date of Observed First Emergent Adult	Total Number of Egg Masses Produced	General Comments	Initials of Culturist
A								
B								
C								
D								
E								
F								

Form R2. Sample Data Form for Temperature and Overlying Water Chemistry Measurements in a Toxicity Test with *Chironomus tentans*.

Sediment Sample Source _____

Date of Test Initiation_____

Toxicologist Conducting Test _____

Test Day	Test Replicate Sampled	Temperature (° C)	Dissolved Oxygen (mg/L)	pH	Hardness (mg/L)	Alkalinity (mg/L)	Specific Conductance (umhos/cm)	Total Ammonia (mg/L)
0								
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Form R3. Data Chart for Performing Reference Toxicant Tests with $CuSO_4$ or KC1 and *Chironomus tentans*.

Brood Stock Source _____ Test Initiation Date _____ Time ____

Age of Test Animals (days post-hatch)_____ Reference Toxicant ($CuSO_4$) or KCl)_____

No. of Animals per Replicate_____ Reference Toxicant Supplier _____

No. of Replicates_____ Reference Toxicant Lot No._____

Dilution Water/Control_____ Reference Toxicant Purity_____

Test Volume_____ Toxicologist_____

Test Type (circle one)[a] SU, SM, RU, RM, FU, FM

	Survival Readings												
	0 h			24 h		48 h		72 h		96 h			
Conc.	pH	D.O.	Temp.	Surv.	pH	Surv.	pH	Surv.	pH	Surv.	pH	D.O.	Temp.

Comments_____

_____

96-hr LC50 =_____
Method of LC50 Estimate_____
Cumulative Mean LC50_____
No. of Tests the Cumulative Mean is Based on_____
Acceptability of Current Test[b] Yes ___ No ___

_____

[a]SU = Static unmeasured, SM = static measured, RU = renewal unmeasured, RM = renewal measured, FU = flow-through unmeasured, FM = flow-through measured.

[b]Based upon two standard deviations around the cumulative mean 96-hr LC50.

Attachment S. General Activity Schedule for Performing a Sediment Toxicity Test with *Chironomus tentans*[a].

Day	Activity
-13	Newly deposited egg masses from the culture unit are assigned for use in the test and placed into hatching dishes.
-12	A larval rearing aquarium is prepared with new substrate.
-11 and -10	Egg masses are examined for hatching success. If successful hatch is occurring, transfer first instar larvae and any remaining unhatched embryos from the crystallizing dishes into the larval rearing aquaria. To begin nutrient enrichment of substrate, add 1.0 mL of concentrated food suspension to the larval rearing aquarium.
-9	Feed 5.0 mL of concentrated food suspension to each larval rearing aquarium. Monitor temperature and dissolved oxygen concentration of overlying water.
-8	(Same as Day -9)
-1	Feed each larval rearing aquarium and monitor temperature and dissolved oxygen, as previously. Add sediment into each of the replicate test beakers, place into exposure system, and activate the automated water renewal system.
0	Remove second instar larvae from the culture chamber substrate. Add 1.0 mL containing 4.0 mg of dry food into each beaker. Transfer 10 larvae into each randomly chosen beaker. Monitor temperature and dissolved oxygen.
1	Add food to each beaker. Monitor water parameters of temperature, dissolved oxygen, pH, hardness, alkalinity, and conductivity.
2	Add food to each beaker. Monitor temperature and dissolved oxygen.
3	Add food to each beaker. Monitor temperature, dissolved oxygen and total ammonia.

4 to 7	(Same as Day 2)
8	Add food to beaker. Monitor temperature, dissolved oxygen and total ammonia.
9	(Same as Day 1)
10	Monitor temperature and dissolved oxygen. Terminate test by collecting the larvae and obtaining dry weight measurements for each replicate.

[a] Activity schedule assumes that a healthy culture has been established, that an exposure system is in place, and that larval rearing substrate and food suspensions have been prepared in advance.

Attachment T. Materials for Culturing of and Conducting Toxicity Tests with *Hyalella azteca*.

Biological Supplies

- *Hyalella azteca* brood stock
- Active dry yeast
- Cerophyl® (dried cereal leaves)
- Trout food pellets
- Algae

Glassware

- Culture chambers (2 L plastic or glass beakers)
- Exposure beakers (300 mL high form)
- Juvenile holding beakers (1 L)
- Wide bore pipets (5 to 6 mm ID)
- Glass disposable pipets
- Burettes (for hardness and alkalinity determinations)
- Graduated cylinders (assorted sizes, 10 mL to 2 L)
- White organism sorting tray

Instruments and Equipment

- Dissecting microscope
- Sieve (e.g., U.S. Standard No. 30 mesh)
- Delivery system for overlying water
- Photoperiod timers
- Photometer
- Temperature controllers
- Thermometer
- Dissolved oxygen meter
- pH meter
- Specific ion meter
- Ammonia electrode (or ammonia test kit)
- Specific conductance meter
- Drying oven
- Dessicator
- Balance (sensitive to 0.01 mg)
- Blender
- Refrigerator
- Freezer
- Light box
- Centrifuge
- Hemacytometer
- Forceps

Miscellaneous

- Ventilation hood for exposure system
- Air supply
- Cotton surgical gauze or cheese cloth
- Stainless steel screen (no. 60 mesh, for test beakers)

Glass hole-cutting bit
Glass glue
Plastic mesh (110 µ mesh opening; Nytex® 110)
Aluminum weighing pans
Fluorescent light bulbs (for culture and toxicity test)
Nalgene® bottles (500 mL, for food preparation and storage)
Deionized water
¼" air line tubing
White plastic dish pan

Chemicals

Detergent (non-phosphate)
Acetone (reagent grade)
Hexane (reagent grade)
Copper sulfate (reagent grade)
Potassium chloride (reagent grade)
Hydrochloric acid (reagent grade)

Attachment U. Some Laboratory Sources of *Hyalella azteca* Cultures.

Laboratory	Contact Person	Phone/Fax/email
Environmental Consulting & Testing 1423 North 8th St., Suite 118 Superior, WI 54880	Steven Poirier	T 800-377-3657 F 715-394-7414
Mid-Continent Ecology Division U.S. Environmental Protection Agency 6201 Congdon Blvd. Duluth, MN 55804	Michael Kahl	T 218-529-5179 F 218-529-5003 E epamdj@du4500.dul.epa.gov
Lake Superior Research Institute University of Wisconsin-Superior Superior, WI 54880	Larry Brooke	T 715-394-8318 F 715-394-8454 E lbrooke@staff.uwsuper.edu
Zoology Department Natural Sciences Building Michigan State University East Lansing, MI 48824	John Giesy	T 517-353-2000 F 517-432-1984 E JGIESY@aol.com
Institute for Environmental Quality Wright State University Dayton, OH 45435	G. Allen Burton	T 937-873-2201 F 937-775-4997 E aburton@wright.edu

Attachment V. Preparation of Food for Feeding *Hyalella azteca* during Culturing and Testing.

The following is a description of the YCT (Yeast-Cerophyl®-Trout food) and algal food preparation. No algal species is specifically mentioned in the algal food preparation. Any green alga will probably work satisfactorily. The procedure is excerpted from: U.S. Environmental Protection Agency. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA 600/4-89/001. Environmental Monitoring and Support Laboratory, Cincinnati, OH.

TABLE V1. YCT PREPARATION

YCT is composed of yeast at a concentration of 5 g/L, Cerophyl® at 10 g/L and fermented trout food at 5 g/L. These three ingredients are combined in equal volumes (1:1:1) to form the final product.

1. Each ingredient is made up as follows:
   A. Yeast
      To be made the same day as the YCT.
      i. Add 2.5 gm active dry yeast to 500 mL dilution water[a].
      ii. Shake vigorously until totally dissolved and use shortly thereafter. Discard excess solution.

   B. Cerophyl®

      To be prepared 24 h before the YCT.
      i. Weigh 5.0 gm Cerophyl®.
      ii. Combine Cerophyl® and 500 mL dilution water[a] in an Erlenmeyer flask.
      iii. Insert a clean bar, cover and stir for 24 hr at medium speed.
      iv. After 24 hr, remove from stir plate, filter through a fine screen (Nitex® 110 mesh).
      vi. Discard the excess and particulates on filter.

   C. Trout Food
      This ingredient must be prepared at least one week in advance, as it must ferment before using. It is best to make a supply ahead of time and freeze it in small batches. Careful planning is needed to avoid being short of this ingredient.
      i. Weigh 5 gm of trout chow pellets (1/8" pellets work well).
      ii. Add 1 L of dilution water[b] to fermentation chamber.
      iii. Place pellets in glass or plastic bottle and aerate, gently rolling the pellets to prevent settling.
      iv. Cover with plastic wrap to decrease evaporation.
      v. Label the container with the date the food should come down (one week from starting date).
      vi. Keep the water level at 1 L by replacing evaporated water each day.
      vii. After one week, shut the air off and filter supernatant through a fine screen (Nitex® 110 mesh). Distribute liquid into smaller containers, label with the current date and freeze.

2. Preparation of YCT

The batch size of YCT may vary, depending upon usage. Batches are made which are used for only two weeks, and new batches should be prepared a day or two in advance to insure a continuous supply.

A. Remove an adequate amount of fermented trout food from the freezer and thaw in a water bath.

B. Shake ingredients well and filter through a fine screen (Nitex® 110 mesh) into a graduated cylinder. Ingredients should be measured out in equal volumes.

C. Combine ingredients in an Erlenmeyer flask and label with the current date.

D. Suspended solid's level must be measured and adjusted to 1800 mg/L before the food is fed.

3. Suspended Solids Monitoring

Solids are monitored on each batch of YCT and adjusted to a constant measure (1800 mg/L) before feeding to keep feeding volumes and food levels consistent.

A. Oven dry labeled weigh pans to a constant weight and weigh.

B. Shake YCT solution well, it is important that the solution be uniform so as to get a good representative sample.

C. Measure 5.0 mL using a 5 mL pipette and dispense into each of two preweighed, oven dried pans.

D. Place pans containing 5 mL YCT in oven and dry completely (at least 4 hr).

E. Weigh pans again and subtract weight of pan alone to get weight of solids in 5 mL YCT.

F. Convert this figure to mg/L and divide by 1800 mg/L to get the dilution factor.

G. Multiply the volume of YCT by the dilution factor to get the final volume and dilute to this final volume. For example:

Pre-weight (g) (oven dried pan alone)	Post-weight (g) (pan & 5 mL YCT dried)	Difference (YCT alone)	Average
1.61665	1.62600	0.009350	
1.62800	1.63750	0.009500	0.009425

Then, 0.009425 g in 5 mL = x mg/L

To find x, multiply 0.009425 x 1000 = 9.425 mg in 5 mL.

Next, divide this quantity by 0.005 liters to get mg/L:

9.425 ÷ 0.005 L = 1885 mg/L

Total Suspended Solids Dilution Factor = 1885 mg/L = 1.05

1800 mg/L

This number (1.05) is multiplied by the volume of YCT prepared to determine final volume obtained after dilution.

H. Repeat the process with 5 mL more of this diluted YCT in pre-weighed pans to confirm suspended solids.

I. If this dilution factor ratio does not work well, it may be best to make a sample dilution of a small aliquot of YCT and check solids levels before diluting the whole batch.

J. Record suspended solids information and mark the solids level of the YCT on the container.

K. Acceptable solids levels are between 1700 and 1900 mg/L (1800 is preferable).

L. Shake well before feeding.

---

[a] 10% Diluted Mineral Water (DMW) is used in cases above. When DMW is not available, a high quality Millipore or distilled water may be substituted.
[b] We use a cut-off 1 gallon Nalgene® jug which is then inverted with a stopper in the neck through which filtered air is supplied.

TABLE V2. ALGAL CULTURE PREPARATION

1. Preparation of Stock Solutions

A. Stock solutions are prepared at a non-specific concentration.

B. Nutrients are added to Millipore water in reagent bottles, mixed until totally dissolved and stored in the refrigerator or cold storage room in the dark. (Stock solutions will remain good for years, barring contamination).

C. Once dissolved, the $NaSiO_3$ stock solution is filter sterilized using a 0.45 µm Millipore filter.

D. Record the date the new stock solutions are prepared. New stock solutions should not be used for 1 month after they are prepared.

E. A 5 mL pipette should be designated and used for each individual stock solution. These pipettes are stored with the stock solutions in a plastic dish pan for each transport.

F. A "pro-pipetter" valved pipette bulb is used for steady and more accurate measurements.

G. The pipette designated for the $NaSiO_3$ stock solution should be

autoclaved or replaced by a new pipette before it is used for a new batch of media. All other stock solution pipettes can be reused indefinitely.

2. Starting Algal Culture

A. Prepare 2 L batches of MBL medium in a 2 L volumetric flask using Woods Hole MBL. Do not add Na_2SiO_3 until after autoclaving.

B. Pour medium into 2 L Erlenmeyer flask, cover with aluminum foil, and autoclave for 15 min.

C. After cooling, add 2 mL of $NaSiO_3.9H_2O$ (1 mL/L of media) using a sterile pipette.

D. Store media at room temperature; prepare more as needed.

E. Transfer media into (previously autoclaved) sterile 2 L separatory funnel.

F. Support funnel with a ringstand using a 10 to 21 cm diameter ring.

G. Inoculate culture media with 2-5 mL of inoculum (see below step N).

H. Place air stem in culture so that the tip is at the stopcock of the flask. This is done to prevent settling of algae.

I. Stopper funnel with foam plug.

J. Cultures are kept at 25 $\pm$ 2° C at a light intensity of approximately 100 ft-c using Grow-lux fluorescent bulbs.

K. Cultures mature in approximately 6-8 days at 25° C under a photoperiod of 16 hr light:8 hr darkness, depending on the amount and concentration of inoculum.

L. When cultures are very green, remove air and transfer culture into a 2 L Erlenmeyer flask.

M. This culture can be stored in the refrigerator if not prepared immediately, or can be centrifuged at this time.

N. New cultures can be started by transferring 2-5 mL of this mature, well mixed culture to sterile media.

O. Approximately four cultures can be started from the same inoculum, after which the next culture should be started from an algal slant to insure purity. Inoculum can be saved from this slant culture to start the next four subsequent cultures.

P. Record data concerning culture and inoculum dates, concentrations and volumes.

3. Starting Algal Cultures From a Slant

Algal slants can be purchased from the Starr Collection at the University of Texas in Austin, Texas or the American Type Culture Collection in Rockville, Maryland.

Slants can be kept and used for several months if stored in a dark refrigerator at 4° C.

All steps, except step G, are followed as in previous procedures. However, between steps D and E do the following:

A. Flame a small wire loop over a bunsen burner and allow to cool.

B. Uncap algal slant and quickly remove a loopful of the algae by pulling the wire loop gently across the surface of the slant so as not to tear up the agar. Try to keep the slant uncapped for as short a time as possible.

C. Flame the mouth of the slant tube to prevent contamination and quickly replace the cap.

D. Immerse the wire loop containing the algae in the MBL medium and swirl until the algae has come off the loop and is in the medium.

4. Preparation of Algae for Feeding

A. Centrifuge mature algal culture in 100 mL tubes at 2000 RPM for 8-10 min.

B. Pour off MBL supernatant. Use diluent[a] in a squirt bottle to resuspend the algal pellet. Dilution water is the same water used for testing and *Ceriodaphnia* culturing.

C. Use only enough dilution water to just break up and suspend the pellet (approximately ¼ the volume of the algal culture which was centrifuged).

D. Transfer resuspended algae into an Erlenmeyer flask.

E. Count and calculate the number of cells per mL using a Coulter counter or hemacytometer[b] and dilute to 35×10^6 cells per mL with diluent.

F. Store algae in refrigerator. Presently, algae is used until it is gone and stock appears to remain viable for several weeks. The algae stock is not viable when it turns yellow in color.

---

[a] 10% Diluted Mineral Water (DMW) is used routinely at ERL-Duluth.

[b] Hemacytometer readings are likely to be higher than Coulter counter readings.

TABLE V3. Algal Media

Prepare stock solutions and use 1 mL of each stock solutions per liter of medium, except add 2 mL of $FeCl_3.6H_2O$ per liter medium.

Macronutrients	Woods Hole MBL[a] gm/L in stock solution
$CaCl_2.2H_2O$[b]	36.76
$MgSO_4.7H_2O$	36.97
$NaHCO_3$	12.60
K_2HPO_4	8.71
$NaNO_3$[b]	85.01
$Na_2SiO_3.9H_2O$	28.42 (add after autoclaving)
Micronutrients	
Na_2 EDTA	4.36
$FeCl_3.6H_20$	3.15
$CuSO_4.5H_2O2$[c]	0.01
$CoCl_2.6H_2O$[c]	0.01
$ZnSO_4.7H_2O$[c]	0.022
$MnCl_2.4H_2O$[o]	0.18
$Na_2MoO_4.2H_2O$[b]	0.006
H_3BO_3[c]	1.0

[a] Nichols, H.W. 1973. In: Handbook of Psychological Methods, J.R. Stein, Ed. Cambridge University Press, London. pp. 7-24.
[b] $CaCl_2$ and $NaNO_3$ can be combined as one stock solution.
[c] Micronutrients can be mixed as single stock solution.

Attachment W. Culture and Test Data Forms for *Hyalella azteca*.

Form W1. Sample Evaluation Form for the Health and Reproduction of a *Hyalella azteca* Culture.

Culture Record
Hyalella azteca

Date: _____ Water Bath Temp. _____

Feeding: Amount YCT _____ Amount Algae _____

Comments:

Culture Renewal

Date: _____ Water Bath Temp. _____

Dissolved Oxygen: _____ (any random tank) pH _____

Culture Jar #	# Adults	# Pairs	Approx. # Young	# Young Saved	# Added	Total

Total # Young Saved: _____ Age: _____ days

Form W2. Sample Data Form for Temperature and Overlying Water Chemistry Measurements in a Toxicity Test with *Hyalella azteca*.

Hyalella azteca Toxicity Test
Overlying Water Chemistry Measurements

Project Name _____

Sediment Sample Source _____

Date of Test Initiation_____

Toxicologist Conducting Test _____

Test Day	Test Replicate Sampled	Temperature (°C)	Dissolved Oxygen (mg/L)	pH[a]	Hardness (mg/L)	Alkalinity (mg/L)	Specific Conductance (umhos/cm)	Total Ammonia (mg/L)
0								
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

[a]Unshaded areas are for measurements.

Form W3. Data Chart for Performing Reference Toxicant Tests with $CuSO_4$ and *Hyalella azteca*.

REFERENCE TOXICANT TESTING FORM

-*Hyalella azteca* and $CuSO_4$-

Brood Stock Source_____ Test Initiation Date_____ Time

Aquarium No._____ $CuSO_4$ Form_____

No. Animals Per Replicate_____ $CuSO_4$ Purity_____

No. of Replicates_____ $CuSO_4$ Supplier_____

Dilution Water/Control_____ $CuSO_4$ Lot No._____

Test Volume_____ Toxicologist_____

Test Type (circle one)[a]: SU, SM, RU, RM, FU, FM

	Survival Readings											
	0 h			24 h		48 h		72 h		96 h			
Conc.	pH	D.O.	Temp.	Surv.	pH	Surv.	pH	Surv.	pH	Surv.	pH	D.O.	Temp.

Comments_____

96-hr LC50 = _____
Method of LC50 Estimate _____
Cumulative Mean LC50 _____
No. of Tests the Cumulative Mean is Based on _____
Acceptability of Current Test[b] Yes ___ No ___

[a]SU = Static unmeasured, SM = static measured, RU = renewal unmeasured, RM = renewal measured, FU = flow-through unmeasured, FM = flow-through measured.

[b]Based upon two standard deviations around the cumulative mean 96-hr LC50.

Attachment X. General Activity Schedule for Performing a Sediment Toxicity Test with *Hyalella azteca*[a].

Day	Activity
-7	Renew mass cultures. Separate the juvenile amphipods from the mass cultures and place juveniles in 1-L beakers with a piece of presoaked cotton gauze and feed. Begin preparing YCT food for the test if not previously prepared.
-6	Feed the juveniles and observe the cultures for survival, monitor temperature and dissolved oxygen.
-5	Same as Day -6, omit dissolved oxygen monitoring.
-4	Same as Day -6.
-3	Same as Day -6, omit dissolved oxygen monitoring.
-2	Same as Day -6.
-1	Same as Day -6; add sediment into each of the replicate test beakers, place into exposure system, and activate the automated water renewal system.
0	Transfer ten 7- to 14-day old juveniles into each randomly chosen beaker. Feed 1.5 mL of YCT into each test chamber. Monitor overlying water temperature and dissolved oxygen.
1	Add 1.5 mL of YCT food to each test chamber. Monitor overlying water characteristics of temperature, dissolved oxygen, pH, hardness, alkalinity, conductivity and ammonia.
2	Add 1.5 mL of YCT food to each test chamber. Monitor overlying water temperature.
3 to 8	Same as Day 2 except monitor dissolved oxygen on even numbered days.
9	Same as Day 1.
10	Monitor temperature and dissolved oxygen. Terminate test by collecting the juveniles with a sieve and observing for survivors. Oven-dry survivors and weigh for growth determination.

[a] Activity schedule assumes that a healthy culture has been established (monitored at least quarterly with a 96-hr reference toxicant test), that an exposure system is in place, and that YCT food suspensions have been prepared in advance.

Attachment Y. Materials for Culturing of and Conducting Bioaccumulation Studies with *Lumbriculus variegatus*.[1]

Biological Supplies

Lumbriculus variegatus starter culture
Trout starter
Helisoma sp. snails (optional)

Glassware

Aquaria (57 L, e.g.)
Pipette (20 cm long, 5 mm I.D. opening, ends fire-polished)
Glass weights (to hold substrate in place)
Exposure chambers (15.8 cm x 29.3 cm x 11.7 cm, W x L x H), 12
Glass bowl (20 cm diameter)
Glass vials (10 mL)
Beakers (500 mL)

Instruments and Equipment

Sieve, fine-meshed (e.g., U.S. Standard No. 35 or 40 mesh)
Water delivery system
Paper shredder, cutter or scissors
Temperature controller
Thermometer
Continuous recording thermometer
Photoperiod timer
Dissolved oxygen meter
Specific ion meter
pH meter
Ammonia electrode (or ammonia test kit)
Drying oven
Desiccator
Freezer
Tissue homogenizer

Miscellaneous

Brown paper toweling
Small dipnets (e.g. 7.6 cm)
Shallow pan (plastic, light-colored)
Shallow pan (glass or stainless steel)
Dissecting probes
Dental picks
Light bulbs
Air Supply

Airstones
Acetone
Hexane
Chloroform
Methanol
Copper sulfate (reagent grade)

---

[1] Does not include the analytical instrumentation, glassware or reagents necessary to analyze for inorganic or organic chemicals that may bioaccumulate.

Attachment Z. Laboratory Sources of *Lumbriculus variegatus* Cultures.

Laboratory	Contact Person	Phone/Fax/email
Environmental Consulting & Testing 1423 North 8th St., Suite 118 Superior, WI 54880	Steven Poirier	T 800-377-3657 F 715-394-7414
Mid-Continent Ecology Division U.S. Environmental Protection Agency 6201 Congdon Blvd. Duluth, MN 55804	Michael Kahl	T 218-529-5179 F 218-529-5003 E epamdj@du4500.dul.epa.gov
Lake Superior Research Institute University of Wisconsin-Superior Superior, WI 54880	Larry Brooke	T 715-394-8318 F 715-394-8454 E lbrooke@staff.uwsuper.edu
Zoology Department Natural Sciences Building Michigan State University East Lansing, MI 48824	John Giesy	T 517-353-2000 F 517-432-1984 E JGIESY@aol.com
Institute for Environmental Quality Wright State University Dayton, OH 45435	G. Allen Burton	T 937-873-2201 F 937-775-4997 E aburton@wright.edu

Attachment AA. Culture and Test Data Forms for *Lumbriculus variegatus*

Form AA1. Evaluation Form for the Health and Reproduction of a *Lumbriculus variegatus* Culture.

Date of Arrival of Brood Stock_____ Culturist_____
Date of Animal Transfer Into Specific Culture Aquaria

Aquarium:	A	B	C	D	E	F
Date :						

Trout Chow Brand and Batch Number_____

Date	Culture Aquarium	Dissolved oxygen (mg/L)	Temp (°C)	Animal Color	Animal Responsiveness	Wet wt. at time of transfer IN	Wet. wt. at time of transfer OUT	Comments
	A							
	B							
	C							
	D							
	E							
	F							
	A							
	B							
	C							
	D							
	E							
	F							

Form AA2. Sample Record Form for Sediment and *Lumbriculus variegatus* Tissue Samples in a Bioaccumulation Study.

Sediment Sample I.D._____ *Lumbriculus* variegatus source_____

Replicate No._____ *L. variegatus* mass added at test start_____

Sediment Collection Date_____ *L. variegatus* mass retrieved at test end _____

Sediment Exposure Start Date_____ Duration of gut clearance period_____

Investigator(s):_____

	Replicate No.			
Annelid Data	1	2	3	4
Wet wt. at end				
Dry wt. at end				
Percent total lipids				
Conc. chemical (wet wt.) (A)				
Conc. chemical (dry wt.) (B)				
Lipid normalized chemical conc., wet wt. basis (C)				
Sediment Data				
Wet wt.				
Dry wt.				
Percent TOC (dry wt. basis)				
AVS (umol/g, dry wt.)				
Conc. of chemical (dry wt.)				
TOC normalized chemical conc., (dry wt.) (D)				
AVS normalized chemical conc., (dry wt.) (E)				
Accumulation factor, AF, for organic compounds (C/D)				
Accumulation factor, AF, for inorganic compounds (B/E)				

Form AA3. Data Chart for Performing Reference Toxicant Tests with $CuSO_4$ and *Lumbriculus variegatus*.

Brood Stock Source _____
Organisms Tested From Culture
Aquarium No. _____
No. of Animals Tested Per Replicate _____
No. of Replicates _____
Method of LC50 Estimate _____

$CuSO_4$ Form _____
$CuSO_4$ Purity _____
$CuSO_4$ Supplier and Lot No. _____
Test Type (circle one)[a]:
SU, SM, RU, RM, FU, FM
Test Initiation Date _____
Toxicologist _____

Number of Mortalities

Exposure Duration (Hr)	Control A	Control B	Exp. 1 A	Exp. 1 B	Exp. 2 A	Exp. 2 B	Exp. 3 A	Exp. 3 B	Exp. 4 A	Exp. 4 B	Exp. 5 A	Exp. 5 B
0												
24												
48												
72												
96												

96 hr. LC50 = _____

Cumulative Mean 96 hr LC50 = _____

Number of Reference Toxicant Tests Used to Determine Cumulative Mean LC50 _____

Acceptability of Current Test[b]
Yes_____ No_____

[a]SU = Static unmeasured, SM = static measured, RU = renewal unmeasured, RM = renewal measured, FU = flow-through unmeasured, FM = flow-through measured.

[b]Based upon two standard deviations around the cumulative mean 96 hr LC50.

Form AA4. Sample Data Form for Temperature and Water Chemistry Measurements in a Bioaccumulation Study with *Lumbriculus variegatus*.

Sediment Sample Source: No. 1 _____ No. 2 _____ No. 3 _____

Date of Test Initiation _____

Toxicologist Conducting Test _____

	Sample Replicate[a]	Temperature (°C)[b]	Dissolved Oxygen[b] (mg/L)	pH[c]	Total Ammonia[c] (mg/L)	Hardness[c] (mg/L as $CaCO_3$)	Alkalinity[c] (mg/L as $CaCO_3$)	Specific Conductance[c] (μmhos/cm)
	1A							
	1B							
	1C							
	1D							
Day _____	2A							
	2B							
(0-28)	2C							
	2D							
	3A							
	3B							
	3C							
	3D							

[a] Sample form is for a study of three sediment samples in quadruplicate. The form may be expanded for larger numbers of sediment samples or increased replication.
[b] To be measured daily.
[c] To be measured twice during the test.