EPA/620/R-95/001
March 1995

ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM (EMAP) METHODS FORMAT GUIDANCE

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EPA Contract No. 68-C1-0022

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DISCLAIMER

The U.S. Environmental Protection Agency through its Office of Research and Development funded and managed the preparation of the guidance described here under Contract No. 68-C1-0022. It has been reviewed by management of the Environmental Monitoring and Assessment Program (EMAP) and the Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati), U.S. Environmental Protection Agency (USEPA). It has further been subjected to the Agency's peer and administrative review process and has been approved for publication. The mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

The U.S. Environmental Protection Agency (USEPA) is developing the Environmental Monitoring and Assessment Program (EMAP) to determine the current status and trends in the condition of our nation's ecological resources on regional and national scales. EMAP's goal is to monitor the condition of the Nation's ecological resources, thereby contributing to decisions on environmental protection and management. EMAP's monitoring efforts are coordinated by seven Coordination Groups: Quality Assurance, Indicators, Information Management, Design and Statistics, Assessment and Reporting, Landscape Characterization, and Methods. These efforts will operate on regional scales over periods of years to decades, and will involve collecting data from seven Resource Groups: Rangelands, Agroecosystems, Forests, Inland Surface Waters, the Great Lakes, Estuaries, and Landscapes. EMAP data will enable policy makers, scientists, and the public to evaluate the success of current policies and programs and to identify emerging problems before they become widespread or irreversible. EMAP's ecological status and trends data will allow decision makers to assess objectively whether or not the nation's ecological resources are responding positively, negatively, or not at all, to regulatory programs.

Methods developed and used by the Resource Groups are in various stages of development. Manuals of field and laboratory methods have been prepared for each stage of EMAP including pilot, demonstration, and full implementation stages. Early in the program, some Resource Groups prepared methods in the style most familiar to them. More recently, using augmented review procedures and having method manuals produced by the Estuaries group to use as a guide, greater uniformity was achieved.

EMAP-Methods has prepared this guidance document to facilitate consistency among the Resource Groups in preparing methods and methods manuals. It provides instructions for preparing methods in the Environmental Monitoring Methods Council (EMMC) format, and for assembling the methods into a manual. EMAP-Methods is also working to develop a methods database on the EPA Wide Area Information Server (WAIS) that will contain the full text of all properly formatted EMAP methods. All EMAP methods will be placed on this server and must be compatible with the server. Therefore, this guidance document provides specifications for consistent organization and naming of electronic files. These electronic file formatting requirements are needed to minimize the effort required to install and maintain methods on the document server.

The "EMAP Methods Format Guidance" has been prepared by Gary Collins, the EMAP-Methods Technical Coordinator, and edited and formatted through Contract #68-C1-0022 with Dyncorp/TAI. The suggested citation for this document is:

> Collins, Gary. 1995. Environmental Monitoring and Assessment Program: Methods Format Guidance. EPA 620/R-95/001. Environmental Monitoring and Assessment Program: U.S. Environmental Protection Agency, Cincinnati, Ohio.

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ACKNOWLEDGMENTS

This document has resulted from several stages of technical review occurring from January 1992 to September 1994. The assistance of EMAP-Center in the preparation of draft documents, especially that of Joanie Currin, is gratefully acknowledged. Helpful comments are acknowledged from reviewers at each stage including William Budde, Sidney Draggan, Richard Garnas, Walter Heck, Marjorie M. Holland, Linda Kirkland, Donald Klemm, Richard Latimer, Dennis McMullen, Glen Miller, Tony Olson, Jerry Parr, David Peck, and Chris Yoder.

EMAP Methods would also like to acknowledge the contributions of several organizations in the preparation of this document. These organizations and their contributions are cited below, listed alphabetically by organization:

EMAP-Estuaries: Contributed Appendix A, "Determination of Butyltin Compounds in Sediments," and Appendix E, "Sediment Collection".

Queens University, Kingston, Ontario, K7L3N6: Sushil Dixit contributed Appendix F, "Diatom Methods".

Technology Applications, Inc., Cincinnati, Ohio, 45268: Dave Craven, Shannon Fink, and Anne Pawlecki contributed Appendix B, "Organic Extraction of Sediment Samples for Analysis of Butyltin Compounds," and Appendix C, "Analysis of Extracts for Butyltin Chloride Compounds by Gas Chromatography with Flame Photometric Detection (GC/FPD)".

Technology Planning and Management Corporation, Durham, North Carolina, 27713: David Strevel contributed the electronic file formatting requirements presented in Section 4.

USEPA, EMSL-Cincinnati, Cincinnati, Ohio, 45268: Brian Hill contributed Appendix D, "Benthic (Sediment) Metabolism".

SECTION 1
INTRODUCTION

1.1 BACKGROUND

1.1.1 The coordination activities being undertaken by EMAP-Methods include providing method format guidance, providing guidance for evaluating EMAP methods, and identifying and organizing EMAP methods in use. EMAP-Methods has the following objectives:

- To produce a standard method format, including written specifications for the style and content of EMAP methods and method manuals;
- To identify and organize the sampling and measurement methods being used by EMAP;
- To establish a full text methods database on the EPA Wide Area Information Server (WAIS) and a database of abstracted versions of methods in the EMAP Information Management System relational database; and
- To develop a method evaluation protocol suited to the requirements of EMAP, to ensure that the methods used by the Resource Groups are consistent and produce comparable data.

1.1.2 Method manuals produced and used by the Resource Groups are in different stages of development. Field and laboratory method manuals have been prepared for pilot, demonstration, and full implementation stages of EMAP. Resource Groups have prepared these methods and manuals in the style most familiar to them. EMAP-Methods has prepared this method format guidance document to meet the objective of providing standardization for method and method manual preparation within EMAP. The method format presented in this guidance, and required for use with EMAP methods, is the Environmental Monitoring Methods Council (EMMC) method format. Specifically, this format is required for use with field collection, field measurement, biological laboratory, and chemical laboratory methods.

1.1.3 To address the objective for the identification and organization of sampling and measurement methods, EMAP-Methods is identifying methods in use by the Resource Groups and is assembling the methods into a hardcopy notebook. As a part of this task, EMAP-Methods is reformatting method manuals, according to the guidance issued in this document, and assembling the methods into the notebook.

1.1.4 The database under development will become a part of the EMAP WAIS. It is intended to facilitate the coordination of methods issues between Resource Groups. Considerable effort has been expended by Resource Groups evaluating essentially identical methods. The database will provide method users a rapid way to determine if an existing method for a given parameter has been used within EMAP. It will provide a way of tracking the introduction of new methods into the program,

as well as method changes through time. Standard documentation is essential for entry and retrieval of methods into and from the database and therefore, certain format specifications provided in the "EMAP Methods Format Guidance" were defined specifically for compatibility with the methods database. These features are intended to assure compliance with the "Twenty Year Rule" that all data and associated metadata be retained and accessible for twenty years.

1.1.5 In addition to providing a standardized method documentation protocol, EMAP-Methods is responsible for developing a method evaluation protocol for the program. Methods used within the EMAP are typically selected from scientific literature or are newly developed. The method evaluation protocol under development will provide guidance to the Resource Groups for the validation and acceptance of methods obtained from various sources. The protocol is intended to assist the Resource Groups in determining if the methods used within EMAP are adequately documented and whether they meet the needs of the program. Because the disciplines within EMAP have different requirements, EMAP-Methods will provide a set of four evaluation protocols, one for each of the following types of methods: field collection, field measurement, biological laboratory, and chemical laboratory methods. Within each of these four evaluation guidance documents, the standard format for EMAP methods presented within this guidance will be addressed again with specific information pertinent to the targeted discipline.

1.2 METHODS FORMAT GUIDANCE

1.2.1 Measurements data used by EMAP are generated by following either field measurement methods or a combination of field collection and laboratory measurement methods. Each method will document how sample collection and/or analysis activities are to be performed. This guidance document provides a method format applicable to all EMAP methods, to facilitate consistency across groups. The specifications provided in this guidance document will be mandatory for use in preparing all EMAP methods and standard operating procedures (SOPs) subsequent to this guidance. This document provides only the minimum documentation requirements for EMAP methods and method manuals. It is not intended to provide guidance for the assessment of method validity.

1.2.2 This document contains guidance on the format and content of manuals of EMAP methods and is appended with examples of methods and standard operating procedures (SOPs), prepared in accordance with the required EMMC format. One method has been appended for each of the identified method types including a chemical laboratory method (with two associated SOPs), a field measurement method, a field collection method, and a biological laboratory method. These appendices have been provided to illustrate the applicability of the method format across the varied disciplines within EMAP. The SOPs associated with the chemical laboratory method are presented to depict the application of the EMMC format to the more narrow focus that is typical of SOPs. The methods and SOPs used as examples are not purported to be perfect or complete in content; they are merely presented to illustrate application of the EMMC method format across disciplines.

1.2.3 Within the guidance for method manual preparation, this document includes a section outlining electronic file formatting requirements. Compliance with the specifications provided is necessary for the successful loading and retrieval of methods documentation through the methods database. EMAP methods documents will be used via two different media. Some users will obtain and read copies of documents on paper; but an even larger number are expected to obtain electronic copies from EPA's on-line servers. The electronic file formatting requirements provided within this guidance have been developed during uploading and retrieval trials on the database under development. These requirements shall be applied to all new EMAP methods.

*************** End of Section 1 ***************

SECTION 2
ELEMENTS OF EMAP METHOD MANUALS

2.1 SUMMARY

2.1.1 Laboratory and field method manuals consist of three parts: 1) the Front Matter; 2) the Body; and 3) the End Matter. The following list illustrates the elements of the method manual in the order in which they should appear:

Elements	Part
• Cover page • Title page • Disclaimer • Foreword • Table of contents • Acknowledgments	FRONT MATTER
• Manual Introduction • Section Introduction • Methods	BODY OF MANUAL
• Glossary • Appendices	END MATTER

2.1.2 This section of the guidance document contains general manual formatting requirements and descriptions of the elements of EMAP method manuals. The front matter, introductions, and end matter merely provide textual continuity when methods are presented in the form of a manual. Therefore, information essential to the performance of a method must never be placed in them. The front matter of this guidance document provide examples of a cover page, title page, disclaimer, foreword, table of contents, and acknowledgments. The manual introduction immediately follows the front matter of a manual. Some resource groups may find it useful to additionally include introductions to groups of methods within the manual. For example, in a chemical methods manual, it may be helpful to have separate sections for inorganic and organic methods, each with its own introduction. Individual methods or sections of methods follow any applicable introduction(s). A method manual ends with a glossary and appendices, if these items are determined to be useful to the manual. The format and content of EMAP methods will be described in Section 3 of this document.

2.2 GENERAL MANUAL FORMATTING REQUIREMENTS

2.2.1 Page Numbering: Number the front matter pages consecutively with lower case Roman numerals. Center the numerals at the bottom of each page, except the cover and title pages. Leave the cover page unnumbered. The title page is counted as page "i" but is not indicated as such with a displayed numeral. Number the body of the manual and any attached appendices consecutively with Arabic numerals centered at the bottom of each page.

2.2.2 Document Control Information: Place one centered line of document control information at the top of each page of a manual, beginning with the first page of text following the table of contents. This information should resemble that shown at the top of this page. The methods appended to this document illustrate appropriate headers for methods in an EMAP manual. In this information header, each independent method must be numbered independently of other methods in the manual. The one-line header should contain the following information:

Resource or Coordination Group, Title, Section No., Month & Year, Page xx of xx

- Provide the name of the Resource Group or Coordination Group, abbreviating, if necessary, to restrict the length of the header to one line.

- Abbreviate the title of the manual within the header of introductory or end matter sections of the manual. Abbreviate the title of an individual method within the header for that method.

- Provide the manual section number, when the manual is divided into sections.

- Provide the month and year that the manual or method was approved.

- Display page numbering within each independent section or method as "Page xx of xx".

2.2.3 Section End: Place an "end of section/method" statement at the conclusion of each manual section or method to be entered into and retrieved from the database, as illustrated below.

*************** End of Section 6 ***************

-or-

************* End of Determination of Butyltin Compounds in Sediments *************

This statement will enable anyone accessing a method through the database to be certain they have retrieved the entire section or method as intended. Each independent section or method within an EMAP method manual should end with a phrase like those illustrated. The main text of this guidance illustrates placement of this phase at the end of each section. The methods and SOPs appended to this document illustrate placement of this phrase at the end of an entire procedure.

2.2.4 Font: Prepare methods and methods manuals in a proportional font, using the same font throughout the entire manual. The preferred font for all methods intended for inclusion in the electronic database is Arial or Times New Roman. If these options are unavailable to any Resource Group, an alternative proportional font should be chosen.

2.2.5 Margins: Define the left and right margins at 1 inch. Define the margins at the top and bottom of the page at 0.5 inch to accommodate the document control header information and page-bottom numbering, without reducing the room available for text on each page.

2.2.6 Line spacing: Space lines with 1 to 1.5 spaces. Line spacing at 1.5 is recommended when subscripts or superscripts are used frequently or any other time when readability is reduced with single spacing.

2.2.7 Headings: Use boldface type, capital letters, or a combination to make section headings and meaningful subheadings distinct from the rest of the text. Altering the font is not an acceptable means of achieving this distinction.

2.2.8 Columns: Present text in a single-column style for all methods and manuals.

2.3 EMAP METHOD MANUAL ELEMENT DESCRIPTIONS

2.3.1 Cover Page: Include the following information on the cover page of any method manual:

- Place the EPA report number and month and year of publication in the top right-hand corner of the page.

- Place the name and address of the issuing organization in the top left-hand corner of the page.

- Limit the title of the manual to ten words or less and place it in the top half of the page. Within the size limitation, Resource Groups may title method manuals as manuals, guides, or other title as preferred.

- Color code EMAP report covers, according to the originating Resource or Coordination Group. Internal reports may be printed with black and white covers, provided they are not distributed outside EPA. Cover pages for method manuals prepared for EMAP use should reflect the color guidelines listed in Table 1.

Table 1: Color Scheme for EMAP Reports

Resource and Coordination Group	Color	Pantone Number
Agroecosystems	Brown	463
Air and Climate	Dark grey	439
Rangelands	Red brown	153
Assessment and Reporting	Red	187
Design and Statistics	Orange	145
Estuaries and Coastal Waters	Blue green	329
Forests	Dark green	364
Great Lakes	Blue	314
Indicator Development	Dark red brown	498
Information Management	Dark blue	294
Landscape Ecology	Green	356
Landscape Characterization	Dark magenta	241
Methods Coordination	Grey green	568
Program Level	Purple	526
Quality Assurance Management	Red purple	2603
Surface Waters	Blue	540
Wetlands	Light green	399

2.3.2 Title Page: Include the following items on the title page:

- Place the EPA report number and the month and year of publication in the top right-hand corner of the page.
- Center the title of the manual in the top half of the page.
- Below the title, list the names of the authors, together with their organizational name and location (not including street address).
- Below the authors, list the names and organizations of manual editors and contributors, with their organizational name and location.
- For grant, contract, or interagency agreement, identify the Project Officer(s), the first organizational subdivision, and the laboratory name and location.
- When a public or private organization originates the work in cooperation with EPA, reflect that information several spaces below the Project Officer's name.
- Place the publisher's full name and address at the bottom of the page.

2.3.3 Disclaimer: Provide disclaimer statements on the page immediately following the title page. Place the disclaimer in the top half of the page. Final documents which contain any information unique to a company, laboratory, or individual, including the use of trade names, should contain a disclaimer such as the following:

> The mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Draft documents that may be released outside the Agency should contain a disclaimer statement as follows:

> This document is a preliminary draft. It has not been formally released by the U.S. Environmental Protection Agency and, at this stage, should not be construed to represent Agency policy. It is being circulated for comments on its technical merit and policy implications.

2.3.4 Foreword: Provide a foreword to the manual on the page following the disclaimer. The foreword should contain information regarding the reasons for undertaking the work described within the manual. The foreword will typically state the types of methods to be found in the particular manual, as well as their significance.

2.3.5 Table of Contents: Provide a table of contents on the next available right-hand page of text following the foreword. The title page and the disclaimer are numbered "i" and "ii" respectively, but these items should not be included in the table of contents. Every element thereafter should appear in the table of contents. The table of contents page(s) should be numbered but not included in the listing. The table should include each main heading and meaningful subheading of the document and the number of the first page on which it appears. Immediately following the table of contents, any lists of exhibits, illustrations, figures, or tables may be included if deemed useful. An acknowledgment page, if used, should be placed at the end of the document's front matter.

2.3.6 Acknowledgments: List any individuals or organizations whose acknowledgment is desired but who does not appear on the title page. Acknowledgments should be limited to peer reviewers and organizations that assisted in the manual's preparation in a substantive way.

2.3.7 Manual Introduction: Place the manual's introduction on the first page of the body of the manual (page 1). Like the foreword, the introduction to the manual should describe the purpose of the manual. Included in the introduction should be discussion on the background, objectives, scope, approach, and references for the manual.

2.3.8 Section Introduction: When manuals are divided into sections, begin each section with an introduction to the methods contained in that section.

2.3.9 Glossary: The glossary, when used, should be considered a partial dictionary where terms that might not be readily known can be listed and explained. List glossary entries alphabetically. Numbering is not required. Accepted definitions may be found in the EMAP Master Glossary:

EMAP. 1993. Environmental Monitoring and Assessment Program: Master Glossary. EPA/620/R-93/013, Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Assessment Program.

2.3.10 Appendices: Because each method is intended to be a free-standing document, appendices are generally not needed in method manuals. Information necessary for the performance of any method within the manual must not be included in an appendix, unless the information is included in both the appendix and the method.

*************** End of Section 2 ***************

SECTION 3
ELEMENTS OF EMAP METHODS

3.1 SUMMARY

3.1.1 Collection or measurement methods differ in the content and level of detail required depending upon the nature of the work to be performed, however, all elements described in the format must be addressed in each type of method used. For illustration of the applicability of the format to the four identified method types, refer to the appendices to this document.

3.1.2 Each EMAP method must be written in the EMMC format, containing the following sections, numbered as shown below and in the appendices to this document:

1. Scope and Application
2. Summary of Method
3. Definitions
4. Interferences
5. Personnel Health and Safety
6. Equipment and Supplies
7. Reagents and Standards
8. Sample Collection, Preservation, and Storage
9. Quality Control
10. Calibration and Standardization
11. Procedure
12. Data Analysis and Calculations
13. Method Performance
14. Pollution Prevention
15. Waste Management
16. References

3.2 GENERAL METHOD FORMATTING REQUIREMENTS

3.2.1 Method Sections: Each method within the manual must contain sections 1 through 16 as listed above, and tables, diagrams, etc. as warranted. The required format was originally prepared for analytical chemistry methods, and therefore, it is recognized that all 16 sections may not apply to all types of methods, however, each of the method sections should be represented in every method. Where a section does not apply to the procedure being described, list the section heading in the proper location, and place "Not Applicable or N/A" below.

3.2.2 Section Numbering: Section numbering is illustrated in the methods appended to this document. The main section headings (first-order) are listed above as they should be numbered. All headings, subheadings, discrete paragraphs, etc., will be numbered: e.g., first-order headings, 1.; second-order headings, 1.1, 1.2, etc.; third-order headings, 1.1.1, 1.1.2, etc.; on through fourth-order.

3.2.3 Indentation: Prepare the text of methods without indentation.

3.2.4 References: Each method must be a free-standing document, in which all information necessary for the method user to perform the method may be found. References within a method should be restricted to associated or source material. Procedural steps or instructions must not be referenced as being found elsewhere. They must be included, in total, within the method.

3.2.5 Tables, Diagrams, Flowcharts, Validation Data: Tables, diagrams, flowcharts, and validation data may be included in a method as a final section (Section 17), as an attachment to the method, or dispersed throughout the method as appropriate. Requirements for electronic file formatting of these items are described in Section 4 of this guidance.

3.3 EMAP METHOD ELEMENT DESCRIPTIONS

3.3.1 Scope and Application: In this section, describe the purpose of the method, with its intended use. The section should include lists of target analytes, organisms, attributes, Chemical Abstract Service Registry Numbers (CASRN) for chemical analytes, and sample matrices to which the method applies. Method sensitivity and Data Quality Objectives should also be addressed in this section.

Exhibit 1: Example of a "Scope and Application" section
from a chemical laboratory method (Appendix A).

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1. SCOPE AND APPLICATION

1.1 This method is for determination of part per billion concentrations of the following analytes in sediments:

Compound	CASRN
Butyltin Trichloride	1118-46-3
Dibutyltin Dichloride	683-18-1
Tributyltin Chloride	1461-22-09

Three butyltin compounds are detected and measured with gas chromatography with flame photometric detection (GC/FPD). Each sample is fortified with surrogate compounds before extraction, and reported analyte concentrations are corrected to reflect 100% recovery of the appropriate surrogate compound.

1.2 Method reporting limits (MRLs) of 5 ng/g for Dibutyltin and Tributyltin, and 12 ng/g for Butyltin were determined by analysis of seven replicates of a Canadian sediment spiked with those method analytes at concentrations of 25 ng/g.

3.3.2 Summary of Method: Provide a brief summary of the method. The purpose of the summary is to provide a succinct overview of the technique to aid the reviewer or data user in evaluating the method and the data. List sample volumes, extraction, digestion, concentration, and other preparation steps employed, measurement methods, analytical instrumentation and detection systems, and quantitative techniques.

Exhibit 2: Example of a "Summary of Method" section
from a field collection method (Appendix E).

2. SUMMARY OF METHOD

2.1 A 1/25 m^2, stainless steel, Young-modified Van Veen Grab sampler is used to collect sediments for benthic analyses. The sampler is constructed entirely of stainless steel and has been Kynar®-coated (similar to Teflon), and is therefore appropriate for collecting sediment samples for both biological and chemical analyses. The top of the sampler is hinged to allow for the removal of the top layer of sediment for chemical and toxicity analyses. This gear is relatively easy to operate and requires little specialized training.

2.2 Once a successful grab has been obtained, the sediment collected is processed according to the protocols described in Section 11 and summarized in Figure 1.

2.3 To minimize the possibility of biasing results from spatial heterogeneity of the sediments, benthic biology grabs are not collected consecutively, but rather interspersed among the chemistry/toxicity grabs. While a biology grab is being processed (sieved), grab samples are collected for chemistry/toxicity.

3.3.3 Definitions: List definitions of terms relevant to this method or those with which the reader may be unfamiliar in this section. For extensive lists of definitions, a glossary may be attached to the end of the method.

Exhibit 3: Example of a "Definitions" section
from a biological laboratory method (Appendix F).

3. DEFINITIONS

3.1 Paleolimnology -- The branch of limnology that deals with describing and interpreting lake histories by studying the information contained in lake sedimentary profiles.

3.2 Diatom -- Algae in the class Bacillariophyceae.

3.3 Frustule or valve -- Refers to the siliceous outer skeleton of the diatom cell (2 valves = a frustule), the characters of which are used in the identification of discreet taxa.

3.4 Digestion -- This procedure refers to the solubilization of organic material by strong acid oxidation.

[This section continues and is presented in full in Appendix F.]

3.3.4 Interferences: Describe any known or potential problems, such as sample or equipment contamination, instrument noise, weather conditions, etc., that may be encountered during the performance of the method. Describe procedures employed to avoid interferences or to reduce their effect on method performance.

Exhibit 4: Example of an "Interferences" section
from a biological laboratory method (Appendix F).

4. INTERFERENCES

4.1 Some times the presence of sand and silt in the sediment sample may interfere with the identification of diatoms. If this is a problem, the lab should use differential settling techniques.

4.2 In dating sediments, high metal concentrations can cause interference with the plating of polonium. This problem should be corrected by increasing the concentration of ascorbic acid.

4.3 Samples containing significant amounts of carbonate will tend to bubble and sputter on the initial addition of acid. In this case, acid addition should be immediately suspended until all evidence of a reaction has ceased.

3.3.5 Personnel Health and Safety: Describe special precautions needed to ensure personnel safety during the performance of the method. Safety issues discussed here should be limited to those that are method-specific and beyond the scope of routine field or laboratory practices. The section should include information regarding specific toxicity of target analytes or reagents.

Exhibit 5: Example of a "Personnel Health and Safety" section
from a field collection method (Appendix E).

5. PERSONNEL HEALTH AND SAFETY

5.1 All sediment grab samplers are dangerous pieces of equipment. Once the device is cocked, it could accidentally trip at any time. The operators must be careful not to place hands or fingers in a position where they could be damaged (or amputated) in the event that the device trips prematurely.

5.2 The sampler is a heavy piece of equipment (especially when full). The operators must take care when deploying or retrieving this gear under adverse weather conditions. A grab sampler swinging wildly at the end of a boom can be very dangerous.

3.3.6 Equipment and Supplies: List all nonconsumable supplies, instruments, or equipment needed to perform the method. Generic language should be used whenever possible; however, when specific equipment is necessary, this should be stated clearly.

Exhibit 6: Example of an "Equipment and Supplies" section from a field measurement method (Appendix D).

6. EQUIPMENT AND SUPPLIES

6.1 Ice chest for floating centrifuge tubes during incubation.

6.2 Floating rack or styrofoam board for holding centrifuge tubes during incubation.

6.3 Grab sampler for sediments.

6.4 50 mL, screw-top, centrifuge tubes.

6.5 YSI Model 58 Dissolved Oxygen (DO) meter with Model 5730 Stirring BOD probe.

6.6 Spare batteries for DO meter.

6.7 Permanent markers for labeling tubes.

6.8 Sample labels and field data sheets.

6.9 Zip-lock sandwich bags to contain replicates from each treatment and site.

6.10 Ice chest with dry ice for sample freezing.

3.3.7 Reagents and Standards: List all reagents and standards required to perform the method. Include preparation instructions and suggested suppliers, as appropriate.

Exhibit 7: Example of a "Reagents and Standards" section from a biological laboratory method (Appendix F).

7. REAGENTS AND STANDARDS

7.1 Nitric acid, hydrochloric acid, sulfuric acid, ascorbic acid, and glacial acetic acid.

7.2 Potassium hydroxide, hydrogen peroxide, acetic anhydride, barium sulpha

7.3 Po-209 yield tracer solution.

3.3.8 Sample Collection, Preservation, and Storage: Provide requirements and instructions for sample collection, preservation, shipment, and storage conditions in this section. When holding times are known, and their effects have been studied, specify them in this section, along with instructions for actions to be taken if holding times are exceeded.

Exhibit 8: Example of a "Sample Collection, Preservation, and Storage" section from a biological laboratory method (Appendix F).

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8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Sediment coring procedures:

8.1.1 Sediment cores from the lakes are taken with a modified K-B gravity corer. The corer is equipped with a modified trigger mechanism (Glew 1989) and adapted to hold a 60 cm long lexon tube with an internal diameter = 6.4 cm and an outer diameter = 6.9 cm. In addition to providing undisturbed cores, the tube also provides sufficient sediment material for sediment dating and for the study of algal and animal microfossils, and pollen. In addition to collect a single sediment core from each of the lakes, each year triplicate cores are taken from three lakes. An attempt should be made to collect sediment cores >45 cm in length, especially from productive lakes, so that background (pre-anthropogenic) conditions can be reached. The cores should be taken from the deep, central area of the lake, and preferably where the bottom is relatively flat. In large lakes, cores should preferably be taken from depths <35 m deep, as gravity coring from very deep areas is more problematic.

8.1.2 Before inserting the core tube in the corer, inspect the top edge of the tube for damage; it is important that the core tube has a smooth, undeformated edge to mate with the seal ring. Check the position of the seal ring in the corer. A small amount of petroleum jelly may be used to retain the seal ring and to ensure a good seal.

8.2 The corer should be lowered slowly in the water, allowing it to sink in the sediment by its own weight, while keeping tension on the rope. Once the corer has penetrated the sediment, a messenger is delivered down the coring line, which triggers the closing of the corer, and effectively seals the top of the core tube. Once triggered, the corer should be gently retrieved to just below the lakewater surface. Then, holding the corer upright by one hand, a sliding plug should be placed at the bottom of the core. When placing the plug into the core tube, the core tube should still be submerged (to avoid the loss of sediment), and so the crew member has to extend his\her arm into the water. The plug used here will be the same one that will be used to extrude the core. See Figure 7 for an illustration of this procedure. A stopper should be placed under the sliding plug to seal the core. The stopper is slightly bigger in size than the sliding plug, and will be removed before the core tube is mounted in the sectioner. While keeping the core tube upright in the boat or raft, the corer will be disengaged from the coring tube, and the top of the core tube is closed by another stopper. Always provide support for the stopper so the sediment does not fall out of the coring tube. In the boat the core should always be kept upright in a stand to keep the tubes vertical.

8.3 The Glew mini corer (Glew 1991) will be used in annually visited lakes and in lakes which are revisited after 4 years because only the top 1 cm samples have to be taken from these lakes. The mini corer operates in a similar fashion to the modified K-B corer, except that it uses a smaller diameter tube and it generally retrieves short cores.

[This section continues and is presented in full in Appendix F.]

3.3.9 Quality Control: Provide a comprehensive summary of the QC requirements of the method. Describe all procedures, samples, and their frequency, as required to fully document the quality of the method.

Exhibit 9: Example of a "Quality Control" section from a field measurement method (Appendix D).

9. QUALITY CONTROL

9.1 All field work conducted during this study will be done by personnel having prior experience with the designated methods, or under the direct supervision of experienced personnel.

9.2 Sediment metabolism will be sampled at each site on all site visits.

9.3 Quality assurance objectives:

9.3.1 Precision -- Precision of measurements is assured by carefully following the method protocols, including calibration of DO meter, electronic balances, and fluorescent microscopes.

9.3.2 Completeness -- Valid data are required from 90% of the sites visited.

9.3.3 Representativeness -- Sediment metabolism is being determined for depositional areas of streams. Replicate samples from these areas in a stream segment (defined by fish survey) are composited and mixed to provide a representative site sample.

9.4 Quality Control Procedures:

9.4.1 Field Operations -- All personnel involved in sediment metabolism determination are trained in the sample collection methods, test set-up and operation, and instrument calibration and use.

9.4.2 Laboratory Operations -- Quality control of non-microscopic samples will be ensured by adherence to protocols and proper use of calibrated instruments. QC of microscopic measures will be accomplished by proper selection of microscopic techniques and stain selection.

9.5 Documentation and Review: Sediment metabolism field data are recorded and checked for completeness and accuracy before leaving the site. Samples returned to the laboratory are logged in and their processing (e.g., mass determination, microbial counts) is tracked until the samples are completed. All data sheets are inspected for completeness, accuracy, and legibility before proceeding to the next sample. Raw data sheets are retained in a file, and the data is entered into an ASCII file.

3.3.10 Calibration and Standardization: Describe the initial method or analytical instrument calibration. Indicate calibration frequency requirements, performance specifications, and corrective actions to be taken when performance criteria are not met. Verification or continuing calibration procedures may be described here or in method section 11.0 (See subsection 3.3.11).

Exhibit 10: Example of a "Calibration and Standardization" section from a chemical laboratory method (Appendix A).

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10. CALIBRATION AND STANDARDIZATION

10.1 GC/FPD Operating Conditions

Column: DB-5
Carrier Gas: Helium
Column Head Pressure: 8-10 psi
Injector Temp: 210°C
Detector Temp: 350°C
Make-up Gas: Helium
Injection Volume: 2 μL

Temperature Program:
65°C initial temp, hold 0 min.
Ramp at 15°/min to 230°C, hold 5 min.
Total run time: 15 min.

10.2 GC/FPD Calibration - A three-point calibration is performed immediately before analyzing a sample set. During analysis of a sample set, calibration is verified every 10 analyses by analyzing the medium concentration calibration solution.

10.2.1 Response factor measurement -- Analyze a 2-μL aliquot of each of three calibration solutions and determine a RF for each analyte relative to the surrogate compound.

$RRF = (A_A \times C_S) \div (A_S \times C_A)$, where

RRF= relative response factor,
A_A = area of analyte peak,
C_S = concentration of surrogate compound,
A_S = area of surrogate compound peak, and
C_A = concentration of analyte.

[This section continues and is presented in full in Appendix A.]

3.3.11 Procedure: Present the specific instructions for using the method. Include all sample processing, instrumental analysis, or observation recording steps.

Exhibit 11: Example of a "Procedure" section
from a field collection method (Appendix E).

11. PROCEDURE

11.1 Protocol for obtaining sediment:

11.1.1 Wash and thoroughly rinse the inside of the grab sampler with seawater from the station being sampled.

11.1.2 Attach the sampler to the end of the winch cable with a shackle and **tighten the pin**. An auxiliary link is also installed to provide added assurance against loss of the equipment. Attach a pinger to the grab.

11.1.3 Attach one set of weights to the sampler. These can be removed, or additional weights added depending on the sediment type. The grab is then cocked.

11.1.4 Lower the grab sampler through the water column such that travel through the last 5 meters is no faster than 1 m/sec. This minimizes the effects of bow wave disturbance to surficial sediments.

11.1.5 Retrieve the sampler and lower it into its cradle on-board. Open the hinged top and determine whether the sample is successful or not. A successful grab is one having relatively level, intact sediment over the entire area of the grab, and a sediment depth at the center of at least 7 centimeters. Grabs containing no sediments, partially filled grabs, or grabs with shelly substrates or grossly slumped surfaces are unacceptable. Grabs completely filled to the top, where the sediment is in direct contact with the hinged top, are also unacceptable. It may take several attempts using different amounts of weight to obtain the first acceptable sample. The more weight added, the deeper the bite of the grab. In very soft mud, pads may be needed to prevent the sampler from sinking in the mud. If pads are used, the rate of descent near the bottom should be slowed even further to reduce the bow wave. Note that weights and pads may be combined to optimize sample collection.

11.1.6 Carefully drain overlying water from the grab.

11.1.7 Enter notes on the condition of the sample into the computer and on the data sheet. Options on smell, texture, etc. are available via menus.

11.1.8 Process the grab sample for either benthic community analysis or chemistry/toxicity testing as described in subsections 11.2 and 11.3.

[This section continues and is presented in full in Appendix E.]

3.3.12 Data Analysis and Calculations: Provide instructions for qualitative and quantitative data analysis. As required by the procedure, include analyte or organism identification criteria, equations, and definitions of constants necessary to calculate results.

Exhibit 12: Example of a "Data Analysis and Calculations" section from a chemical laboratory method (Appendix A).

12. DATA ANALYSIS AND CALCULATIONS

12.1 Analyte Identification - Target analytes are identified by comparison of retention time data obtained by analysis of a blank or sample extract with retention time data from analysis of standards. For identification, a sample extract component peak must be within the appropriate retention time window established for the column. The analytical software automatically identifies analyte peaks and calculates their concentrations based on integrated peak area counts and the appropriate RF established during calibration.

12.2 Each measured analyte concentration corrected for surrogate internal standard recovery is calculated by the equation:

$C_a = (A_a \times M_{sc} \times F \times V_f) \div (A_{sc} \times RRF \times M_s \times V_i)$; where,

Ca = target analyte concentration (ng/g) in dry sample
A_a = area of target analyte peak
A_{sc} = area of surrogate compound peak
M_{sc} = mass (ng) of surrogate compound added to the dry sample
RRF = mean response factor from initial calibration
M_s = dry mass (g) of extracted sample aliquot
F = dilution factor, if needed
V_f = final extract volume (µL)
V_i = volume (µL) injected

12.3 The measured concentration of the surrogate compound, tetrabutyltin, is calculated by the equation:

$C_{sc} = (A_{sc} \times F \times V_f) \div (RF_{sc} \times M_s \times V_i)$; where,

C_{sc} = measured concentration of surrogate compound in extract
A_{sc} = area of surrogate compound peak
F = dilution factor, if needed
RF_{sc} = mean area units per nanogram of surrogate compound from initial calibration
M_s = dry mass (g) of extracted sample aliquot
V_f = final extract volume (µL)
V_i = volume (µL) injected

12.4 When measured concentrations of sample extract components exceed the upper limit of the calibration standards, the extract is diluted and analyzed after dilution. Concentration data from the initial analysis are reported for compounds within the calibration range before extract dilution; concentration data from the second analysis are reported for compounds exceeding the calibration range during initial analysis.

3.3.13 Method Performance: Provide method performance data for the method. Include precision and bias statements, detection limits, and limitations of the method.

Exhibit 13: Example of a "Method Performance" section from a biological laboratory method (Appendix F).

13. METHOD PERFORMANCE

13.1 As with past EPA funded PIRLA-II and EMAP-SW research, all efforts will be made to keep abreast with ecologically valid, up-to-date methods of diatom models. Ordination techniques for ecological analyses have been designed (ter Braak 1987) and described within the context of other ordination techniques (Jongman et al. 1987). In addition, there are now a number of computer intensive methods (e.g. Monte Carlo simulation, bootstrapping, etc.) which will be utilized to develop reliable error estimates for inferences (Birks et al. 1990a,b). Time to time experts will be consulted to review experimental design and assist with data analysis as a prudent measure to ensure appropriate state-of-the-art analyses. QA of the environmental data (e.g. chemistry, map information) must also be rigorous.

13.2 Sediment dating:

13.2.1 Duplicate analyses are performed regularly. For example, an estimate of the precision for a recent work, where the average coefficient of variation for 11 sets of duplicates is computed, shows the average coefficient of variation was 3.2%. This is roughly equal to the uncertainty introduced by the counting statistics. Usually 100s to a few thousands counts of polonium are collected. For 1000 counts, the uncertainty is 3%.

13.2.2 When calculating absolute accuracy, it is important to point out that some uncertainty may be associated with the use of a standard solution which has an uncertainty of about 5%. However this error does not contribute to uncertainty in the analysis of sediment accumulation rates or dates because the relative values are used in these calculations rather than the absolute values.

13.2.3 Accuracy of the analyses: In each batch of samples two standard reference materials are analyzed for Pb-210; certified by the NBS (National Bureau Standards) and CANMET (Canada Mineral Energy and Technology) in roundrobin interlaboratory comparisons. This allows to evaluate the accuracy of each set of samples. For example, the value (0.62 + 0.045) obtained for one standard (CANMET CLV-1) recently was close to the accepted value of 0.66 + 0.023, indicating that the results are consistent with those of other laboratories.

13.2.4 Lower limit of detection:

13.2.4.1 The lower limit of detection is set by process and detector backgrounds. In all samples that have been analyzed to date, the Pb-210 activity in sediments is at least 10 times the lower detection limit.

13.2.4.2 With each set of samples, process backgrounds are analyzed, and detector backgrounds are measured at least once a month. A figure showing the long-term stability in our backgrounds is presented below [Not included in this exhibit]. The process backgrounds are very rarely greater than the counter background. To calculate the lower limit of detection the background for a two day count is calculated and the definition of LLD is set as three times the background standard deviation. The average values of counting efficiency and radiochemical yield are used. The LLD for various detectors used in the lab range from 0.001 to 0.005 Bq/g. Pb-210 concentrations in sediments are usually 1.0 to 0.02 Bq/g.

3.3.14 Pollution Prevention: Describe practices employed to minimize or prevent pollution that may be attributable to the method. The most recent revision of the EMMC format has added this section to the method format. The methods used for illustration throughout this guidance have not yet been revised to adequately present the information needed in this section. Therefore, only guidelines are provided for consideration when preparing the pollution prevention section of a method. Although these guidelines, taken from Method 350.1 (EPA/600/R-93/100), were written specifically for chemical laboratory methods, they may be generalized across disciplines whenever pollutants (chemicals, solvents, fuels, etc.) are used or generated during the performance of a collection or measurement method.

- Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

- The quantity of chemicals and reagents purchased should be based on expected use during shelf lives and on disposal costs of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

- For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction," available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036.

3.3.15 Waste Management: Describe proper disposal of waste and samples specific to the method. The most recent revision of the EMMC format has added this section to the method format. The methods used for illustration throughout this guidance have not yet been revised to adequately present the information needed in this section. Therefore, only guidelines are provided for consideration when preparing the waste management section of a method. Although these guidelines, taken from Method 350.1 (EPA/600/R-93/100), were written specifically for chemical laboratory methods, they may be generalized across disciplines whenever wastes are generated during the performance of a collection or measurement method.

- The U.S. Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations.

- Excess reagents, samples, and method process wastes should be characterized and disposed of in an acceptable manner. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, by complying with the letter and spirit of any waste discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

- For further information on waste management, consult the "Waste Management Manual for Laboratory Personnel," available from the American Chemical Society at the address listed in subsection 3.3.14.

3.3.16 References: List source documents or publications in this section.

Exhibit 14: Example of a "References" section
from a field measurement method (Appendix D).

16. REFERENCES

Anderson, J.P.E., R.A. Armstrong, and S.N. Smith. 1981. Methods to evaluate pesticide damage to the biomass of the soil microflora. Soil Biology and Biochemistry 13:149-153.

Barkay, T. and H. Pritchard. 1988. Adaptation of aquatic microbial communities to pollutant stress. Microbiological Sciences 5:165-169.

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*************** End of Section 3 ***************

SECTION 4
ELECTRONIC FILE FORMATTING REQUIREMENTS

4.1 SUMMARY

EMAP manuals will be loaded in their entirety on to the EPA Wide Area Information Server (WAIS) and made available over the Internet. This section of the guidance presents the requirements for electronic file arrangement and formatting for all of the manual elements. The requirements for the creation of electronic files presented here are necessary to permit loading, retrieval, and viewing of methods documentation, while minimizing incompatibilities and editorial effort. Due to requirements of the WAIS, file size must be controlled. This requires that a manual be divided into several files, following the guidelines presented in subsection 4.2.

4.2 ELECTRONIC FILE FORMATTING REQUIREMENTS

4.2.1 Word Processing: Prepare all methods documentation using WordPerfect for DOS. The documents of concern to EMAP-Methods will be created using the EPA Agency standard, WordPerfect for DOS, version 5.0 or 5.1. WordPerfect for Windows may become an acceptable format in the future. However, the standard word processing tool must currently be WordPerfect for DOS, because, while some EPA personnel now have WordPerfect for Windows available to them, most do not. Documents authored with WordPerfect for Windows cannot be retrieved, viewed, or printed by users of WordPerfect for DOS. Documents authored with WordPerfect for DOS, however, can be viewed on-screen or printed by users operating either WordPerfect for DOS or WordPerfect for Windows.

4.2.2 File Size: Organize each method document into files of a size that users will find convenient for electronic searching and retrieval over EPA's network or the Internet. This can best be accomplished by limiting the size of each file. Extremely large files can be difficult and time consuming to retrieve. EMAP-Methods recommends that a document stored in a single electronic file be no longer than 100 pages. Additionally, individual electronic files should be no larger than 500,000 bytes. The electronic file size limitation is needed because documents with fewer pages, but containing a lot of graphics, will become more difficult to manage as the electronic file size is increased.

4.2.3 File Organization: When preparing a method that will be stored on EMAP's electronic servers, place the text and all associated graphics, tables, equations, and other "special" WordPerfect formats in a single DOS or Unix file. Users retrieving methods through the network should be able to retrieve all of the information associated with a single method by retrieving a single file. Arranging a method into multiple files is not an acceptable way to prepare the document for electronic storage. The servers in use cannot maintain multi-file associations while storing and transmitting the documents back to users. When preparing a manual of methods for storage on the electronic servers, the entire manual should be organized into a series of files of appropriate size. Sections of methods may be combined into a single file, as long as this can be achieved within the specified file size limitation.

4.2.4 Front Matter Organization: Place all of the front matter elements of a manual in one file; however, do not include the graphic cover page for electronic publication. Provide the remainder of the front matter in a single file with the title page as the first page in the electronic version of the document.

4.2.5 File Naming Convention: A simple file naming convention has been developed during the process of loading initial methods documents into the database. This convention has been developed because the file names given to elements of a manual are of concern to the editor organizing the document for loading to the electronic servers. Additionally, these DOS or Unix filenames are visible to users retrieving EMAP's electronic documents. Consistency in the arrangement and naming of these electronic files will enable both editors and method users to identify file contents with ease. The following list outlines the recommended file naming convention.

- Name the file containing all of the manual's front matter "FRONT.WP."
- Name the introduction to the manual, if applicable, "INTROD.WP."
- Organize individual methods or sections of methods into DOS files named in sequential order, such as "SECT01.WP, SECT02.WP. SECT03.WP," etc.
- When the body of the manual must be divided into sections, each containing multiple files, assign names such as "SECT0101.WP, SECT0102.WP," etc.
- Organize appendices into DOS files named in sequential order, such as "APPNDXA.WP, APPNDXB.WP, APPNDXC.WP," etc.
- If a glossary is a part of the document, name the glossary file "GLOSS.WP". Name a references section "REF.WP"; and an acronyms section, "ACRONYMS.WP."

The filename extension "WP" has been used to indicate that the document was created in WordPerfect for DOS. When the use of WordPerfect for Windows becomes an option, the extension will be "WDP". These extensions are important for document retrieval to the user's PC or workstation. Some client retrieval packages trigger the calling of "viewer" software based on the filename extension. Files with extensions of "WP" will be sent to a WordPerfect for DOS viewer, while those with an extension of "WDP" will be sent to WordPerfect for Windows.

4.3 Electronic File Formatting Illustration: Exhibit 15 has been provided to illustrate the use of the file size, organization, and naming requirements described in subsection 4.2. This exhibit contains a comprehensive listing of the electronic files that comprise the "Handbook of Laboratory Methods for Forest Health Monitoring." This listing contains the name of each electronic file, a description of its contents, and its file size. As shown in the exhibit, the document and file size limitations presented in subsection 4.2 can normally be easily achieved within a method manual.

Exhibit 15: Example of the contents of an electronically submitted laboratory method manual

Filename	File Content	Document Size (pages)	File Size (bytes)
FRONT.WP	Front Matter	6	10415
INTROD.WP	Manual Introduction	2	26953
SECT0101.WP	Introduction to Section 1	7	38100
SECT0102.WP	Sample Receipt and Tracking	6	26319
SECT0103.WP	Mineral Sample Processing and Rock Fragment Determination	9	56746
SECT0104.WP	Bulk Density Determination from Core Samples	5	33168
SECT0105.WP	Organic Soil Processing and Biomass Determination	10	46980
SECT0106.WP	Sample Batching, Shipping, and Archiving	8	39024
SECT0201.WP	Introduction to Section 2	17	119690
SECT0202.WP	Air-to-Oven Dry Moisture Content	6	52290
SECT0203.WP	Particle Size Analysis	9	62737
SECT0204.WP	Electrical Conductivity	7	59402
SECT0205.WP	pH	9	71490
SECT0206.WP	Cation Exchange Capacity	14	103174
SECT0207.WP	Exchangeable Cations	16	112449
SECT0208.WP	Exchangeable Acidity and Aluminum	15	104981
SECT0209.WP	Mineralizable Nitrogen	10	74370
SECT0210.WP	Extractable Phosphorous by Bray 1	8	72096
SECT0211.WP	Extractable Sulfate	11	89399
SECT0212.WP	Total Carbon and Nitrogen	9	42925
SECT0213.WP	Total Sulfur	7	37557
SECT0214.WP	Total Elemental Content of Forest Floor Samples	2	9333
SECT0301.WP	Introduction to Section 3	6	25449
SECT0302.WP	Receipt and Tracking of Foliar Samples	6	37073
SECT0303.WP	Drying, Comminution, and Homogenization of Foliar Samples	5	17715
SECT0304.WP	Sample Batching, Shipping, and Archiving	6	51282
SECT0401.WP	Introduction to Section 4	16	122342

SECT0402.WP	Methods for the Elemental Analysis of Foliar Tissue and Forest Floor Litter Materials	7	33759
SECT0403.WP	Mercury Analysis in Foliage and Forest Floor Litter by Cold Vapor ICP	7	33633
SECT0404.WP	Methods for Total Arsenic by Vapor Generalization ICP for Forest Floor Litter Material	8	35742
SECT0405.WP	Methods for Total Nitrogen Analysis of Foliar and Forest Floor Litter	6	19571
SECT0501.WP	Introduction to Section 5	6	24221
SECT0502.WP	Receipt and Tracking of Lichen Samples	7	54600
SECT0503.WP	Weighing, Drying, Batching, and Shipping for Dendrochronological Examination	6	31253
SECT0504.WP	Dendrochronological Measurements of Stemwood	7	38876
SECT0505.WP	Sample Batching, Shipping, and Archiving for Elemental Analysis	7	44152
SECT0601.WP	Introduction to Section 6	6	24765
SECT0602.WP	Receipt and Tracking of Lichen Samples	5	28282
SECT0603.WP	Taxonomic Identification of Lichen Communities	6	38850
SECT0604.WP	Lichen Sample Cleaning, Batching, Shipping, and Archiving for Elemental Analysis	7	57446
SECT0701.WP	Introduction to Section 7	16	107841
SECT0702.WP	Methods for the ICP-MS Elemental Analysis of Stemwood and Lichen Samples	14	60924
SECT0801.WP	Introduction to Section 8	1	4339
SECT0802.WP	Root Pathogens and Mycorrhizae Laboratory	6	19515

********** End of Section 4 **********

APPENDICES

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APPENDIX A: Example of a Chemical Laboratory Method

DETERMINATION OF BUTYLTIN COMPOUNDS IN SEDIMENTS

1. SCOPE AND APPLICATION

1.1 This method is for determination of part per billion concentrations of the following analytes in sediments:

Compound	CASRN
Butyltin Trichloride	1118-46-3
Dibutyltin Dichloride	683-18-1
Tributyltin Chloride	1461-22-09

Three butyltin compounds are detected and measured with gas chromatography with flame photometric detection (GC/FPD). Each sample is fortified with surrogate compounds before extraction, and reported analyte concentrations are corrected to reflect 100% recovery of the appropriate surrogate compound.

1.2 Method reporting limits (MRLs) of 5 ng/g for Dibutyltin and Tributyltin, and 12 ng/g for Butyltin were determined by analysis of seven replicates of a Canadian sediment spiked with those method analytes at concentrations of 25 ng/g.

2. SUMMARY OF METHOD

2.1 A dried 20-g aliquot of homogenized sediment sample is serially extracted with 0.2% tropolone in methylene chloride on a roller mill. The extract is concentrated by Kuderna-Danish (K-D) evaporation and solvent exchanged to hexane. The extract is then hexylated with Grignard reagent; afterward, the excess Grignard reagent is neutralized with hydrochloric acid. The hexylated extract is then dried and concentrated. Finally, a silica/alumina clean-up column is used to remove interferences.

2.2 With GC/FPD, butyltin compounds are identified by comparing GC retention times of sample components to those for standards. FPD peak areas are used to measure compound concentrations.

3. DEFINITIONS

3.1 Surrogate compound(s) -- A known quantity of one or more pure compounds, which are extremely unlikely to be found in any sample, is added to a sample aliquot before extraction and is measured with the same procedures used to measure sample components. The purpose of a surrogate compound is to monitor method performance with each sample. With this method, measured sample component concentrations are corrected to reflect 100% recovery of the appropriate surrogate compound.

3.2 Laboratory reagent blank (LRB) -- 20-g of clean sand that is spiked with surrogate compounds and extracted and analyzed with a set of samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or analytical system.

3.3 Laboratory fortified sample matrix (LFM) -- An aliquot of an environmental sample to which known quantities of method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. Background concentrations of analytes in the sample are determined in a separate aliquot, and measured LFM values are corrected for background concentrations.

3.4 Stock standard solution -- A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.5 Primary dilution standard solution -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.6 Calibration standard -- A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. Calibration solutions are used to calibrate the instrument response with respect to analyte concentration.

3.7 Standard reference material (SRM) -- A sample containing a known amount of some or all of the analytes of interest as determined by a consensus of results from a multilaboratory study; NIST marine sediment PACS-1 is used for this method.

4. INTERFERENCES

4.1 Solvents, reagents, glassware, and other sample processing hardware may produce contamination artifacts and/or elevated baselines that interfere with interpretation of chromatograms. Purification of solvents by distillation in all-glass systems may be necessary if reagent blanks are contaminated; high-purity, distilled-in-glass solvents are commercially available.

4.2 Phthalates are common laboratory contaminants that are used widely as plasticizers in plastic labware and have been found as contaminants in sodium sulfate. Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. Because PTFE can absorb certain compounds, it must be carefully rinsed with appropriate solvents.

4.3 Because non-analyte sample components coextracted from samples can interfere with identification and measurement of analytes, sediment sample extracts are subjected to cleanup procedures to minimize these interferences.

5. PERSONNEL HEALTH AND SAFETY

5.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined. However, each chemical compound should be treated as a potential health hazard, and exposure should be minimized. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. These procedures for the safe handling of chemicals should be made available to and followed by all personnel involved in these analyses. Additional information on laboratory safety can be found in the three references listed in Section 16.

6. EQUIPMENT AND SUPPLIES

6.1 125 mL PTFE screw-cap bottles.

6.2 Glass conical funnels.

6.3 Kuderna-Danish (K-D) apparatus:

6.3.1 Concentrator tube - 10-mL, graduated (Kontes K-570050-1025 or equivalent) with a ground glass stopper (19/22 joint).

6.3.2 Evaporation flask - 500-mL (Kontes K-570050-0500 or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012).

6.3.3 Snyder column - three-ball macro (Kontes K-503000-0232 or equivalent).

6.3.4 PTFE boiling chips - extracted with $MeCl_2$ and heated at 450°C for at least 1 hour.

6.4 Buchner funnels.

6.5 125 mL filter flasks.

6.6 Water bath - heated, with concentric ring cover, capable of temperature control (±2°C), installed in a fume hood.

6.7 Sample vials - amber glass, 2-mL with PTFE-lined screw caps; 2-mL crimp-top autosampler vials.

6.8 Analytical balance - capable of weighing 0.1 mg.

6.9 Nitrogen evaporation device - Equipped with a water bath that can be maintained at 35-40°C. The N-Evap (Model #111) by Organomation Associates, Inc., is suitable.

6.10 Balance - capable of weighing 100 g to the nearest 0.01 g.

6.11 Disposable Pasteur pipets - sealed with aluminum foil and annealed at 450°C for several hours; rinsed with solvents before use.

6.12 Drying oven.

6.13 Annealing oven - capable of reaching 450°C.

6.14 Chromatography column - borosilicate glass with PTFE stopcock, 300 mm X 10 mm I.D.

6.15 Syringes - 10-µL and 100-µL for preparation of standards.

6.16 Centrifuge tubes - 50 mL with PTFE-lined screw caps.

6.17 Ceramic burundums.

6.18 Roller mill.

6.19 Gas chromatograph - equipped with a DB-5 column and a flame photometric detector. The GC should have temperature programming capability and a splitless injection port for capillary column.

6.19.1 DB-5 Column - 30 m x 0.25 mm I.D., 25 µm coating, 5% phenyl, 95% methyl polysiloxane fused silica capillary column.

6.19.2 Data system - Nelson Intelligent Interface, Series 760. Nelson Analytical software, Version 5.1, is used to acquire and store data, identify peaks, and measure peak height/areas.

7. REAGENTS AND STANDARDS

7.1 Reagents:

7.1.1 n-hexane and $MeCl_2$ (pesticide quality, distilled-in-glass).

7.1.2 Silica gel - 100-200 mesh, Aldrich #21,447-7, Grade 923.

7.1.3 Hydrochloric acid - concentrated, make 6N HCl with reagent water.

7.1.4 Alumina oxide - standard grade, 150 mesh.

7.1.5 Sodium sulfate - reagent grade, granular anhydrous, rinsed with $MeCl_2$ (20 mL/g) and conditioned at 450°C for at least one hour.

7.1.6 Tropolone - 0.2% solution with methylene chloride.

7.1.7 Hexylmagnesium bromide - 0.5 M in diethyl ether - Grignard reagent, purchase in solution.

7.1.8 Filter paper - 4.25 cm.

7.2 Stock standard solutions - Prepare from materials of known purity and composition, or purchase as solutions or mixtures with certification of purity, concentration, and authenticity. If the compound purity is ≥96%, the uncorrected weight can be used to calculate the concentration of the standard. Store standards in the dark at -20°C to -10°C in screw-capped vials with PTFE-lined lids.

7.2.1 Prepare a 5000 µg/mL stock of each of the three target compounds and the surrogate, tetrabutyltin, by dissolving 0.050 g neat material in hexane and diluting to the mark in a 10 mL volumetric flask. Transfer each solution to a vial with a PTFE-lined screw cap and seal cap with PTFE tape. Store at 4°C. when not in use.

7.2.2 Stock standard solutions should be replaced after 1 year, or whenever comparison with quality control check samples indicates a change in concentration.

7.3 Primary dilution standards:

7.3.1 Butyltin primary dilution standard - Using a syringe, add a volume of 500 µL each of the butyltin trichloride, dibutyltin dichloride, and tributyltin chloride stock standard solutions to approximately 7 mL hexane in a 10 mL volumetric flask. Dilute to the mark with hexane. This solution contains each target analyte at a concentration of 250 µg/mL.

7.3.2 Surrogate primary dilution standard - Add a volume of 500 µL tetrabutyltin stock standard solution to approximately 7 mL hexane in a 10 mL volumetric flask. Dilute to the mark with hexane. This solution contains tetrabutyltin at a concentration of 250 µg/mL.

7.4 Working standards:

7.4.1 Surrogate spike solution - To a 50-mL volumetric flask containing about 30 mL of hexane, add 5.0 mL of surrogate compound stock solution. Dilute to the mark with hexane. The resulting solution contains 25 µg/mL of tetrabutyltin.

7.4.2 Matrix spike solution - To a 50-mL volumetric flask containing about 30 mL of hexane, add 5.0 mL each of the three target compound stock standard solutions. Dilute to the mark with hexane. The resulting solution contains each analyte at a concentration of about 25 µg/mL.

7.5 GC/FPD calibration solutions:

7.5.1 For instrumental analysis, 200 µL of each of the two primary dilution standards are combined and diluted to a final volume of 1.0 mL in hexane. This standard must be taken through the Grignard reaction, column clean-up and concentration steps of the extraction procedure. The resulting 1.0 mL standard will be used as the high concentration calibration standard at a concentration of 50 µg/mL.

7.5.2 Prepare the medium concentration calibration standard at a concentration of 10 µg/mL by diluting 100 µL of the high concentration standard to a final volume of 500 µL in hexane.

7.5.3 Prepare the low level calibration standard at a concentration of 5 µg/mL by diluting 50 µL of the high level standard to a final volume of 500 µL in hexane.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Sediment samples should be stored in the dark and frozen at -20°C until extraction. Appropriate holding times have not been established for frozen sediments, but guidelines generally agreed upon are 6 to 12 months for samples held at -20°C.

8.2 Analysis of extracts within 40 days of preparation is desirable.

9. QUALITY CONTROL

9.1 A laboratory reagent blank (LRB) is analyzed with each batch of ≤20 samples. If the measured concentration of an analyte or potentially interfering compound is ≥MRL, analyses must be halted until the source of the contamination is identified and eliminated; associated samples should be re-extracted.

9.2 Duplicate aliquots of a selected sample are fortified with known amounts of all target analytes and analyzed with each set. An acceptable measured concentration is 50%-120% of the fortified concentration. The relative percent difference (RPD) between duplicates should be ≤30%. When percent recovery is <50%, the RPD can be used to assess consistency; if RPD is acceptable, the sample set is considered to be acceptable. When both recovery and RPD are outside limits, sample set acceptance is judgmental.

9.3 Surrogate compound recovery - Acceptable surrogate compound recovery is 30% -150% of fortified concentration. When surrogate recoveries are outside these limits, the sample should be reinjected; if recoveries are still outside limits, the sample should be re-extracted.

9.4 Standard reference material (SRM) Analysis - With each sample set, an aliquot of PACS-1 is extracted and analyzed along with samples. The measured concentration of each component is calculated, and each measured concentration is corrected for surrogate compound recovery. The goal is corrected values that are ±30% of accepted "true" values, but one analyte may fail to meet this criterion if mean recovery of all analytes is ±30%. Because some component results may consistently differ from the accepted SRM values; control charts are maintained to indicate acceptable performance (i.e., within laboratory control limits) on a continuing basis. If SRM results are not acceptable, associated samples are re-extracted.

10. CALIBRATION AND STANDARDIZATION

10.1 GC/FPD operating conditions:

Column: DB-5
Carrier Gas: Helium
Column Head Pressure: 8-10 psi
Injector Temp: 210°C
Detector Temp: 350°C
Make-up Gas: Helium
Injection Volume: 2 µL

Temperature Program:
65°C initial temp, hold 0 min.
Ramp at 15°/min to 230°C, hold 5 min.
Total run time: 15 min.

10.2 GC/FPD calibration - A three-point calibration is performed immediately before analyzing a sample set. During analysis of a sample set, calibration is verified every 10 analyses by analyzing the medium concentration calibration solution.

10.2.1 Response factor measurement -- Analyze a 2-µL aliquot of each of three calibration solutions and determine a RF for each analyte relative to the surrogate compound.

$$RRF = (A_A \times C_S) \div (A_S \times C_A)$$, where

RRF = relative response factor,
A_A = area of analyte peak,
C_S = concentration of surrogate compound,
A_S = area of surrogate compound peak, and
C_A = concentration of analyte.

10.2.2 Calculate the mean RRF and RSD for each analyte. If RSD is >15%, recalibration is necessary.

10.2.3 For the surrogate compound, calculate the mean detector response in terms of peak area per quantity injected. This term is used to calculate the surrogate compound recovery in each sample.

$$RF = A_S \div C_S$$

10.2.4 Retention time windows (RTWs):

10.2.4.1 The RTW is the retention time interval (in minutes) assigned to each target analyte and surrogate compound; it provides the basis for automated peak identification with the analytical software. Each RTW must be wide enough to reliably identify each target analyte present in a sample at or above the detection limit but narrow enough to discriminate between the analyte and closely eluting interferences. Calibration standards are used to establish RTWs empirically.

10.2.4.2 For each analyte, calculate the mean RT and the associated SD from data obtained by analyzing at least three calibration solutions throughout a 72 hour period. Calculate each RTW as the range of the mean RT $\pm$3 SD.

10.2.4.3 The RTWs must be recalculated if analytical software fails to identify and measure a target analyte in a check standard, a new GC column is installed, a column is shortened during routine maintenance, or column head pressure is adjusted.

11. PROCEDURE

11.1 Sediment sample extraction and concentration:

11.1.1 Remove any water that has separated from the sediment. Remove non-representative material (e.g., twigs, leaves, shells, rocks, and any material larger than 1/4 in.). Mix by stirring.

11.1.2 Determine percent moisture - Weigh about 5 g of wet sediment to the nearest 1 mg in a tared disposable aluminum dish. Distribute sample on the dish to maximize surface area. Dry sample overnight in 105°C oven. Weigh dry sediment and calculate percent moisture.

$$\%\ \text{Moisture} = [\ (M_w - M_d) \div M_w\ ] \times 100$$

where, M_w is wet sample weight and M_d is sample weight after drying.

11.1.3 Dried sediment to be used in extraction - Determine the wet weight that is equivalent to 20 g dry weight with the following equation:

$$\text{Wet Weight} = 20\ \text{grams} \div [\ 1 - (\%\ \text{Moisture}/100)\ ]$$

Weigh this amount of wet sediment into a PTFE bottle. Place bottle in hood and dry the sediment overnight at room temperature.

11.1.4 Tropolone/methylene chloride extraction:

11.1.4.1 Add 1 mL of the surrogate spiking solution to each 20 g sediment sample, 10 g SRM (PACS-1) sample, QC sample, and LRB. Add 1 mL matrix spiking solution to each matrix spike sample.

11.1.4.2 Add about five burundums and 50 mL 0.2% tropolone in methylene chloride to each PTFE bottle.

11.1.4.3 Roll bottle on roller mill for 3 hours.

11.1.4.4 Filter the sample through a Buchner funnel lined with filter paper, collecting solvent in a K-D flask. Save solvent. Return sediment and filter paper to the PTFE bottle.

11.1.4.5 For each sample, repeat steps described in subsections 11.1.4.2-11.1.4.4, collecting solvent in original flask with saved solvent.

11.1.5 K-D evaporation and solvent exchange:

11.1.5.1 Add PTFE boiling chips to K-D flask. Pre-wet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus in a hot water bath, at 65-75°C, so that the concentrator tube is partially immersed in the hot water. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood.

11.1.5.2 Concentrate the extract to approximately 10 mL. Add 30 mL n-hexane and evaporate to 5 mL.

11.1.5.3 Transfer the contents of the concentrator tube to a PTFE-capped 50 mL centrifuge tube. Add an additional 20 mL hexane.

11.1.6 Grignard reaction:

11.1.6.1 Add 4 mL of Grignard reagent (hexylmagnesium bromide) to the hexane extract. Place the tube in a water bath at 70°C and allow reaction to continue for 4 hours.

11.1.6.2 Remove the tube from the bath and allow the reaction mixture to cool. Neutralize the excess Grignard reagent by adding 5 mL of 6 M. hydrochloric acid. Be careful to add acid slowly; this is an exothermic reaction.

11.1.6.3 Shake sample vigorously and allow phases to separate.

11.1.6.4 Stopper a glass conical funnel with glass wool and add approximately 5 g sodium sulfate. Place funnel over a K-D flask. Dry organic phase through the sodium sulfate by transferring with a Pasteur pipet into funnel and collecting eluate in flask.

11.1.6.5 Add 15 mL of 3:1 hexane:methylene chloride to the aqueous phase, shake vigorously, and allow the phases to separate.

11.1.6.6 Dry the organic phase through the sodium sulfate funnel as in subsection 11.1.6.4, collecting eluate in the original K-D flask.

11.1.6.7 Repeat steps described in subsections 11.1.6.5 and 11.1.6.6 once more.

11.1.6.8 Attach Snyder column to K-D flask, and concentrate to approximately 5 mL.

11.1.7 Silica/alumina clean-up and final extract preparation:

11.1.7.1 Prepare clean-up column by adding approximately 5 g of silica gel to chromatographic column. Add approximately 5 g of alumina oxide. Wash the column with 15 mL hexane and discard solvent.

11.1.7.2 Transfer sample to the column, collecting the eluate in a K-D flask. Wash the column with an additional 50 mL hexane, collecting in the K-D flask.

11.1.7.3 Attach Snyder column and concentrate the extract to a volume of 5-10 mL.

11.1.7.4 Remove the K-D concentrator tube from the water bath and place it in the N-EVAP concentrator. Reduce extract at ambient temperature with a gentle stream of nitrogen to a final volume of 1.0 mL.

11.2 GC/FPD determination of butyltin compounds:

11.2.1 Preparation of sample set - Collect calibration standards and extracts. This set consists of a 1.0-mL concentrated extract of each of the following: one aliquot of SRM (PACS-1), a laboratory reagent blank, two spiked aliquots of a selected sample, and $\leq$15 sediment samples.

11.2.2 Set up GC operating conditions as described in subsection 10.1. Enter appropriate method parameters and RTWs, as described in subsection 10.2.4, into the GC software according to manufacturer instructions. Proceed to inject extracts.

11.2.3 An injection sequence is analyzed in the following order: three calibration standards, LRB, SRM, sample, spike, spike duplicate, samples. Calibration check standards are analyzed after every tenth injection.

11.2.4 Calibration check standards - Analyte concentrations are measured using procedures described in subsection 12.1. Percent recovery (calculated concentration divided by known concentration) should be 75%-125% for each analyte. If not, instrument recalibration may be required.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Analyte identification - Target analytes are identified by comparison of retention time data obtained by analysis of a blank or sample extract with retention time data from analysis of standards. For identification, a sample extract component peak must be within the appropriate retention time window established for the column. The analytical software automatically identifies analyte peaks and calculates their concentrations based on integrated peak area counts and the appropriate RF established during calibration.

12.2 Each measured analyte concentration corrected for surrogate internal standard recovery is calculated by the equation:

$$C_a = (A_a \times M_{sc} \times F \times V_f) \div (A_{sc} \times RRF \times M_s \times V_i)$$; where,

Ca = target analyte concentration (ng/g) in dry sample
A_a = area of target analyte peak
A_{sc} = area of surrogate compound peak
M_{sc} = mass (ng) of surrogate compound added to the dry sample
RRF = mean response factor from initial calibration
M_s = dry mass (g) of extracted sample aliquot
F = dilution factor, if needed
V_f = final extract volume (µL)
V_i = volume (µL) injected

12.3 The measured concentration of the surrogate compound, tetrabutyltin, is calculated by the equation:

$$C_{sc} = (A_{sc} \times F \times V_f) \div (RF_{sc} \times M_s \times V_i)$$; where,

C_{sc} = measured concentration of surrogate compound in extract
A_{sc} = area of surrogate compound peak
F = dilution factor, if needed
RF_{sc} = mean area units per nanogram of surrogate compound from initial calibration
M_s = dry mass (g) of extracted sample aliquot
V_f = final extract volume (µL)
V_i = volume (µL) injected

12.4 When measured concentrations of sample extract components exceed the upper limit of the calibration standards, the extract is diluted and analyzed after dilution. Concentration data from the initial analysis are reported for compounds within the calibration range before extract dilution; concentration data from the second analysis are reported for compounds exceeding the calibration range during initial analysis.

13. METHOD PERFORMANCE

Data acquired for components of PACS-1(sediment):

ANALYTE	Mean[a] Meas. Conc.	SD	NIST Conc.	% Bias
Butyltin Trichloride	3.97	1.12	2.8	-29%
Dibutyltin Dichloride	6.64	1.53	11.6	-43%
Tributyltin Chloride	9.51	2.08	12.7	-25%

[a] n= 12

14. POLLUTION PREVENTION

The Chemical Hygiene Plan for U.S. EPA Laboratory Operations should be consulted for chemical handling, clean-up, and decontamination procedures. References for procedures to follow for disposal of chemicals are listed in Section 15.

15. WASTE MANAGEMENT

Personnel should follow procedures for chemical disposal outlined in the EPA-Cincinnati Safety Office's "Waste Packaging Guidelines" and "Laboratory Waste Management Guidelines".

16. SELECTED REFERENCES

Carcinogens - Working with Carcinogens, Dept. of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health Publication #77-206 (August 1977).

OSHA Safety and Health Standards, General Industry, OSHA 2206, 29 CFR 1910 (revised January 1976).

Safety in Academic Chemistry Laboratories, American Chemical Society Publications, Committee on Chemical Safety, 3rd Edition (1979).

********** End of Determination of Butyltin Compounds in Sediments **********

APPENDIX B: Extraction SOP Associated with the Chemical Laboratory Method

ORGANIC EXTRACTION OF SEDIMENT SAMPLES FOR ANALYSIS OF BUTYLTIN COMPOUNDS

1. SCOPE AND APPLICATION

1.1 This method describes the extraction of butyltin compounds from sediment samples. The following compounds are extracted with this procedure:

Compound	CASRN	MRL
Butyltin Trichloride	1118-46-3	12 ng/g
Dibutyltin Dichloride	683-18-1	5 ng/g
Tributyltin Chloride	1461-22-09	5 ng/g
Tetrabutyltin	1461-25-2	(Surrogate)

1.2 This method applies to the extraction of sediment for analysis of butyltins by gas chromatography with flame photometric detection (GC/FPD).

1.3 This procedure also applies to the preparation of butyltin standards for use in the extraction of marine sediment samples and for use in the instrumental analysis of the resultant extracts.

2. SUMMARY OF METHOD

2.1 A 20 gram equivalent dry sediment sample is serially extracted with 0.2% tropolone in methylene chloride on a roller mill. The extract is concentrated by Kuderna-Danish (K-D) evaporation and solvent exchanged to hexane. The extract is then hexylated with Grignard reagent; afterward, the Grignard reagent is neutralized with hydrochloric acid. The hexylated extract is then dried and concentrated. Finally, a silica/alumina clean-up column is used to remove interferences.

2.2 This procedure provides the instructions for preparing standards for use in instrumental analysis and extractions. Stock standards are prepared from neat materials. Primary dilution standards are diluted from the stocks. Calibration and spiking standards are prepared from the primary dilution standards. Stock, primary dilution, and calibration standards are prepared by chemists performing instrumental analysis. Surrogate and matrix spiking standards are prepared by extraction personnel.

3. DEFINITIONS

3.1 Stock standard: A concentrated solution containing a single analyte, which is used to prepare primary dilution standards.

3.2 Primary dilution standard: A solution of one to several analytes prepared from stock standard solutions, used to prepare calibration and spiking standards.

3.3 Calibration standard: A solution prepared from primary dilution standards which is used to calibrate the instrument response.

3.4 Surrogate spike standard: A standard containing all of the surrogate standard compounds for analysis. All samples and QC samples are spiked with this standard prior to extraction.

3.5 Matrix spike standard: A standard containing all target analytes, which is spiked into a matrix spike sample and duplicate prior to extraction.

3.6 Laboratory reagent blank (LRB): A sand blank, which is spiked with surrogate and then carried through the procedure with a sample set.

3.7 Matrix spike sample/ duplicate (LF1, LF2): Duplicate aliquots of a selected sediment sample which are spiked with the surrogate spike standard and matrix spike standard, then processed through the method with the original sample.

3.8 Certified reference material (CRM): An analytical performance test sample (NIST Marine Sediment PACS-1).

4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to false positive analyte detection on the GC/FPD.

4.2 Glassware cleaning to minimize contaminant interferences: Clean glassware by water washing with detergent and rinsing with tap, followed by D.I. water. Solvent rinse, in order, with acetone, methylene chloride, and hexane. Do not solvent rinse Teflon bottles - dry them at 105°C for 0.5-1 hour.

5. PERSONNEL HEALTH AND SAFETY

5.1 Butyltin Chlorides are corrosive, therefore appropriate protective gloves and lab coats must be worn to prevent the possibility of skin contact.

6. EQUIPMENT AND SUPPLIES

6.1 125 mL Teflon bottles (Nalgene).

6.2 Ceramic burundums.

6.3 Kuderna-Danish apparatus:

6.3.1 10 mL concentrator tubes, graduated.

6.3.2 3-ball snyder column.

6.3.3 500 mL evaporative flask.

6.4 Chromatographic column with Teflon stopcock, 300 mm x 10 mm I.D.

6.5 250 mL filter flask.

6.6 Buchner funnel.

6.7 Centrifuge tubes, 50 mL with Teflon-lined screw caps.

6.8 Glass conical funnels.

6.9 Water bath - capable of heating to 70°C.

6.10 Nitrogen evaporation device - N-EVAP.

6.11 Roller mill.

6.12 Drying oven.

6.13 Volumetric flasks - 10 mL and 100 mL.

6.14 Syringes - 100, 500, and 100 µL.

7. REAGENTS AND STANDARDS

7.1 Anhydrous sodium sulfate.

7.2 Tropolone.

7.3 Methylene chloride, pesticide grade.

7.4 Hexane, pesticide grade.

7.5 6 N. Hydrochloric acid.

7.6 Hexylmagnesium bromide - 0.5 M. in diethyl ether - Grignard reagent, purchased in solution.
7.7 Alumina oxide, standard grade, 150 mesh.

7.8 Silica gel - Grade 923, 100-200 mesh.

7.9 Glass wool.

7.10 Disposable glass Pasteur pipets - 1 mL.

7.11 Teflon boiling chips.

7.12 1.5 mL glass vials with Teflon-lined caps.

7.13 Aluminum foil.

7.14 Filter paper - 4.25 cm.

7.15 Tetrabutyltin, standard grade.

7.16 Tributyltin chloride, standard grade.

7.17 Dibutyltin dichloride, standard grade.

7.18 Monobutyltin trichloride, standard grade.

7.19 Standard solution preparation:

7.19.1 Preparation of all standards: When preparing stock standards from neat materials, begin by taring a volumetric flask. Weigh the specified amount of neat material into the volumetric flask. Add solvent to volume. When preparing standards as dilutions, begin by adding solvent to the volumetric flask. Add specified volume of stock standard or standard solution. Add solvent to volume.

7.19.2 Preparation of stock standards: Prepare individual butyltin stocks, one each of target analytes and tetrabutyltin, the surrogate standard, at a concentration of 5000 µg/mL by adding 0.050 gram neat material to hexane in a 10 mL volumetric flask.

7.19.3 Preparation of primary dilution standards:

7.19.3.1 Butyltin primary dilution standard: Prepare the primary dilution standard containing tributyltin chloride, dibutyltin dichloride, and monobutyltin trichloride, each at a concentration of 250 µg/mL in hexane, by adding 0.5 mL of each of the stock standards to a 10 mL final volume.

7.19.3.2 Surrogate standard primary dilution standard: Prepare the primary dilution standard containing tetrabutyltin at a concentration of 250 µg/mL in hexane by adding 0.5 mL of the stock standard to 10 mL.

7.19.4 Preparation of surrogate spike: Prepare the spiking solution of tetrabutyltin at a concentration of ~25 µg/mL in hexane by adding 5.0 mL of standard prepared in subsection 7.19.3.2 to a final volume of 50 mL.

7.19.5 Preparation of the matrix spike: Prepare the matrix spiking solution at a concentration of ~25 µg/mL in hexane by adding 5.0 mL of standard prepared in subsection 7.19.3.1 to a final volume of 50 mL.

7.19.6 Preparation of calibration standards (for use during the analysis of sample extracts according to SOP No. OR-006):

7.19.6.1 For instrumental analysis, 200 µL of each of the primary dilution standards are combined to a final volume of 1.0 mL in hexane. This standard must then be taken through the Grignard Reaction, column clean-up and concentration steps described in subsections 11.4 and 11.5. The resulting 1.0 mL standard will be used as the high level calibration standard at a concentration of ~50 µg/mL.

7.19.6.2 Prepare the mid level calibration standard at a concentration of ~10 µg/mL by diluting 100 µL of the high level standard to a final volume of 500 µL in n-hexane.

7.19.6.3 Prepare the low level calibration standard at a concentration of ~5 µg/mL by diluting 50 µL of the high level standard to a final volume of 500 µL in n-hexane.

8. SAMPLE COLLECTION, PRESERVATION, and STORAGE

8.1 Sediment samples are stored frozen until thawed for extraction. Immediately after an extraction aliquot is removed from original storage container, the sediment is refrozen. The holding time from sample collection to extraction for the frozen sediments is one year.

8.2 Extracts are stored in Teflon-lined screw-capped amber vials at 4°C until analysis. The extract holding time, from extraction to analysis, is 40 days.

8.3 All standards are stored in Teflon-lined screw-capped amber vials at 4°C. Stock standards are replaced after one year and primary dilution standards are replaced every six months. Calibration, surrogate spike, and matrix spike standards are replaced monthly.

9. QUALITY CONTROL

9.1 The extraction, Grignard reaction, cleanup, and concentration steps described may be conducted collectively for up to 15 samples plus quality control (QC) samples, which together comprise a sample set. Attachment A [Not included in this Appendix] provides a flowchart of the procedure. Processing a sample set through this procedure will require two laboratory associates (chemists and/or chemical technicians) and should be completed within three days. Ideally, each major, sequential step in this procedure should be accomplished for all samples within a set before proceeding to the next step. With each sample set, the following QC samples must be processed through the extraction and cleanup procedures:

1 Laboratory reagent blank
1 Standard Reference Material
(NIST Marine Sediment PACS-1)
1 Matrix spike sample (LF1)
1 Matrix spike duplicate sample (LF2)

9.2 Standards must be verified for accuracy before use. New standards are compared to the old standard by comparison of the response or area when the two standards are analyzed together.
9.2.1 Standards must agree within 25%.

9.2.2 Standards that fail validation are reanalyzed against the old standard. If criteria fail a second time, an EPA reference standard must be analyzed, and the two standards compared to the reference. If the new standard fails it must be remade. If the old standard fails, it must be immediately discarded.

9.2.3 Spiking standards are quantitated against the GC calibration curves for validation. Quantitated values must agree within 25% for the spike to be used for extractions.

9.2.4 When criteria for validation are met, the new standard replaces the old standard, which is then discarded.

9.2.5 Standards must be compared to EPA reference materials annually. If criteria are not met new standards must be prepared.

9.3 Documentation procedures:

9.3.1 Any problems which may occur during analysis must be documented on a nonconformance memo and necessary corrective action applied.

9.3.2 A daily record of extraction and cleanup activities must be kept in a bound laboratory notebook. The record should document the analyst, the date, a narrative of the procedures, sample IDs (expressed as Lab IDs), reagent control numbers of solvents and reagents, masses of samples used in the extraction. Include any observations or occurrences that may bear upon analytical performance (e.g. extract color).

9.3.3 Using notebook entries, complete the Extraction Summary form for each completed sample set. Immediately notify the Group Leader that the form is ready for review. Make two photocopies of the reviewed form, attaching one to the lefthand page in the bound notebook opposite the page containing the last data entry for the sample set. Store the second copy in the QC sample log. The original will be retained in the sample set's data package/report.

9.3.4 Percent moisture and wet weight determinations are documented on a Percent Moisture Determination form. This form must be reviewed by the Group Leader upon completion, prior to the start of the extraction of a sample set. Keep one copy of this form in the Percent Moisture notebook, which is stored in the extraction lab, Room 451. The original form should accompany the extraction summary original.

9.3.5 Preparation of stock standards is recorded on the Organic Standards Preparation Form.

9.3.5.1 Standards Prep Forms are maintained in loose-leaf notebooks in the extraction laboratories and the instrument labs.

9.3.5.2 Each standard is assigned a unique number, starting with the room number of the lab the notebook is stored in, followed by the next available number in the logbook.

9.3.6 Preparation of primary dilution, calibration, and spiking standards are recorded on the Organic Standards Dilution Form.

9.3.6.1 Standard dilution forms are stored and named in the same manner as the stock standards. In the case of multi-level standards, each level will share the same identification number followed by a suffix- high, mid, low, or single letter identifiers.

9.3.7 Standard validation is recorded on the Standard Validation Form.

9.3.7.1 Original standard validation forms are stored in loose-leaf notebooks in the laboratory where the validation was performed. The raw instrument data is filed behind the form.

10. CALIBRATION AND STANDARDIZATION

N/A

11. PROCEDURE

11.1 Sample preparation:

11.1.1 Determine percent moisture of sediment - Weigh about 5 grams of wet sediment to the nearest mg. in a tared disposable aluminum dish. Distribute sample on the dish to maximize surface area. Dry sample overnight in drying oven at 105°C. Reweigh. Calculate percent moisture.

11.1.2 Weigh wet sample equivalent to 20 grams dry sediment into 125 mL Teflon bottle. Add surrogate internal standard and matrix spiking solution when appropriate.

11.2 Extraction with tropolone/methylene chloride:

11.2.1 Add 50 mL 0.2% tropolone in methylene chloride to Teflon bottle containing sediment. Add 4-6 burundums.

11.2.2 Roll bottle on roller mill for 3 hours.

11.2.3 Filter the sample through a Buchner funnel, collecting solvent in K-D flask. Save solvent. Return the sediment and filter paper to the Teflon bottle.

11.2.4 Repeat the steps described in subsections 11.2.1 through 11.2.3, collecting solvent in original flask, with saved solvent.

11.3 K-D Evaporation and solvent exchange:

11.3.1 Add Teflon boiling chips to K-D flask. Pre-wet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus in a hot water bath, at 65-75°C, so that the concentrator tube is partially immersed in the hot water. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood.

11.3.2 Concentrate the extract to approximately 10 mL. Add 30 mL hexane and evaporate to 5 mL.

11.3.3 Transfer the contents of the concentrator tube to a Teflon-capped 50 mL centrifuge tube. Add an additional 20 mL hexane.

11.4 Grignard reaction:

11.4.1 Add 4 mL of hexylmagnesium bromide (Grignard reagent) to the hexane extract. Place the tube in a water bath at 70°C and allow reaction to continue for 4 hours.

11.4.2 Remove the tube from the bath and allow the reaction mixture to cool. Neutralize the excess Grignard reagent by adding 5 mL. of 6 M. hydrochloric acid. Be careful to add acid slowly - this is an exothermic reaction.

11.4.3 Shake sample vigorously and allow phases to separate.

11.4.4 Stopper a glass conical funnel with glass wool and add approximately 5 grams sodium sulfate. Place funnel over K-D flask. Dry organic phase through the sodium sulfate by transferring with a Pasteur pipet into funnel and collecting eluate in K-D flask.

11.4.5 Add 15 mLs of 3:1 hexane:methylene chloride to the aqueous phase, shake vigorously, and allow the phases to separate.

11.4.6 Dry the organic phase through the sodium sulfate funnel as in subsection 11.4.4, collecting eluate in K-D flask.

11.4.7 Repeat the steps described in subsections 11.4.5 and 11.4.6.

11.4.8 Attach Snyder column to K-D flask, and concentrate to approximately 5 mL.

11.5 Clean-up and preparation of final extract:

11.5.1 Prepare clean-up column, add approximately 5 grams of silica gel to chromatographic column. Add approximately 5 grams of aluminum oxide. Wash the column with 15 mL hexane and discard solvent.

11.5.2 Pour sample through the column, collecting eluate in a K-D flask. Wash the column with an additional 50 mL hexane poured through the column.

11.5.3 Attach Snyder column and concentrate to the volume of 5-10 mLs. Blow down extract under nitrogen to a final volume of 1 mL.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Percent moisture:

$$\% \text{ Moisture} = [(M_w - M_d) \div M_w] \times 100$$

where, M_w is the sediment wet weight, and M_d is the weight after drying.

12.2 Wet weight determination:

$$\text{Wet Weight} = 20 \text{ grams} \div [1 - (\% \text{ Moisture}/100)]$$

13. METHOD PERFORMANCE

Data acquired for components of PACS-1(sediment):

ANALYTE	Mean[a] Meas. Conc.	SD	NIST Conc.	% Bias
Butyltin Trichloride	3.97	1.12	2.8	-29%
Dibutyltin Dichloride	6.64	1.53	11.6	-43%
Tributyltin Chloride	9.51	2.08	12.7	-25%

[a] n= 12

14. POLLUTION PREVENTION

The Chemical Hygiene Plan for U.S. EPA Laboratory Operations should be consulted for chemical handling, clean-up, and decontamination procedures. References for procedures to follow for disposal of chemicals are listed in Section 15.

15. WASTE MANAGEMENT

Personnel should follow procedures for chemical disposal outlined in the EPA-Cincinnati Safety Office's "Waste Packaging Guidelines" and "Laboratory Waste Management Guidelines".

16. REFERENCES

Detection of Butyltin Chloride Compounds in Sediments Using High Resolution Gas Chromatography and Flame Photometric Detection, Dick Carr, QARD, EMSL Cincinnati, Cincinnati, OH.

SOP No. OR-006, Determination of Butyltin Compounds in Sediment by Gas Chromatography with Flame Photometric Detection (GC/FPD), Anne Pawlecki, Technology Applications, Inc., Cincinnati, OH.

SOP No. OR-011, Organic Glassware Cleaning, Dave Craven, Technology Applications, Inc., Cincinnati, OH.

*************** End of SOP No. OR-005-00 ***************

APPENDIX C: Analytical SOP Associated with the Chemical Laboratory Method

ANALYSIS OF EXTRACTS FOR BUTYLTIN CHLORIDE COMPOUNDS BY GAS CHROMATOGRAPHY WITH FLAME PHOTOMETRIC DETECTION

1. SCOPE AND APPLICATION

1.1 This SOP describes the analytical conditions and procedures used for gas chromatographic detection and quantitation of butyltin compounds. Also included are guidelines for data evaluation, quality assurance criteria, and procedures for the reporting of data.

1.2 This procedure applies to the following compounds:

Compound	CASRN	MRL
Butyltin Trichloride	1118-46-3	12 ng/g
Dibutyltin Dichloride	683-18-1	5 ng/g
Tributyltin Chloride	1461-22-09	5 ng/g
Tetrabutyltin	1461-25-2	(Surrogate)

2. SUMMARY OF METHOD

2.1 This SOP provides the analytical conditions and procedures for chromatographic separation detection and measurement of Butyltin compounds in hexane extracts, prepared in accordance with SOP No. OR-005. Detection is performed using a gas chromatograph equipped with a flame photometric detector (GC/FPD). Data are collected and stored with Nelson Analytical Software, and the data are processed with Public Domain Software.

3. DEFINITIONS

3.1 Certified reference material (CRM): An analytical performance test sample (NIST Marine Sediment PACS-1), with known concentrations, etc.

3.2 Laboratory reagent blank (LRB): A sand blank which is spiked with surrogate standard, extracted, and analyzed with a sample set.

3.3 Matrix spike sample and matrix spike duplicate (LF1 and LF2): Two aliquots of a selected sediment sample which are spiked with surrogate standard and matrix spike standard, then extracted, and analyzed with a sample set.

3.4 Surrogate internal standard (SIS): A standard which is spiked into all samples, LRBs, matrix spike samples, CRMs, prior to extraction. In analyzing extracts, the SIS response is used for an internal standard calibration and sample quantitation.

4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware. These interferences could lead to false positive results in GC/FPD detection.

4.2 Glassware cleaning to minimize contaminant interferences: Clean glassware by water washing with detergent and rinsing with tap, followed by D.I. water. Solvent rinse, in order, with acetone, methylene chloride, and hexane.

5. PERSONNEL HEALTH AND SAFETY

5.1 Butyltin Chloride compounds are corrosive - therefore, appropriate protective gloves and clothing must be worn when handling samples or standards to prevent skin contact.

6. EQUIPMENT AND SUPPLIES

6.1 Gas chromatograph: Varian Model 3700 GC, equipped with a flame photometric detector.

6.1.1 Column: DB-5 fused silica capillary column (30 m x 0.32 mm I.D., 0.25 µm film thickness), J&W Scientific.

6.2 Data system:

6.2.1 Nelson Intelligent Interface.

6.2.2 IBM compatible personal computer

6.3 Software:

6.3.1 Nelson Analytical Software, Version 5.1 is used to acquire and store data, for peak identification and for peak quantitation.

6.3.2 Public Domain Software, Version 11 is used for sample quantitation and report generation.

6.4 Syringes: 10 µL appropriate for use in performing manual injections onto the GC.

7. REAGENTS AND STANDARDS

7.1 Butyltin calibration standards at a minimum of three concentration levels, at 5, 10, and 50 µg/mL, prepared in accordance with SOP No. OR-005.

7.2 Hexane, pesticide grade.

8. SAMPLE COLLECTION, PRESERVATION, and STORAGE

8.1 Sample extracts are stored in screw-cap vials, sealed with Teflon tape, at 4°C. The extract holding time, from extraction to analysis, is 40 days.

8.2 All standards are stored in Teflon-lined screw-capped amber vials at 4°C. Stock standards must be replaced every year, and primary dilution standards every six months. Calibration standards are replaced monthly.

9. QUALITY CONTROL

9.1 This SOP has defined types of QC samples and parameters (Section 3). This section defines the quality figures derived from the QC samples, control/acceptance criteria and the procedures by which control must be demonstrated and tracked. A set of extracts consists of 1.0 mL concentrates of each sediment sample and each of the following QC samples:

a) certified reference material (1)
b) marine/estuarine sediment samples (15 or fewer)
c) laboratory reagent blank (1)
d) matrix spike sample (1)
e) matrix spike duplicate (1)

9.2 Laboratory reagents blanks (LRBs): LRBs are analyzed to assure that all reagents, glassware, and analytical systems are free from contamination. Each sample set will include an LRB extraction. LRB concentrations of analytes of interest must be less than 3 times the MDL established for the analysis. When concentrations are above this level, analysis must be halted until the source of the contamination is identified and eliminated. The sample set involved should be re-extracted.

9.3 Matrix spike and matrix spike duplicate samples (LF1 and LF2): With each sample set, one sample is selected for spiking. Two aliquots of the sample are spiked with known amounts of all target analytes to assess method performance on the sample matrix.

9.3.1 The percent recovery limit for all spiked compounds is that recovery must be greater than 50% of the spiked amount.

9.3.2 The limit for relative percent difference (RPD) between the duplicates is 30%.

9.3.3 When percent recoveries are outside limits, RPD may still be used to assess consistency. If RPD is within limits, the sample set will pass. When both recoveries and RPD's are outside limits, passing or rejecting a sample set is at the discretion of the analyst and the group leader.

9.4 Certified reference material (CRM): With each sample set, one sample matrix certified reference material is extracted and analyzed.

9.4.1 Individual analyte recovery limits are to be +/- 35% of certified and non-certified values for target analytes present in the CRM.

9.4.2 Average recovery of the analytes should be +/- 30%.

9.4.3 Control charts are maintained for CRM recoveries. For analytes that are consistently outside recovery limits, analytical control must be demonstrated and maintained.

9.4.4 Sample sets whose CRM recoveries do not meet the above stated criteria are to be re-extracted.

9.5 Surrogate internal standard recoveries: Surrogate internal standards which are spiked into a sample prior to extraction must meet recovery limits of 30 - 150%. Samples with recoveries outside this limit must first be re-injected. When re-injection confirms that recovery is outside limits, the sample must be re-extracted.

9.6 Documentation procedures:

9.6.1 Any problems which may occur during analysis must be documented on a nonconformance memo and necessary corrective action applied.

9.6.2 Each instrument must have an injection log in which all instrumental runs are documented. Included in the log are the analyst's name, the date and the time of each injection, the sample name, the data file name, the injection volume and the standards used for calibration.

9.6.3 Each instrument must have an instrument maintenance log. The log must document preventative maintenance on the instrument, repairs made, gas cylinder changes, the analyst performing the maintenance and the date.

9.6.4 Data are compiled into a package for review by the Group Leader in accordance with SOP No. OR-009.

10. CALIBRATION AND STANDARDIZATION

10.1 GC/FPD operating conditions:

Column: DB-5
Carrier Gas: He
Column Head Pressure: 8-10 psi
Injector Temp.: 210°C
Detector Temp.: 350°C
Make-up Gas: Helium
Injection Volume: 2 µL

Temp. Program: 65°C initial temp., hold 0 min.,
Ramp at 15° C/min. to 230°C., hold 5 min.
Total Run Time: 5 min.

10.2 A three point (minimum) calibration must be generated prior to the analysis of samples. During subsequent analyses, the calibration must be verified by running periodic calibration check standards at a rate of one following every tenth injection. An internal standard (IS) calibration technique referenced to the response of the surrogate internal standard (SIS) is employed for all measurements. The initial calibration standards and the calibration check standards also serve as a benchmark for determination of appropriate retention time windows.

10.3 Process calibration standards through the instrumental procedure in the injection sequence as follows:

High level standard
Mid level standard
Low level standard

10.4 Calculate the relative response factor (RRF) for each analyte at each concentration level using the equation below:

$$RRF = (A_S \times C_{SIS}) \div (C_S \times A_{SIS})$$

where,

A_S = area counts of the target analyte
A_{SIS} = area counts of the surrogate internal standard
C_S = concentration of the target analyte (ng/mL)
C_{SIS} = concentration of the surrogate internal standard (ng/mL)

10.5 Use the RRF's calculated at each standard concentration to compare the average, the standard deviation (SD) and percent relative standard deviation (% RSD) for each analyte.

10.5.1 A % RSD $\leq$ 15% is the criterion for linearity of FPD response within the calibration range of each analyte. A % RSD that is greater than 25% for any target analyte will necessitate recalibration.

10.6 Calculate the absolute response factor (RF) for the surrogate internal standard, Tetrabutyltin, at each concentration level using the equation below:

$$RF = A_{SC} \div C_{SC}$$

10.7 Determine the average RF for the surrogate internal standard. This term will be used to calculate the absolute surrogate internal standard recovery in each sample. Criterion for absolute surrogate recovery is specified in subsection 9.5.

10.8 Retention time windows (RTWs):

10.8.1 The retention time window is the retention time interval (in minutes) assigned to each target analyte (and internal standard) within the analytical software which provides the basis for automated peak identification. The RTW must be wide enough to reliably (without exception) identify a target analyte that is present in the sample at, or above, the detection limit, yet narrow enough to discriminate between the analyte and closely eluting interferences. Calibration standards are used to provide an empirical basis for selecting the RTW.

10.8.2 Calculate the mean retention time and the standard deviation in retention time for each analyte from among a minimum of 3 calibration standards analyzed throughout a 72 hour period.

10.8.3 The RTW is taken to be the width of 3 standard deviations above and below the mean retention time.

10.8.4 RTWs must be recalculated when any one of the following conditions occur:

a) Failure of analytical software to identify and measure a target analyte in a check standard.
b) Installation of a new GC column or when column ends are cut as part of routine maintenance.
c) Following adjustment to column head pressure.

11. PROCEDURE

11.1 Preparation of extracts for GC injection:

11.1.1 Collect sample set extracts for butyltin analysis including up to 15 sample extracts and required QC sample extracts.

11.1.2 Collect calibration standards itemized in subsection 10.3.

11.2 GC analysis:

11.2.1 Start up the FPD (Refer to manufacturer's instrument installation manual for lighting the detector). Turn on the inlet pressure and air. Slowly turn on the hydrogen flow while simultaneously hitting the ignitor switch. Check for moisture coming out of the detector chimney to ensure that the detector is lit. Once lit, turn on the make-up Helium gas and the power to the FPD detector.

NOTE: To turn the detector off after a run: Turn off the hydrogen flow. Turn off the inlet pressure, air, and make-up gas. Turn off the power to the FPD last. Leave the splitter flow in the "off" position.

11.2.2 Enter appropriate method parameters and RTWs into the Nelson Analytical software per manufacturer instructions. Inject 2 µL of a "sample" onto the instrument. 45 seconds into the GC run, turn the splitter flow to "remote." Be certain to turn the splitter flow "off" before making the next injection.

11.2.3 Proceed to analyze samples by performing manual injections onto the GC. The vial sequence begins with calibration standards, followed by actual samples, QC samples and calibration check standards in the injection sequence described below:

Samples	Cumulative Injections
High level std.	1
Mid level std.	2
Low Level std.	3
LRB	4
CRM	5
Samples 1-8	6-13
Mid level calibration check	14

NOTE: QC Injections should be inserted into the sequence as follows: Include the matrix spike and duplicate as one of the first 10 samples. Run in order: sample, matrix spike sample, matrix spike sample duplicate.

11.2.4 An injection sequence may be allowed to continue as shown - injecting mid-level calibration check standards following every ten sample (or QC) injections - until calibration check standard fails to meet acceptance criteria (subsection 11.3).

11.3 Check standard acceptance criteria:

11.3.1 Concentrations of all analytes of interest are quantitated in the calibration check standards using the internal standard calibration described in subsection 12.4. Percent recoveries (calculated concentration divided by known concentration) must be within 75 to 125%. When calibration check standards fall outside this limit, instrument recalibration may be required.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Analyte identification - Target analytes are identified by comparison of retention time data obtained by analysis of a blank or sample extract with retention time data from analysis of standards. For identification, a sample extract component peak must be within the appropriate retention time window established for the column. The analytical software automatically identifies analyte peaks and calculates their concentrations based on integrated peak area counts and the appropriate RF established during calibration.

12.2 Analyte concentrations are calculated using area counts for target analyte and surrogate internal standard peaks identified by Nelson Analytical software, the average RRF for each analyte obtained from the calibration data, and the sample dry weight extracted. Calculations described in this section have been automated by a method facility of the Public Domain Software. The analyst should be thoroughly trained in computer application of this facility, described in SOP No. OR-008-00, before initiating data reduction.

12.3 Sample concentration of target analytes corrected for surrogate internal standard recovery is calculated by the equation:

$$C_s = (A_s \times M_{sis} \times F_a) \div (A_{sis} \times RRF \times M_s)$$

where,
C_s = target analyte concentration in dry sample (ng/g)
A_s = area counts of target analyte peak
A_{sis} = area counts of surrogate internal standard
M_{sis} = mass of surrogate internal standard added to the dry sample (ng)
RRF = average relative response factor
M_s = dry weight of sample (g)
F_a = extract aliquot factor (if applicable).

12.4 The absolute concentration of the surrogate internal standard, Tetrabutyltin, is calculated by the equation:

$$C_{sis} = (A_{sis} \times F_a) \div (RF_{sis} \times M_s)$$

where,

C_{sis} = uncorrected analytical concentration of surrogate internal standard in dry sample
A_{sis} = area counts of surrogate internal standard peaks
RF_{sis} = average absolute response factor of surrogate internal standard

13. METHOD PERFORMANCE

Data acquired for components of PACS-1(sediment):

ANALYTE	Mean[a] Meas. Conc.	SD	NIST Conc.	% Bias
Butyltin Trichloride	3.97	1.12	2.8	-29%
Dibutyltin Dichloride	6.64	1.53	11.6	-43%
Tributyltin Chloride	9.51	2.08	12.7	-25%

[a] n= 12

14. POLLUTION PREVENTION

The Chemical Hygiene Plan for U.S. EPA Laboratory Operations should be consulted for chemical handling, clean-up, and decontamination procedures. References for procedures to follow for disposal of chemicals are listed in Section 15.

15. WASTE MANAGEMENT

Personnel should follow procedures for chemical disposal outlined in the EPA-Cincinnati Safety Office's "Waste Packaging Guidelines" and "Laboratory Waste Management Guidelines".

16. REFERENCES

Detection of Butyltin Chloride Compounds in Sediments Using High Resolution Gas Chromatography and Flame Photometric Detection, Dick Carr, QARD, EMSL Cincinnati, Cincinnati, OH.

SOP No. OR-005, Organic Extraction of Sediment Samples for Analysis of Butyltin Compounds, Dave Craven, Technology Applications, Inc., Cincinnati, OH.

SOP No. OR-011, Organic Glassware Cleaning, Dave Craven, Technology Applications, Inc., Cincinnati, OH.

SOP No. OR-008, Data Processing with Public Domain Software, Shannon Fink, Technology Applications, Inc., Cincinnati, OH.

SOP No. OR-009, Organic Data Package Preparation, Shannon Fink, Technology Applications, Inc., Cincinnati, OH.

*************** End of SOP No. OR-006-00 ***************

APPENDIX D: Example of a Field Measurement Method

BENTHIC (SEDIMENT) METABOLISM

1. SCOPE AND APPLICATION

1.1 Ecosystems are complex, self-regulating, functional units defined by rates and processes, such as energy flow or material cycling. Functional indicators are those metrics that measure energy flow and material transformation within the ecosystem.

1.2 The method outlined here is designed for headwater to mid-order streams, though it may be adapted for larger rivers or lakes.

2. SUMMARY OF METHOD

2.1 The method measures changes in dissolved oxygen (DO) concentrations of the overlying water within microcosms containing small amounts (ca. 10 mL) of sediments as a means of assessing benthic microbial community activity. Sediments are collected from depositional habitats along a study reached defined by 40X channel width. Following incubation, DO is remeasured and the sediments saved for ash free dry mass (AFDM) analysis. Respiration, the decline in DO within each microcosm, is adjusted for AFDM, yielding a measure of community respiration/g AFDM. Organic carbon turnover time can be calculated from the empirical relationship between organic carbon (estimated as AFDM X 0.5) concentration of the sediment and oxygen consumption.

3. DEFINITIONS

3.1 AFDM - ash free dry mass

3.2 DO - dissolved oxygen

4. INTERFERENCES

Conditions which may interfere with this method are unknown. The method has been performed successfully in waters containing high metal concentrations, high conductivity, and moderate salinity.

5. PERSONNEL HEALTH AND SAFETY

No special safety precautions are required of users of this method.

6. EQUIPMENT AND SUPPLIES

6.1 Ice chest for floating centrifuge tubes during incubation.

6.2 Floating rack or styrofoam board for holding centrifuge tubes during incubation.

6.3 Grab sampler for sediments.

6.4 50 mL, screw-top, centrifuge tubes.

6.5 YSI Model 58 Dissolved Oxygen meter with Model 5730 Stirring BOD probe.

6.6 Spare batteries for DO meter.

6.7 Permanent markers for labeling tubes.

6.8 Sample labels and field data sheets.

6.9 Sandwich bags with zippered closure to contain replicates from each treatment and site.

6.10 Ice chest with dry ice for sample freezing.

7. REAGENTS AND STANDARDS

7.1 Formalin for preserving bacterial identification samples (optional).

7.2 Chemicals for inhibition/enhancement of microbial community during substrate-influenced respiration (optional).

8. SAMPLE COLLECTION, PRESERVATION, and STORAGE

8.1 Collect and composite 0.3 L of fine-grained, surface sediments from all depositional habitats within the stream reach defined by Physical Habitat assessment.

8.2 Fill ice chest 2/3 full with stream water and record temperature and dissolved oxygen (DO).

8.3 Thoroughly mix composited sediments.

8.4 Place approximately 10 mL of sediment in each of 5 labeled, 50 mL screw-top centrifuge tubes.

8.5 Fill each tube to the top (no head space) with stream water from the ice chest and seal.

8.6 Incubate in closed ice chest for 2 hours.

8.7 Measure DO in each tube.

8.8 Respiration is the difference between initial and final DO.

8.9 Decant overlying water and save sediment.

8.10 Seal tubes and place on dry ice as soon as possible.

8.11 Store frozen for laboratory analysis.

8.12 If you are collecting samples for sediment toxicity tests (Test Sites and Research Team), save remaining composite sediment sample by double-bagging in labeled, zip-
lock freezer storage bags.

8.13 Store sediment toxicity sample chilled (but not frozen!) for laboratory analysis.

8.14 NOTE: All samples must be carefully labeled with the appropriate bar-code and adhesive tags!!

8.15 Ship samples to: (Address)

9. QUALITY CONTROL

9.1 All field work conducted during this study will be done by personnel having prior experience with the designated methods, or under the direct supervision of experienced personnel.

9.2 Sediment metabolism will be sampled at each site on all site visits.

9.3 Quality assurance objectives:

9.3.1 Precision -- Precision of measurements is assured by carefully following the method protocols, including calibration of DO meter, electronic balances, and fluorescent microscopes.

9.3.2 Completeness -- Valid data are required from 90% of the sites visited.

9.3.3 Representativeness -- Sediment metabolism is being determined for depositional areas of streams. Replicate samples from these areas in a stream segment (defined by fish survey) are composited and mixed to provide a representative site sample.

9.4 Quality control procedures:

9.4.1 Field Operations -- All personnel involved in sediment metabolism determination are trained in the sample collection methods, test set-up and operation, and instrument calibration and use.

9.4.2 Laboratory Operations -- Quality control of non-microscopic samples will be ensured by adherence to protocols and proper use of calibrated instruments. QC of microscopic measures will be accomplished by proper selection of microscopic techniques and stain selection.

9.5 Documentation and Review: Sediment metabolism field data are recorded and checked for completeness and accuracy before leaving the site. Samples returned to the laboratory are logged in and their processing (e.g., mass determination, microbial counts) is tracked until the samples are completed. All data sheets are inspected for completeness, accuracy, and legibility before proceeding to the next sample. Raw data sheets are retained in a file, and the data are entered into an ASCII file.

10. CALIBRATION AND STANDARDIZATION

10.1 Each day, before leaving lab, motel, or base station, the following procedures should be completed to calibrate the Dissolved Oxygen Meter (YSI model 58, with YSI model 5730 stirring BOD probe):

10.1.1 Check meters batteries to ensure that meter and stirring probe are operational.

10.1.2 Check probe membrane to ensure that it is not frayed or torn and that there are no bubbles under the membrane. If membrane is not intact or has entrapped bubbles, it should be replaced according to the manufacturer's directions.

10.2 Upon arriving at each site:

10.2.1 Zero meter according to manufacturer's directions.

10.2.2 Calibrate meter using the water-saturated atmosphere method described in the meter's operations manual.

11. PROCEDURE

11.1 Dry sediments at 60°C for 5 days.

11.2 Weigh sediment samples to nearest 0.0001 g.

11.3 Ash samples at 525°C for 30 minutes.

11.4 Re-wet (to re-hydrate clays) samples and dry again at 60°C for 3 days.

11.5 Re-weigh sediment to determine ash free dry mass (AFDM).

12. DATA ANALYSIS AND CALCULATIONS

12.1 Respiration relative to the amount of organic carbon (estimated from AFDM) yields an estimate of organic carbon turnover, a metric that can be compared across sites and regions, and one which may be adjusted to temperature using Arrhenius plots. These plots calculate the energy of activation (E_a), which may be compared across diverse regions:

$E_a = \ln R \div (1 \div \text{Temperature}, ^\circ K)$

where ln R = the natural log of respiration.

12.2 AFDM Determination:

AFDM = [(Pan + sample) - pan weight] - [(Pan + ash) - pan weight]

13. METHOD PERFORMANCE

N/A

14. POLLUTION PREVENTION

N/A

15. WASTE MANAGEMENT

For normal respiration measurements, no waste is produced. Water may simply be decanted into the stream from which it came.

16. REFERENCES

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*************** End of Benthic (Sediment) Metabolism ***************

APPENDIX E: Example of a Field Collection Method

SEDIMENT COLLECTION

1. SCOPE AND APPLICATION

Sediment is collected at each station for a variety of analyses. Three samples are collected for benthic species composition, abundance, and biomass determination. Additional sediment grabs are collected for chemical and grain size analyses, and for use in acute toxicity tests.

2. SUMMARY OF METHOD

2.1 A 1/25 m^2, stainless steel, Young-modified Van Veen Grab sampler is used to collect sediments for benthic analyses. The sampler is constructed entirely of stainless steel and has been Kynar®-coated (similar to Teflon), and is therefore appropriate for collecting sediment samples for both biological and chemical analyses. The top of the sampler is hinged to allow for the removal of the top layer of sediment for chemical and toxicity analyses. This gear is relatively easy to operate and requires little specialized training.

2.2 Once a successful grab has been obtained, the sediment collected is processed according to the protocols described in Section 11 and summarized in Figure 1.

2.3 To minimize the possibility of biasing results from spatial heterogeneity of the sediments, benthic biology grabs are not collected consecutively, but rather interspersed among the chemistry/toxicity grabs. While a biology grab is being processed (sieved), grab samples are collected for chemistry/toxicity.

3. DEFINITIONS

N/A

4. INTERFERENCES

Procedures to prevent contamination of samples during sample collection are listed in Section 11 of this method.

5. PERSONNEL HEALTH AND SAFETY

5.1 All sediment grab samplers are dangerous pieces of equipment. Once the device is cocked, it could accidentally trip at any time. The operators must be careful not to place hands or fingers in a position where they could be damaged (or amputated) in the event that the device trips prematurely.

5.2 The sampler is a heavy piece of equipment (especially when full). The operators must take care when deploying or retrieving this gear under adverse weather conditions. A grab sampler swinging wildly at the end of a boom can be very dangerous.

6. EQUIPMENT AND SUPPLIES

6.1 Young-modified Van Veen grab sampler, stainless steel, Kynar-coated.

6.2 Computer, equipped with a bar code reader.

6.3 500 µm mesh sieve.

6.4 Sieve box.

6.5 Whirl Packs.

6.6 Cooler with ice.

6.7 Cut-off 60cc syringes.

6.8 125 mL plastic jars.

6.9 250 mL plastic jars.

6.10 500 mL glass bottles.

6.11 1 gallon plastic containers.

6.12 mm ruler.

6.13 Teflon stirring paddle.

6.14 Stainless steel spoon.

7. REAGENTS AND STANDARDS

Samples collected for benthic community analyses must be preserved in 10% buffered, stained formalin immediately upon collection and sieving. A 100% formalin stock (37% formaldehyde solution) must be made. One hundred mL of this stock are added to each one-liter sample jar, resulting in a final concentration of 10%. The stock is made by adding ¼ teaspoon Rose Bengal Stain and 8 heaping tablespoons of Borax to two gallons of full strength formalin.

8. SAMPLE COLLECTION, PRESERVATION and STORAGE

Sample collection, preservation, and storage activities are described in Section 11.

9. QUALITY CONTROL

9.1 Precautions:

9.1.1 The interior surfaces of the grab sampler (including the underside of the hinged top) must be washed and thoroughly rinsed prior to use to assure that no sediment remains from the previous station.

9.1.2 Prior to use, all Teflon and stainless steel supplies which are to come into contact with samples must also be properly cleaned.

9.1.3 Care must be taken to assure that the chemistry samples do not become contaminated. This requires great care in extracting the sample, homogenizing it, and placing it in the proper container. If it is raining when the sample is collected, all activities should be conducted under a tarp to prevent contamination of the sample by rain water.

9.1.4 Great care must be taken to avoid atmospheric contamination from engine exhaust. The boats engines and the Briggs and Stratton must be turned off whenever the sample is exposed to the atmosphere.

9.1.5 The grab must be suspended off the deck at all times to avoid contamination.

9.1.6 Care should be taken to assure that the sediment saved for chemical and toxicological analyses is collected only from the top two cm of the grab.

9.1.7 Assure that the proper bar code labels are affixed to all samples.

9.2 Quality Control Samples: At selected sites, "blanks" for chemistry will be obtained. An empty glass chemistry jar is left open whenever the sample is exposed, mimicking the treatment it would receive if a sample was to be placed in it. The jar is then sealed and the sample number recorded. This jar is then treated in the same fashion as all other chemistry samples.

9.3 Contingency Plans: It is recognized that at certain stations the sediment type will prevent the collection of sediment samples. If a single "acceptable" grab sample cannot be obtained after five attempts, or if ≥70 percent of the attempts are unsuccessful, then additional attempts are abandoned and no sediment samples collected. All other samples should be collected. This must be noted in the computer and the Field Coordinator notified during the next scheduled call-in.

10. CALIBRATION AND STANDARDIZATION

N/A

11. PROCEDURE

11.1 Protocol for obtaining sediment (See Figure 1):

11.1.1 Wash and thoroughly rinse the inside of the grab sampler with seawater from the station being sampled.

11.1.2 Attach the sampler to the end of the winch cable with a shackle and **tighten the pin**. An auxiliary link is also installed to provide added assurance against loss of the equipment. Attach a pinger to the grab.

11.1.3 Attach one set of weights to the sampler. These can be removed, or additional weights added depending on the sediment type. The grab is then cocked.

11.1.4 Lower the grab sampler through the water column such that travel through the last 5 meters is no faster than 1 m/sec. This minimizes the effects of bow wave disturbance to surficial sediments.

11.1.5 Retrieve the sampler and lower it into its cradle on-board. Open the hinged top and determine whether the sample is successful or not. A successful grab is one having relatively level, intact sediment over the entire area of the grab, and a sediment depth at the center of at least 7 centimeters. Grabs containing no sediments, partially filled grabs, or grabs with shelly substrates or grossly slumped surfaces are unacceptable. Grabs completely filled to the top, where the sediment is in direct contact with the hinged top, are also unacceptable. It may take several attempts using different amounts of weight to obtain the first acceptable sample. The more weight added, the deeper the bite of the grab. In very soft mud, pads may be needed to prevent the sampler from sinking in the mud. If pads are used, the rate of descent near the bottom should be slowed even further to reduce the bow wave. Note that weights and pads may be combined to optimize sample collection.

11.1.6 Carefully drain overlying water from the grab.

11.1.7 Enter notes on the condition of the sample into the computer and on the data sheet. Options on smell, texture, etc. are available via menus.

11.1.8 Process the grab sample for either benthic community analysis or chemistry/toxicity testing as described in subsections 11.2 and 11.3.

11.1.9 Repeat steps described in subsections 11.1.4 through 11.1.8 until all samples are collected. To minimize the chance of sampling the exact same location twice, after three grabs are taken (whether successful or not), move the boat 5 meters downstream by letting out the appropriate length of anchor line.

```
                    +))))))))))))))))))))))),
                    *  OBTAIN  SEDIMENT   *
                *    GRAB SAMPLES      *
                .)))))))))))0))))))))))) -
                 +)))))))))2))))))))),
                 * EVALUATE  EACH FOR *
                 *   ACCEPTABILITY     *
                 .)))))))))0))))))))) -
              +))))))))))))))))2))))))))))))))),
              * LOG NOTES ON APPEARANCE OF GRAB   *
              .))))))))))))))))0))))))))))))))) -
        BENTHIC BIOLOGY        *           CHEMISTRY/TOXICITY
           +))))))))))))))))))2) 0))))))))))))))))),
     +))))))))2))))))))),   +)))))))2))))))))),   +)))))))2))))),
     * MEASURE DEPTH AT *  *REMOVE 2 CM PLUG*  *  REMOVE TOP *
     *  CENTER OF GRAB  *  * FROM EACH GRAB *  *     2 CM    *
     .))))))))0))))))))) -  * FOR AVS SAMPLE *  .)))))))0))))) -
           *          .)))))))0)))))))) -   +))))))2))))),
           *             +))))2))),        *  PLACE IN  *
           *             * CHILL  *       * S.S.  PAN  *
           *             .)))))))) -       .))))))0))))) -
           *                      +))))))))2))))))),
           *                      * BETWEEN GRABS,*
           *                      *  KEEP ON ICE  *
           *                      .))))))))0)))))) -
+))))))))))))2)))))))))),  +)))))),    +))))))))))))))2))))))))),
* EXTRUDE SEDIMENT FROM *  *STORE *    *    CONTINUE UNTIL    *
* CORE & SAVE FOR GRAIN /))1 ON   *    *   SUFFICIENT QUANTITY *
*   SIZE ANALYSIS     *  * ICE  *    *    HAS BEEN COLLECTED *
.))))))))))))0)))))))))) -  .)))))) -    .))))))))))))))0)))))))) -
 +))))))))))2))))))))))),           +))))))))))))))2))))))))),
 *   DUMP REMAINING     *            * STIR SEDIMENT FOR 10 MIN*
 * SEDIMENT INTO BUCKET *            *      TO HOMOGENIZE      *
 .))))))))))))0)))))))))) -          .))))))))))))))))0))))))))) -
   +)))))))))2))))),               +))))))))))))))))2)))))),
   * SIEVE THROUGH *            +)))1 PLACE 250 cc IN GLASS *
   * 0.5 mm SIEVE  *           *   *  JAR FOR CHEMISTRY    *
   .))))))))))0))))) -          *   .)))))))))))))))))0)))))) -
+))))))))))))2))))))))))))),      * +))))))))))))))))))2))))))))),
 *   RINSE ORGANISMS INTO *      /)1 100 cc FOR METALS IN JAR   *
 * SAMPLE CONTAINER <700cc*      * .))))))))))))))))))0))))))))) -
.)))))))))))0)))))))))))) -      * +))))))))))))))))))2))))))))),
           *               /)1 PLACE 3000 cc IN PLASTIC   *
           *               * *    JAR FOR TOXICITY       *
           *               * .))))))))))))))))))0))))))))) -
  +)))))))))2))))))))))))),      * +))))))))))))))))))2))))))))),
  *PRESERVE WITH FORMALIN*     /))1PLACE 100 cc IN WHIRL PACK *
  *    FILL TO RIM      *      * *  FOR GRAIN SIZE ANALYSIS  *
  .)))))))))0)))))))))))) -     * .))))))))))))))))))0))))))))) -
   +))))))))2)))),            *    +))))))))))))))2))))))))),
   * CLEAN SIEVE *            *    *     PLACE ON ICE      *
   .))))))))))))) -           .)))))1     DO NOT FREEZE    *         .))))))))))))))))))))))) -
```

Figure 1. Sediment collection flowchart.

11.1.10 Because of spatial heterogeneity of the sea bottom, samples processed for benthic community analyses should be interspersed among those processed for chemistry and toxicity, *i.e.*, three consecutive samples should not be processed for community analyses.

11.2 Field processing of samples for benthic community assessment (See Figure 1):

11.2.1 Assign a sample number to the sample, affix the label to the sample jar, and scan the number into the computer using the bar code reader.

11.2.2 Measure the depth of the sediment at the middle of the sampler and record the value on the data sheet. The depth should be ≥7 cm. Record descriptive information about the grab, such as the presence or absence of a surface floc, color and smell of surface sediments, and visible fauna in the computer.

11.2.3 Insert a cut-off 60 cc syringe into a random location within the sampler and extract a core sample. Extrude the sediment from the core tube into a "Whirl Pack." If an insufficient amount of sediment has been extruded to fill the Whirl Pack half-way, repeat until the pack is full. Place an appropriate bar code label on the Whirl Pack. Record the sample number on the Whirl Pack, and store for later analysis to determine the relative proportion of silt and clays versus sands. The sample should be stored on ice (NOT dry ice) as the sample should be refrigerated at 4°C, not frozen.

11.2.4 Process the remainder of the grab for benthic community analyses. Dump the sediments into a basin and then into a 500 µm mesh sieve. Place the sieve into a table (sieve box) containing water from the sampling station. Agitate the tray in the sieve box thus washing away sediments and leaving organisms, detritus, sand particles, and pebbles larger than 500 µm. This method minimizes mechanical damage to fauna that is common when forceful jets of water are used to break up sediments. A gentle flow of water over the sample is acceptable. Extreme care must be taken to assure that no sample is lost over the side of the sieve.

11.2.5 Drain the water from the sieve box and gently rinse the contents of the tray to one edge. Using either your fingers or a spoon, GENTLY scoop up the bulk of the sample and place it in the plastic screw-top bottle from which the sample number was scanned in subsection 11.2.1 (which should be placed in the sieve or a bucket in case some of the sample spills over). Rinse the outside of the sample jar into the sieve, then, using a funnel, rinse the contents into the jar. The jar should be filled no higher than the 700 ml mark. If the quantity of sample exceeds 700 ml, place the remainder of the sample in a second, unlabeled container. Using a waterproof marker, write the sample number on the second container and tape the two together. Note in the computer that the sample consists of more than one container.

11.2.6 Carefully inspect the sieve to ensure that all organisms are removed using fine forceps (if necessary) to transfer fauna from the sieve to the bottle containing the proper sample number.

11.2.7 Ten percent buffered formalin is used to fix and preserve samples. A 100 % buffered, stained stock formalin solution should be mixed according to the recipe in Section 7. One hundred mL of the formalin should be added to each sample jar, and a teaspoon-full of borax

added to assure saturation of the buffer. **Fill the jar to the rim with seawater to eliminate any air space**. This eliminates the problem of organisms sticking to the cap because of sloshing during shipment. Gently invert the bottle to mix the contents and place in the dark. If the sample occupies more than one container, tape all the sample bottles containing material from that grab together.

11.2.8 Prior to sieving the next sample, use copious amounts of forceful water and a stiff brush to clean the sieve, thereby minimizing cross-contamination of samples

11.3 Field Processing of Sediments for Chemistry and Toxicity Testing: In addition to the three grabs collected for benthic community analyses, additional grabs are collected for chemical analyses and toxicity testing. The top two cm of these grabs are removed, homogenized, and split for chemistry and toxicity testing. Because of contamination concerns these samples are removed and processed in the order described in subsections 11.3.1 through 11.3.9. A clean stainless steel spoon and an uncontaminated syringe are used to remove sediments from grab samples for these analyses. All items must be washed with Alconox and rinsed with ambient seawater before use. **NOTE:** All engines (outboards and Briggs and Stratton) must be turned off whenever the sample is exposed to the air to reduce the hazard of atmospheric contamination.

11.3.1 For Acid Volatile Sulfides - Use an uncontaminated 60 cc syringe to extract the top two centimeters of material from undisturbed surficial sediment. An appropriate number of plugs must be taken from each chemistry grab in order to fill a 125 ml plastic jar completely (normally one plug/grab). In between grabs the jar must be sealed and placed in a cooler on ice. **Care must be taken to prevent oxidation of the sample.** Fill the sample container completely, leaving no head space. This sample must be refrigerated immediately.

11.3.1.1 Remove the top two cm of sediment using the stainless steel spoon. Place the sediment removed in a stainless pot and place the pot in a cooler on ice (NOT dry ice). The sample must be stored at 4°C, NOT FROZEN.

11.3.1.2 Repeat this procedure, composite the sediment in the same stainless pot until a sufficient quantity of sediment has been collected for all samples. Stir sediment homogenate after every addition to the composite to insure adequate mixing. Keep the container covered and in the cooler between grabs.

11.3.1.3 Homogenize the sediment by stirring with a Teflon paddle for 10 minutes.

11.3.2 For Organics - Using a stainless steel spoon, carefully place 250 cc of sediment in a 500 ml glass bottle for chemical analysis. **Care must be taken to assure that the inside of the bottle, bottle cap, and the sample are not contaminated.** If not already in place, affix the label supplied with the bottle containing the lot number (this need not be recorded anywhere). Record the sample number, wrap the jar in "bubble wrap" to protect it from breakage, and place the sample on ice (NOT dry ice). To reduce the possibility of breakage, the sample should be stored at 4°C, not frozen.

11.3.3 For Metals - Using a stainless steel spoon, place approximately 100cc of sediment into a pre-cleaned plastic (HDPE) sampling jar. Record the sample number and keep on ice at 4°C.

11.3.4 For Sediment Chemistry QA - At one previously designated station per crew, three additional samples need to be collected for each of the following: 125 ml AVS, 250 ml organics and 125 ml metals for duplicate analyses (1) and for analysis by a referee laboratory (2). Four plugs must be taken from each of chemistry grabs to fill each of the AVS bottles at a constant rate following directions in subsection 11.3.1. QA samples for organics and metals are collected from the same composite as per the directions in subsections 11.3.5 and 11.3.6. The Field Coordinator will notify the crew at which station these samples need to be collected. In addition, one glass sample jar should be left open on the deck whenever the organics sample is exposed. This will serve as a blank.

11.3.5 For Sediment Grain Size - Attach an appropriate bar code label to a Whirl-Pack, and fill approximately half-way for sediment grain size analysis, and record the sample number. Store this sample on ice (NOT dry ice).

11.3.6 For Sediment Toxicity - Using the stainless steel spoon, fill approximately 85% of the 1 gallon plastic container for toxicity testing with sediment (minimum volume required is 3000 ml). Record the sample number on the bottle, and place the sample on ice (NOT dry ice). The sample must be stored at 4°C, NOT FROZEN.

12. DATA ANALYSIS AND CALCULATIONS

N/A

13. METHOD PERFORMANCE

The collection of sediments can be hindered by rough seas, strong currents, or substrate type. The use of additional weights or pads may be necessary to obtain acceptable samples under a wide variety of conditions.

14. POLLUTION PREVENTION

A formalin solution, a suspected carcinogen, is used to preserve samples for benthic community analyses. If spilled on the deck it should be wiped up immediately with paper towels, and the residual washed overboard. Paper towels should be discarded as described in Section 15.

15. WASTE MANAGEMENT

Any and all waste or excess formalin solution must be returned to ERL-N for proper disposal by the ECHS Officer. Paper products contaminated with formalin should be contained in air-tight plastic bags and can be discarded with routine garbage.

16. REFERENCES

N/A

*************** End of Sediment Collection ***************

APPENDIX F: Example of a Biological Laboratory Method

DIATOM METHODS

1. SCOPE AND APPLICATION

1.1 A large body of knowledge exists concerning the use of diatoms as indicators of environmental change (e.g. Lowe 1974; Smol 1990; Dixit et al. 1992). Diatoms are unique among aquatic organisms for the monitoring needs of EMAP-SW. They are abundant and have high species richness in most aquatic habitats. Many diatom taxa have somewhat different environmental requirements and tolerances. These optima and tolerances can be quantified and used in large scale environmental monitoring programs.

1.2 Powerful inference models have been developed using sedimentary diatom assemblages. This is generally done by calibrating the surface sediment diatom assemblages with measured environmental variables. Calibration models can be applied to diatom assemblages deposited in the past, and thus a historical assessment can be made about shifts in environmental quality. Because historical monitoring data are rarely available, sedimentary diatoms are being used increasingly for environmental assessments of aquatic systems. Using this approach, it will be possible to follow environmental trends in time by periodically sampling (e.g. every 4 years) the surface sediments of the study lakes. Because diatom cell walls are siliceous, they are well preserved in lake sediments. These attributes make diatom community analysis a powerful tool for EMAP-SW.

1.3 In EMAP-SW, the recent diatom assemblages (top 1 cm of sediment, representing the last few years of sediment accumulation) are calibrated for significant environmental variables and various models and indices are developed to characterize lakewater conditions. By analyzing the bottom samples of the sediment cores (i.e. pre-industrial) it will be possible to provide a retrospective view of the lakes prior to major anthropogenic impact (i.e. background or "reference" conditions). As identified from the PIRLA-II (Cumming et al. 1992) and the 1991 EMAP-SW lakes study (Dixit and Smol, 1994), by using this approach it would be possible to identify the proportion of lakes that have remained in steady-state or have changed with respect to selected environmental variables. Many environmental variables will be evaluated with the goal of selecting those that have the greatest influence on diatom assemblages in any specific region. Computer intensive techniques have been developed that provide error estimates for each sample (Birks et al. 1990a,b). This information is important for EMAP-SW because it is necessary to separate natural variation from anthropogenic-induced changes.

2. SUMMARY

Methods for diatom research presented here have been evaluated and standardized in previous research projects (Charles and Whitehead 1986; Charles and Smol 1990). Nevertheless, improvements are actively sought and tested (e.g. Glew 1988; Birks et al. 1990a). These methods were recommended to EMAP-SW when the lake sampling started in 1991 in the northeast (Dixit and Smol 1991). The methods rely heavily on the paleolimnological protocols that were developed for three large, multi-institution paleolimnological research projects which investigated

the effects of acid rain on aquatic resources in the United States (PIRLA-I, Charles and Whitehead 1986; and PIRLA-II, Charles and Smol 1990), and the Surface Water Acidification Programme (SWAP) of Great Britain and Scandinavia (Battarbee et al. 1990). Comments are restricted to coring, sediment core subsampling and archiving, dating, and diatom and pollen methods; however, the list of methodologies from the PIRLA project (Charles and Whitehead 1986) also includes: lake selection, watershed and climatic history, charcoal, chrysophytes, chironomids, total metals, sequential chemical extraction of metals, total sulfur, total carbon, total hydrogen, total nitrogen, sulfur isotopes, water chemistry, variability study, and Cladocera. The reader is also referred to amendments made to the PIRLA-I protocols during the PIRLA-II project (Smol et al. 1989), which are also relevant to EMAP-SW.

3. DEFINITIONS

3.1 Paleolimnology - The branch of limnology that deals with describing and interpreting lake histories by studying the information contained in lake sedimentary profiles.

3.2 Diatom - Algae in the class Bacillariophyceae.

3.3 Frustule or valve - Refers to the siliceous outer skeleton of the diatom cell (2 valves = a frustule), the characters of which are used in the identification of discreet taxa.

3.4 Digestion - This procedure refers to the solubilization of organic material by strong acid oxidation.

3.5 Weighted averaging (WA) - This is a statistical method which can be used for analyzing environmental gradients with biological response variables (e.g. diatoms and chrysophytes). The optimum of each species along a gradient (e.g. total phosphorus) is estimated as the average of all TP values for lakes in which the taxon occurs, weighted by the taxon's relative abundance (WA regression). Reconstructions calculate a predicted environmental value for an algal assemblage based on the optima of the species and their abundances (WA calibration). This method is an approximation of the more formal procedure of maximum likelihood regression and calibration. The theory has been developed and elaborated mainly by ter Braak (1986, 1987).

3.6 WACALIB - This is a FORTRAN program by Line and Birks (1990) that implements regression calibration based on weighted averaging.

4. INTERFERENCES

4.1 Some times the presence of sand and silt in the sediment sample may interfere with the identification of diatoms. If this is a problem, the lab should use differential settling techniques.

4.2 In dating sediments, high metal concentrations can cause interference with the plating of polonium. This problem should be corrected by increasing the concentration of ascorbic acid.

4.3 Samples containing significant amounts of carbonate will tend to bubble and sputter on the initial addition of acid. In this case, acid addition should be immediately suspended until all evidence of a reaction has ceased.

5. PERSONNEL HEALTH AND SAFETY

5.1 Since the composition of the sediment material has not been discerned, disposable gloves should be used when handling sediment.

5.2 When transferring crucibles or samples from the drying oven, wear gloves to protect hands from high temperature.

5.3 Nitric acid is an extremely hazardous reagent. As a strong acid oxidizer it can cause severe burning of exposed skin and clothing. At room temperature, concentrated nitric acid produces intense fumes when exposed to open air.

5.4 Any concentrated nitric acid containers open to the air must be contained within a positive-draw chemical hood at all times.

5.5 Personnel are required to wear safety glasses and protective gloves at all times when handling concentrated nitric acid.

5.6 When samples are received by the diatom preparation laboratory, personnel must be informed if any preservatives have been used in the samples (e.g. formaldehyde, Lugol's solution, glutaraldehyde, etc.). Unexpected, violent and/or noxious reactions can occur during the cleaning procedure if nitric acid is mixed with other chemical substances.

6. EQUIPMENT AND SUPPLIES

6.1 Modified K-B gravity corer (Glew 1989) and extruder. See Figures 1 and 2.

6.2 Glew mini corer (Glew 1991) and extruder. See Figure 3.

6.3 Lexon coring tubes and rubber stoppers.

6.4 Positive-draw chemical hood.

6.5 Microwave apparatus (CEM Model MDS-2100): 0-950 watts (1% intervals); controlled and monitored temperature (fiber optics) and pressure; programmability for different cycles of temperature, pressure, and time at various temperature and pressure combinations; rotating turntable. See Figure 4.

6.6 Closed microwave digestion vessel: vessel liner (Teflon PFA); vessel liner cover (Teflon PFA); vessel cap (plastic, microwave "invisible"); vessel body (plastic, microwave "invisible"); rupture membrane (Teflon PFA); vent stem (PTFE). See Figure 5.

6.7 Digestion vessel for temperature and pressure control and monitoring: Vessel liner, vessel cap, vessel body and rupture membrane as in 6.6; vessel liner cover (Teflon PFA) with exhaust, temperature and pressure ports; vent stem (PTFE); thermowell (Pyrex with Teflon coating). See Figure 6.

6.8 100 ml tall glass beakers and 20 ml glass vials with conical liners.

6.9 Faucet siphon apparatus and plastic stirring rods and spatula.

6.10 5 ml (0.2 ml gradients) and 1 ml (0.01 ml gradients) plastic syringes.

6.11 1 dm (3.9 ml) glass vials with screw cap and 20 ml, glass vials with plastic screw cap and cone liner.

6.12 Wash bottle, laboratory markers, adhesive labels, transparent tape (2 cm width), plastic disposable gloves, diamond scribe, safety glasses, and acid impervious hand protection.

6.13 Analytic balance (sensitivity = 0.01 gm).

6.14 Drying oven (to 90 ºC).

6.15 Porcelain crucibles, low, wide-form, 100-ml+.

6.16 Subsampling and sediment weight data sheets.

6.17 Research microscope with camera attachment.

6.18 Immersion oil.

6.19 Photographic supplies (e.g. film, developing paper etc.).

6.20 Glass slides and round cover glass (size 22 mm, no 1).

6.21 Battarbee (1973) evaporation trays.

6.22 Distilled water (DW).

6.23 Alpha spectrometers (equipped with gold foil surface barrier detectors).

7. REAGENTS AND STANDARDS

7.1 Nitric acid, hydrochloric acid, sulfuric acid, ascorbic acid, and glacial acetic acid.

7.2 Potassium hydroxide, hydrogen peroxide, acetic anhydride, barium sulphate.

7.3 Po-209 yield tracer solution.

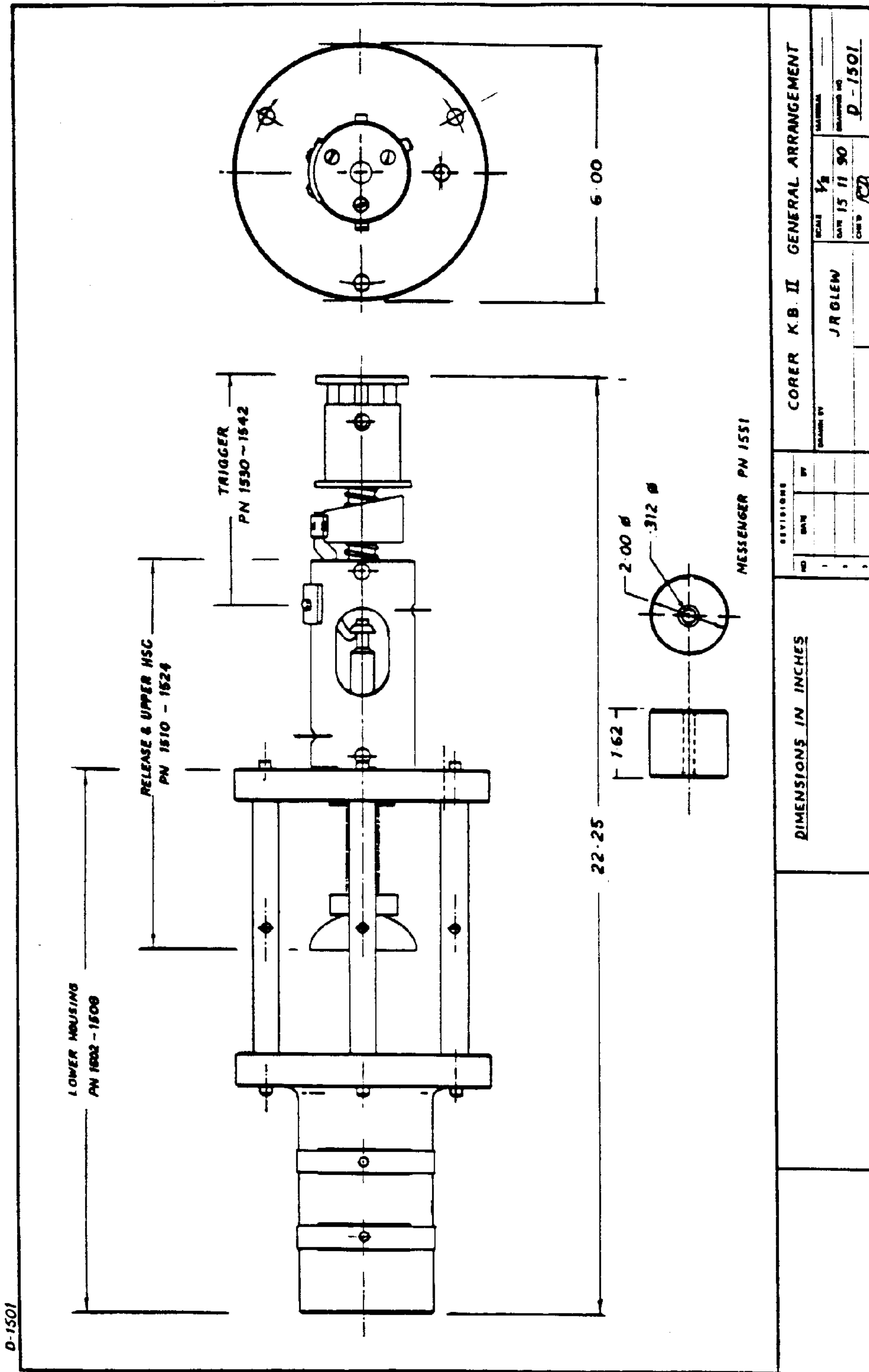

Figure 1: K-B gravity corer.

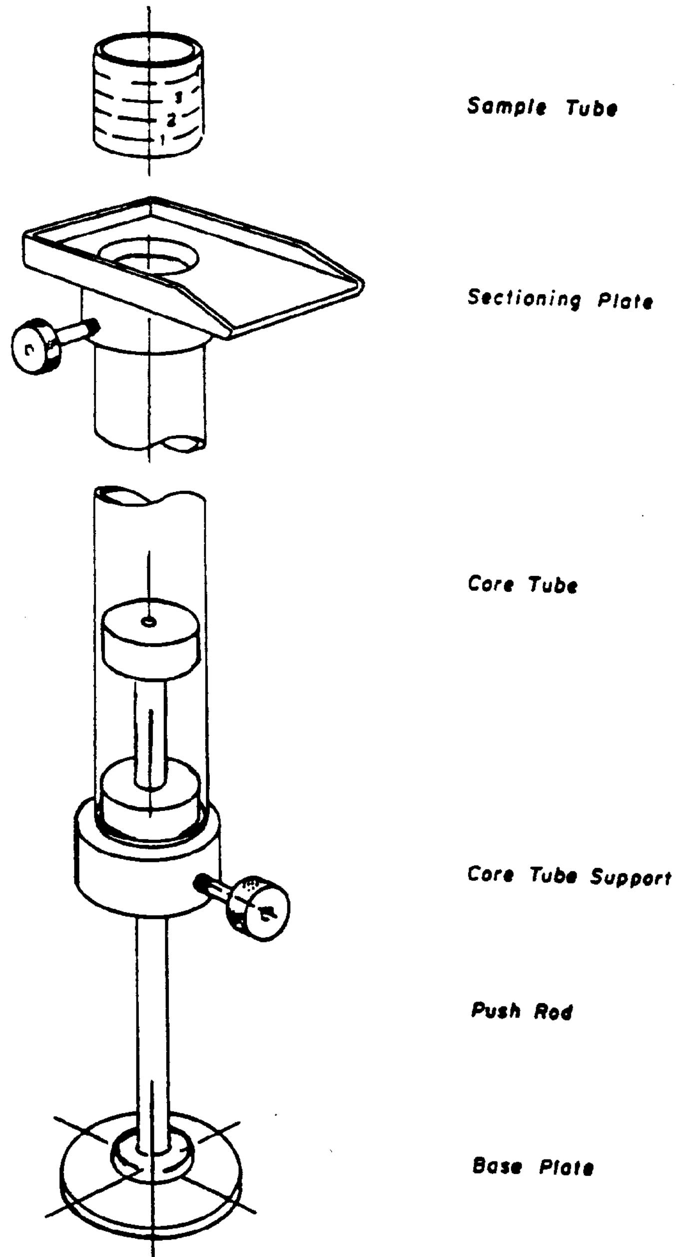

Figure 2: Extruder

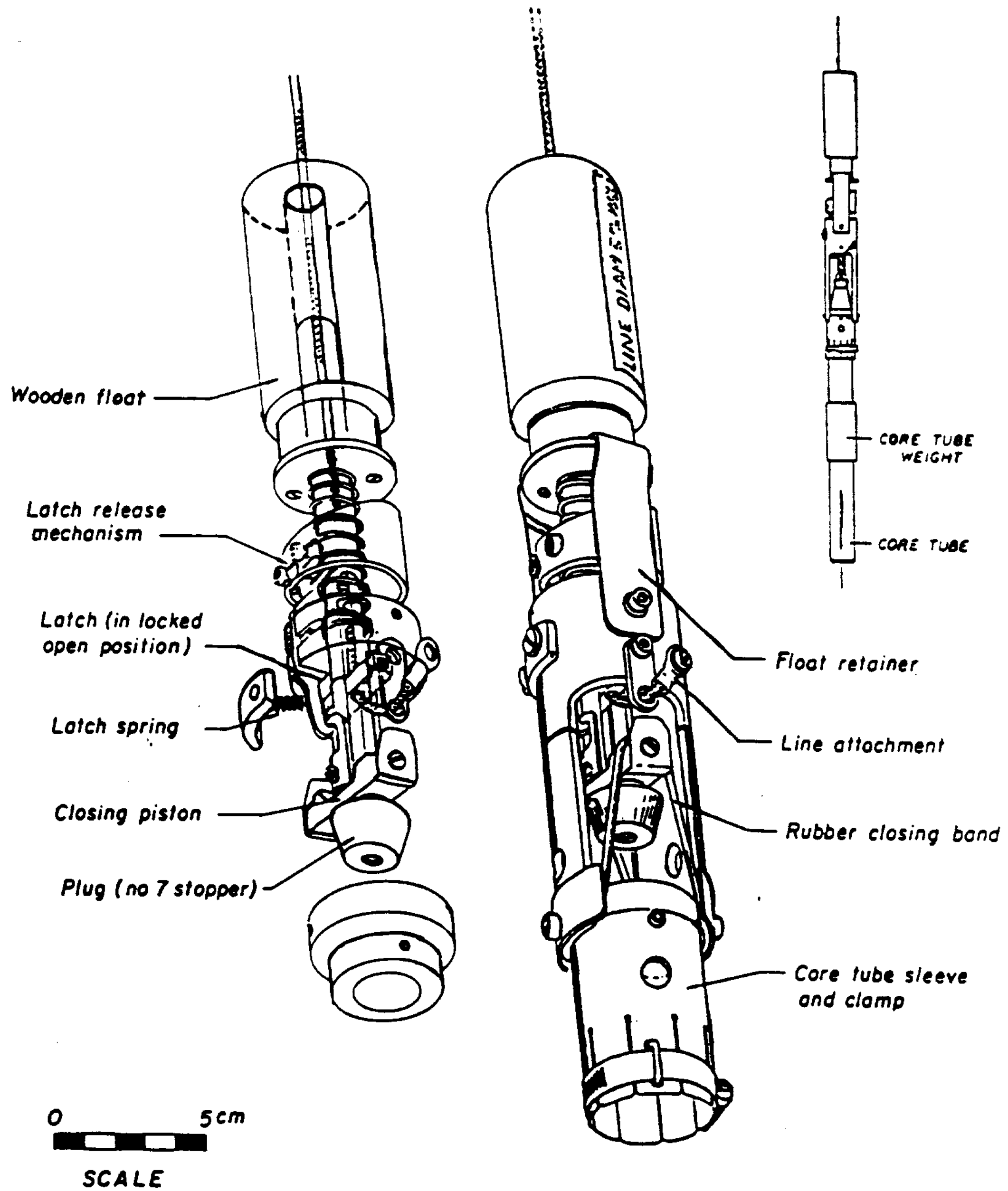

Figure 3: Glew mini corer.

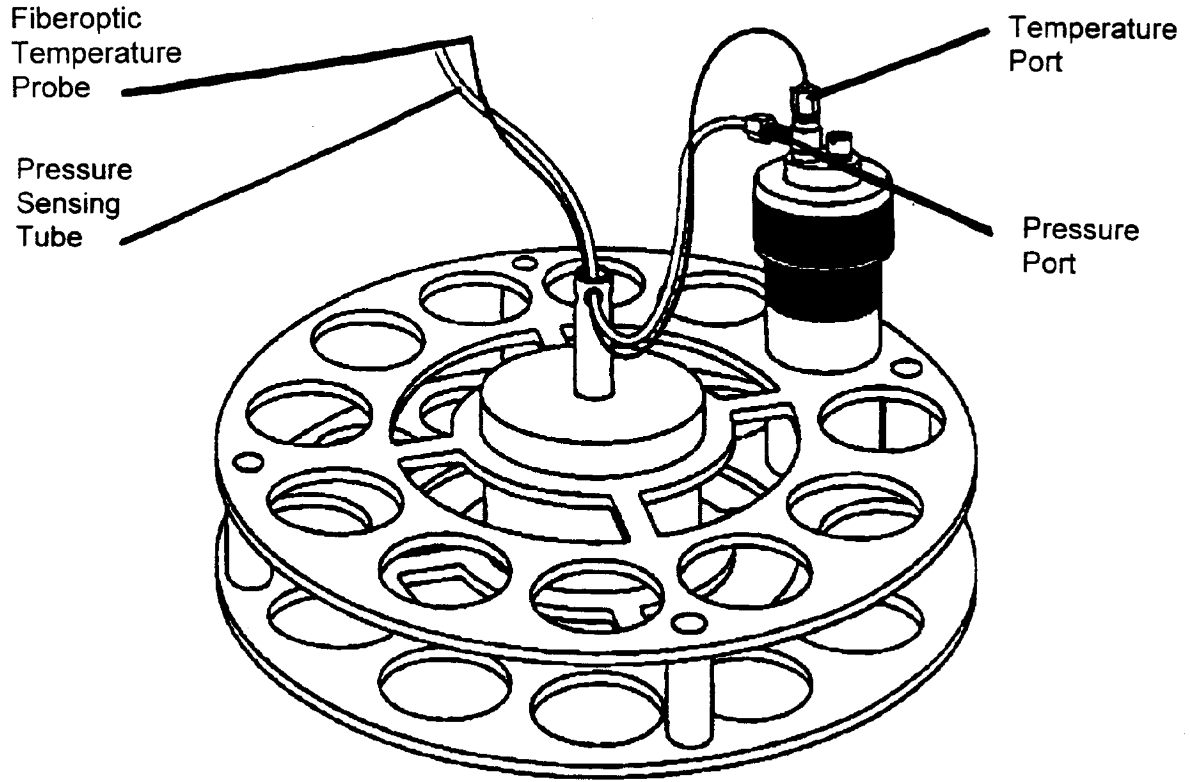

Figure 4: Routing of pressure sensing tube and fiberoptic temperature probe.

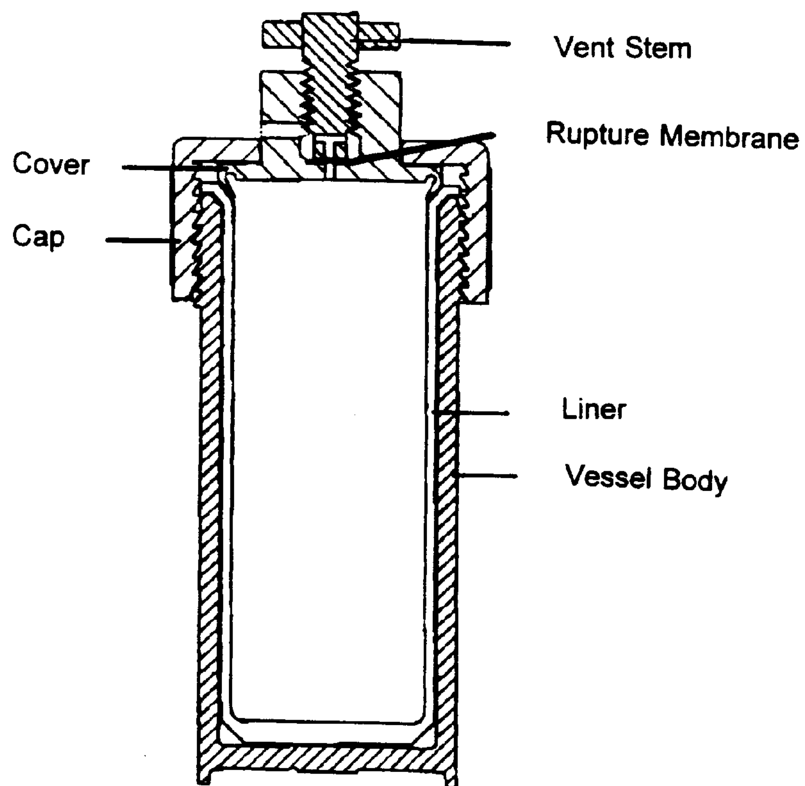

Figure 5: Microwave digestion vessel.

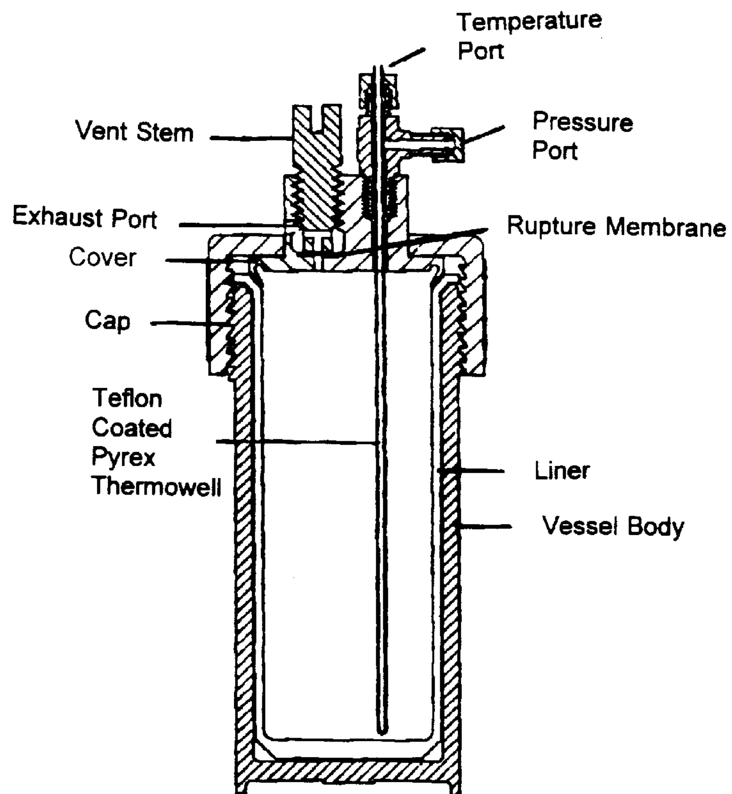

Figure 6: Microwave digestion vessel with temperature and pressure controls.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Sediment coring procedures:

8.1.1 Sediment cores from the lakes are taken with a modified K-B gravity corer. The corer is equipped with a modified trigger mechanism (Glew 1989) and adapted to hold a 60 cm long Lexon tube with an internal diameter = 6.4 cm and an outer diameter = 6.9 cm. In addition to providing undisturbed cores, the tube also provides sufficient sediment material for sediment dating and for the study of algal and animal microfossils, and pollen. In addition to collect a single sediment core from each of the lakes, each year triplicate cores are taken from three lakes. An attempt should be made to collect sediment cores >45 cm in length, especially from productive lakes, so that background (pre-anthropogenic) conditions can be reached. The cores should be taken from the deep, central area of the lake, and preferably where the bottom is relatively flat. In large lakes, cores should preferably be taken from depths <35 m deep, as gravity coring from very deep areas is more problematic.

8.1.2 Before inserting the core tube in the corer, inspect the top edge of the tube for damage; it is important that the core tube has a smooth, undeformated edge to mate with the seal ring. Check the position of the seal ring in the corer. A small amount of petroleum jelly may be used to retain the seal ring and to ensure a good seal.

8.2 The corer should be lowered slowly in the water, allowing it to sink in the sediment by its own weight, while keeping tension on the rope. Once the corer has penetrated the sediment, a messenger is delivered down the coring line, which triggers the closing of the corer, and effectively seals the top of the core tube. Once triggered, the corer should be gently retrieved to just below the lakewater surface. Then, holding the corer upright by one hand, a sliding plug should be placed at the bottom of the core. When placing the plug into the core tube, the core tube should still be submerged (to avoid the loss of sediment), and so the crew member has to extend his\her arm into the water. The plug used here will be the same one that will be used to extrude the core. See Figure 7 for an illustration of this procedure. A stopper should be placed under the sliding plug to seal the core. The stopper is slightly bigger in size than the sliding plug, and will be removed before the core tube is mounted in the sectioner. While keeping the core tube upright in the boat or raft, the corer will be disengaged from the coring tube, and the top of the core tube is closed by another stopper. Always provide support for the stopper so the sediment does not fall out of the coring tube. In the boat the core should always be kept upright in a stand to keep the tubes vertical.

8.3 The Glew mini corer (Glew 1991) will be used in annually visited lakes and in lakes which are revisited after 4 years because only the top 1 cm samples have to be taken from these lakes. The mini corer operates in a similar fashion to the modified K-B corer, except that it uses a smaller diameter tube and it generally retrieves short cores.

8.4 Sediment cores will be sectioned on the boat soon after they are retrieved. The following steps are involved in core sectioning:

8.4.1 Measure the length of the core using a meter stick.

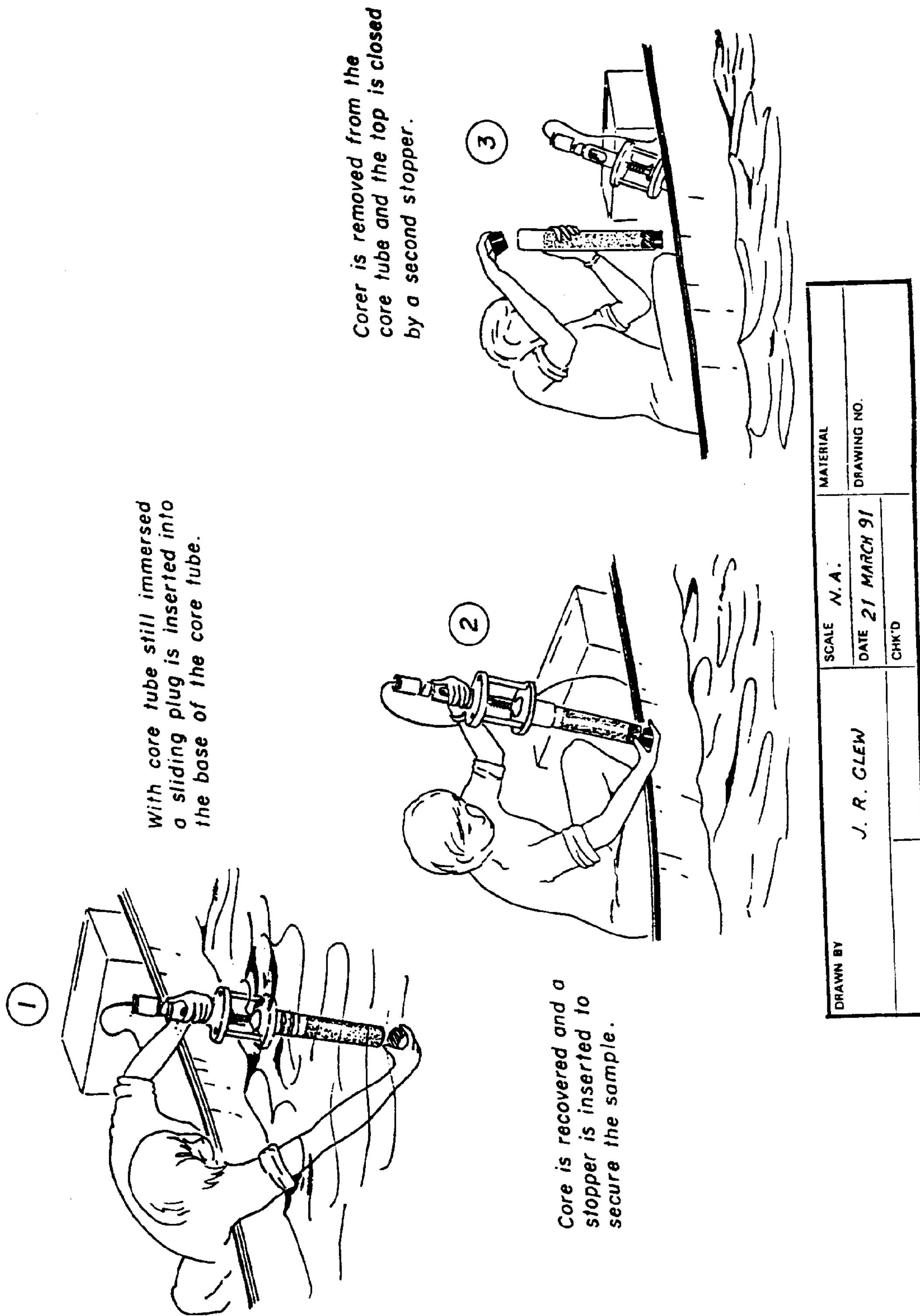

Figure 7: Method of securing a core.

8.4.2 Note the depth and nature of any interesting changes visible in the stratigraphy.

8.4.3 With the extruder push rod retracted completely, remove the lower stopper and insert the core tube in the clamping sleeve; tighten the sleeve clamp.

8.4.4 Remove the upper stopper and attach the tray; water can now be siphoned off the sample or alternatively it may be displaced by extrusion (next step).

8.4.5 Push the core tube down over the push rod to drive the core up to the top of the tube. With the sample at the level of the tray, place the calibrated cylinder over the core and push the upper 1 cm of sediment into the cylinder. Slide off the cylinder and bag the sample.

8.4.6 Extrude and discard the center portion of the core. Using the calibrated cylinder (as in subsection 8.4.5) sample the lower 1 cm sediment. To avoid contamination, the lower sample should be taken 1 cm above of the bottom of the core.

8.4.7 Place the sediment samples in tightly sealed Whirl-Pack bags (18 ounce size). The labeling of each bag will include: lake name, sample depth, and the date when the sample was collected. No preservatives or chemicals are added to sediment samples.

8.4.8 From field, sediment samples will be sent to the lab for subsampling and archiving according to the applicable procedures described in Charles and Whitehead (l986). Samples are stored in small plastic bags in plastic insulated boxes at cold temperature (2-5°C; not frozen) to prevent evaporation. Complete records are maintained to identify and track sediment samples. These include field and coring data forms, as well as custody forms. Copies of these documents are appended to this document.

9. QUALITY CONTROL

9.1 Coring QA:

9.1.1 Coring QA is designed to evaluate and assess variability due to differences in sediment stratigraphy at different locations within a lake, and differences due to the coring process itself. The design of a QA/QC program will depend on the size and objectives of a study. For example, Charles et al. (1991) addressed problems related to the variability of surface sediments from different locations in a lake, and the reliability of a single sediment core from the deep basin of an acidic lake. Davis et al. (1990) compared stratigraphic interpretations from triplicate cores from acidic lakes in northern New England. Anderson (1989) undertook a study of multiple sediment coring in a recently eutrophied lake system. Kreis (1986, 1989) specifically addressed potential sources of variability encountered during coring and sectioning for the PIRLA-I project and Cumming et al. (1990, 1992) for the PIRLA-II project. Researchers generally show that single sediment cores collected for the deep water basins of lakes are adequate for overall measures of diatom community change. In EMAP-SW the question of spatial variability is further evaluated by taking triplicate cores from three lakes during each field sampling program. These cores will be taken from the deep basin. Tops and bottom samples of these cores will be analyzed.

9.1.2 Preventative maintenance: Coring equipment should be cleaned and inspected before and after each use. In addition, identical back-up corers and extruders should always be transported to the study area. John Glew has already made 13 modified K.B. corers, 1 Glew mini corer, and appropriate extruders for the EMAP-SW. These equipments have been successfully used during the 1991, 1992, and 1993 field sampling in the northeast.

9.2 Diatom QA:

9.2.1 EMAP-SW sediment digestion method for diatoms was developed at the Academy of Natural Sciences of Philadelphia, and has been used since 1992 for the preparation of diatoms for taxonomy, community analysis and archival purposes. The size of samples, amount of acid, boiling times and settling times have been adjusted to insure a near complete recovery of diatom frustules from samples while allowing for the efficient use of the work day. It should be understood that this procedure does not result in 100% recovery of all diatoms contained within the original sample, especially for samples with lightly silicified forms. However, the variance introduced by this cleaning procedure has been shown to be significantly less than the variance to be expected from replicate field samples. Observed loss of material at the boiling or transfer stages of this procedure compromises quantitative samples. Samples which experience such loss should be discarded immediately if sufficient material has been retained to allow for a fresh sample. If the compromised sample represents the only material available from the station and date in question, the procedure should be completed, and the nature of spillage or loss should be clearly documented.

9.2.2 Diatom frustules are microscopic, generally falling in the fine silt size range, therefore, during digestion and slide preparation the possibility for contamination of samples is great. Laboratory rooms where raw or processed samples are handled should be kept as clean as possible. Lab bench surfaces should be kept clean and free of debris. Techniques similar to those used for sterile experiments (bacteriological plating, etc.) should be followed to minimize the risk of cross contamination of samples. Where feasible, disposable pipettes, stirrers, etc. should be used.

9.2.3 New glassware and digestion vessel liners should be washed and/or rinsed prior to use. Used glassware should be vigorously scrubbed, washed and rinsed with DW to prevent contamination. Previously used digestion vessel liners should be washed with a detergent and mild brush (beware of abrasives, however) and rinsed with DW. Note: at times tap water, because of algal blooms and use of diatomaceous earth filters, may contain diatoms.

9.2.4 Three top and three bottom sediment samples from three lakes will be analyzed to provide data on within lake variability. Triplicate subsamples from both top and bottom samples of one core will be analyzed to assess within core and processing variability. Replicate slides of three separate samples will be analyzed by three counters to assure quality and consistency of taxonomy.

9.2.5 A photographic log of difficult diatom taxa will be maintained, which can be exchanged with other diatom taxonomists. The new photomicrographs will be used in conjunction with those already prepared for the PIRLA-I Diatom Iconograph (Camburn et al. 1984-1986) which

depict many specimens from the eastern United States, the northern Great Lakes States, and Florida, and the PIRLA-II Iconograph for the Adirondacks (Camburn in prep.). Dr. Charles Reimer at ANSP will provide special assistance for difficult taxa. Both Queen's and ANSP contain all of the up-to-date taxonomic literature, including a very extensive photographic and slide library. The ANSP collection is the largest in North America, and the Queen's collection is almost certainly the largest in Canada.

9.2.6 After the slides are counted for diatoms, one set of slides and a corresponding aliquot of dried sediment will be curated at the ANSP Diatom Herbarium. Computer data files describing the diatom slides and material (e.g. location, project, counter, etc.) will be added into Herbarium data base. Accession numbers for the slides will be provided along with adding cards to the card files. These curated EMAP-SW slides will be available to fellow diatom researchers. One complete set of diatom slides will be sent to the EPA Laboratory responsible for coordinating the diatom study and one set of slides will be retained at the lab doing diatom analysis.

9.2.6 Preventative maintenance: Research microscopes should be routinely maintained before and after each use; professional maintenance in response to serious difficulties (for example cleaning an objective internally) is done by the product vendors on an "as needed" basis.

9.3 Dating QA: Once received, the samples are logged, in pen, into a page-numbered lab notebook. The contract information and sample information is logged in as follows:

9.3.1 Date when samples are received and by whom.

9.3.2 The customer's name, address, phone number, FAX, and Email address.

9.3.3 The number of samples received.

9.3.4 The markings of the samples received.

9.3.5 The samples are assigned a number or other such marking system used in the lab.

9.3.6 The requested completion date, and the computer disk number and file name to be used for the work.

9.3.7 A section in the notebook notes all materials and supplies purchased against the order number as well as all technical and professional hours charged against the work.

9.3.8 Strike-overs and overwrites are initialled by the person assigned to the analysis.

9.3.9 Counting results are noted in the notebook and computer file. Repeat sample counts or repeat analysis are recorded. Out of every 10-14 samples, one sample is analyzed in duplicate, and if the result exceeds the norm, a second duplicate is done.

9.3.10 A set of procedural blanks is used to monitor the background. Counter backgrounds are also run over the weekends. Duplicate standard samples are done with each set of samples.

9.3.11 The glassware used for Pb-210 analysis is used at random for the samples, blanks, and standards. The glassware is only used for Pb-210 analysis.

9.3.12 All counting blanks, backgrounds, and standard sample results are recorded with the data files as well as in their respective summary files so a summary graph can be provided appropriate to the analysis dates of samples.

9.3.13 Except where noted, no counter background corrections are applied as these values are 1% of the lowest Pb-210 concentrations for the majority of samples analyzed in the lab.

9.3.14 The analysis report is reviewed by two professionals in accordance with lab policy.

9.3.15 Preventative maintenance: Equipments used for sediment dating work are continuously maintained; problems are recognized if and when calibrations of standard samples deteriorate, and remedial action is taken before any new samples are analyzed. A logbook of all maintenance and equipment changes is kept.

10. CALIBRATION AND STANDARDIZATION

In all Pb-210 analyses, standard additions are made and the activity of the standard and the unknown is determined simultaneously by alpha spectroscopy. As a result, the entire analysis has a built-in calibration. In addition, instrument calibrations for each batch of samples with standards of known activity are tested. The efficiency of chemical procedures for each sample is monitored.

11. PROCEDURES

11.1 Preparation of Subsamples: This procedure covers subsampling lake sediments for diatom, pollen and Pb-210 dating analyses and for archival purposes, and determination of wet and dry weight of sediment. The method is based largely on the PIRLA-I and PIRLA-II projects. In EMAP-SW the top and bottom samples are used for Pb-210 dating and diatom analyses, and bottom samples are used for pollen analyses.

11.1.1 Prior to subsampling, the type of subsamples taken from each sample must be determined. This is necessary to assure that the material in the sample is used efficiently.

11.1.2 Obtain samples from storage, place adhesive labels (2.5-cm x 10-cm; can be pre-printed) on each sample bag to record subsample dates and amounts and initials of person taking subsample(s). Note nature and condition of each sample (e.g. color, texture, presence of clumps of algae and other organic and inorganic particles).

11.1.3 Prepare several sets of syringes (1 and 5-ml) by cutting off the tip portion (i.e., cut at the 0 mark).

11.1.4 Using a permanent laboratory marker, label each crucible with a unique number. Soak crucibles in a bath of distilled water and laboratory detergent (e.g. Alconox) for 10 minutes and wash with a sponge or bristle brush. Rinse with DW and place in a drying oven set at 90°C. After crucibles are dry, turn off drying oven and allow crucibles to cool to room temperature (20°C).

11.1.5 Prior to adding sediment, weigh each crucible and record on sediment weight data sheet. Using plastic spatulas and/or stirring rods, remove enough sediment from the sample bag to result in approximately 1 g when dried at 90°C. Note: the amount of water in sediment samples is variable; this step becomes easier with experience.

11.1.6 Place sediment into a pre-weighted crucible and weigh the crucible with the wet sample and record on the sediment weight data sheet. After placing aluminum foil over the top of the crucible, put the crucible with the wet sample in a drying oven (90°C). The sample should dry in 12 to 36 hrs. When dry, allow crucible and dry sample to come to room temperature (20°C). Remove the aluminum foil and immediately weigh and record the weight of the crucible and dry sample. Transfer the dried sediment to 20-ml pre-pre-labeled glass vials. When transferring, scrape the sides of the crucible and break up the sediment with a pestle or stirring rod (the sample does not need to be crushed to fine particle size - this step is performed at the dating lab).

11.1.7 For diatom work remove 5 ml sediment from the sample bags using the 5 ml syringe and transfer the samples carefully to the 20-ml glass vials. Etch (diamond scribe) the sample identifier (lake ID and tracking number) on the side of the vial and with the medium point laboratory marker label the vial cap. Use distilled water to wash excess material from the syringe to vial. Clean and rinse each syringe in distilled water before reusing on another sample.

11.1.8 For pollen work remove 2 ml of wet sediment into 1 dm glass vials using 5-ml syringe. Use distilled water to wash excess material from the syringe to vial. Tape a small adhesive label with sample identifier and sample location by wrapping the transparent tape around the vial so that it overlaps (this will help minimize the possibility that the label will be lost).

11.2 Diatom Analysis: Slide Preparation and Counting: In order to perform diatom community analysis on the species level, it is necessary to prepare diatom samples prior to slide mounting. The cleaning procedure is necessary to remove both extracellular and intracellular organic matter from the siliceous valves (frustules) of the diatoms as well as to rid the sample of extraneous organic material that may be present. Sediment sample digestion for diatom analysis will follow the basic protocols outlined in Charles and Whitehead (l986) to make quantitative slides, with exception that digestions will be done using a specifically designed microwave (CEM model MDS-2100) with enclosed vessels and temperature and pressure monitoring and control systems instead of a hot plate.

11.2.1 The first step is to insure adequate concentration of the sample so that sufficient diatoms will be processed and available for analysis. On the other hand, samples must be of reasonable size for the cleaning procedure to be effective in removing most organics. A technician's skill in judging the proper sample size grows with experience.

11.2.2 Approximately 1 ml of wet sediment is transferred to a 100 ml beaker. About 10 ml of distilled water is added to bring the sample in disaggregation.

WARNING: THE FOLLOWING PROCEDURE IS TO BE PERFORMED ONLY IN A POSITIVE-DRAW CHEMICAL HOOD. TECHNICIANS ARE REQUIRED TO WEAR SAFETY GLASSES AND PROTECTIVE GLOVES!

11.2.3 Place the sample into the liner portion of a labeled microwave digestion vessel (record vessel number on preparation form). To the sample, in the vessel liner, add concentrated nitric acid, the amount equal to the amount of sample. If the sample contains a very high amount of organic material, more acid can be added (routinely the sample is in a 10 ml water matrix requiring the addition of 10 ml nitric acid). Initially, acid should be added very slowly and with great caution anticipating that an unexpected reaction may take place. After determining that there is no possibility of a violent reaction, the remainder of the acid can be slowly and cautiously added to the samples.

11.2.4 Preparation, assembly of digestion vessels and connecting to the microwave apparatus:

11.2.4.1 Before the assembly of the digestion vessels, new rupture membranes must be seated in the vessel liner covers as illustrated in Figure 6. Make sure that there is only one rupture membrane seated in each vessel liner cover.

11.2.4.2 Once the rupture membrane is seated, thread the vent stem back into the vessel liner cover until hand tight. Do not apply excess pressure or use a wrench to tighten the vent stem!

11.2.4.3 Place the vessel liner cover (with rupture membrane and vent stem installed) on top of the vessel liner. Thread the vessel cap onto the vessel body (by turning in a clockwise direction) until hand tight. Insert the vent tube into the small exit port located in the side of the vessel cover extending above the vessel cap.

11.2.4.4 Place the complete vessel assembly into a turntable, orienting the vent tube towards and into the collection vessel at the center of the turntable. For the remaining vessels repeat steps in subsections 11.2.4.1 through 11.2.4.4.

11.2.4.5 Place turntable with vessels into the microwave apparatus cavity on it's drive lug. Turn on microwave apparatus and rotate the turntable. After confirming the operation of the turntable, rotate so that the vessel with the temperature and pressure sensors is at 12:00 (as looking into the microwave cavity).

11.2.4.6 Bleed and connect pressure sensing line: Using keypad controls and menu system, rotate turntable to 9:00. Turn pressure valve (outside left of microwave cavity) to open. Tap pressure sensing line to get air bubbles to connection end. With the syringe filler (outside left of microwave cavity), flush air bubbles out of the pressure sensing line. Connect pressure sensing line to the pressure port on the temperature and pressure vessel liner cover (use only hand pressure!) and place pressure sensing line in center post of the turntable. Turn pressure valve to

neutral. Connect fiberoptic temperature probe: Without bending, place fiber optic probe (note: it is glass!) into the thermowell of the temperature and pressure vessel liner cover and connect (use only hand pressure for the connection!); thread probe into center of the turntable, similar to the pressure sensing tube.

11.2.4.7 Configuring the microwave apparatus: From the main menu, choose either to load a preexisting program or to enter new operating conditions (and possibly a new program) from the keypad. In the view mode, use the arrow keys and numeric keypad to set the following operating conditions: Cycle 1 - 25% power, 20 PSI, 5 min @ pressure; Cycle 2 - 62% power, 40 PSI, 5 min @ pressure; Cycle 3 - 87% power, 60 PSI, 20 min @ pressure; Adjust power settings for number of vessels by reducing 3% for each vessel less than a full tray (12 vessels); Record additional information in the "Sample Information" screen.

11.2.4.8 Make sure printer is connected and on, then print out operating conditions and sample information as displayed.

11.2.5 Start the microwave apparatus; the program will take about 60 min.

11.2.6 After temperature and pressure conditions are back to normal levels (30-45 min): Place the vessel with the temperature and pressure controls at 9:00. Vent the vessel with the temperature and pressure controls by turning (counter- clockwise) the vent screw. Carefully take out the fiberoptic probe and place in one of the holes near the top of the microwave cavity. Remove pressure sensing line and place away from the turntable. Remove turntable from microwave cavity and place in chemical hood; carefully vent each of the vessels.

11.2.7 Remove vessel caps, vessel liner covers and transfer sample to a tall 100 ml beaker, washing liner with DW from wash bottle; fill beaker with DW; be sure to record vessel and beaker numbers on preparation form!

11.2.8 Decanting procedure: After 8 hours, the supernatant is siphoned off without disturbing any of the material that has settled to the bottom. In the siphoning process, the tip of the siphon should be placed just beneath the water's meniscus and moved slowly down as the water level drops to prevent loss of material through water column turbulence. As much water as possible should be removed without disturbing the sediment material. This settling and siphoning procedure is repeated at least three more times or until a pH above or similar to that of the DW (above 6.0) has been achieved.

11.2.9 Transfer procedure: The cleaned material left in the bottom of the beakers after the final siphoning is then carefully swirled and poured into a 20 ml glass vial which has been previously labeled (sample number, station (sub-station and date with a diamond scribe)). Using a wash bottle containing DW, remaining material adhering to the beaker sides is washed into the vial, and the volume of each vial is brought to 20 ml. The vial is capped and set aside for the slide making procedure.

11.2.10 An aliquot of clean diatom sample is diluted with DW and poured into evaporation trays containing four circular (22 mm diameter) glass cover slips, similar to those described by Battarbee (1973). Samples are left to evaporate in a dust and disturbance free environment. Upon drying diatom coated coverslips are mounted on glass slides using Hyrax^R mounting medium. Four slides are made for each sediment sample processed.

11.2.11 At least 500 diatom valves are identified and counted from each of the samples under oil immersion at magnifications of 1000X or higher. Identifications are made to the lowest taxonomic level (e.g. variety). Scanning electron microscopy should be used when required to confirm taxonomic identifications.

11.3 Pollen Analysis: All bottom samples are analyzed for pollen to determine the abundance of ragweed pollen.

11.3.1 Approximately 1 ml of sediment is placed in a 15 ml polypropylene centrifuge tube and successively treated with 10% HCl, 10% KOH (placed in a boiling water bath for 20 minutes), glacial acetic acid, and acetolysis solution (concentrated H_2SO_4 and acetic anhydride (1:20 v/v, placed in a boiling bath for 3 minutes).

11.3.2 The resulting slurry is mounted onto a microscope slide. For each sediment sample a minimum of 350 pollen grains are enumerated at 400X magnification using bright field optics. The relative abundance of ragweed pollen to other pollen will be used to determine if the bottom sediments are in fact "pre-cultural".

11.4 Radioisotope Dating: The bottom sediment samples of EMAP-SW project are dated following the method of the Atomic Energy of Canada Limited, Chalk River, Ontario.

11.4.1 The bottom sediment samples are dated by measuring Pb-210 and Ra-226. The limit of sediment core dating by the Pb-210 method is reached when the activity of Pb-210 is equal to that of Ra-226 at the same depth. The concentrations of Pb-210 in sediment samples is measured by assay for the concentration of Po-210. Secular equilibrium between the Pb-210 and Po-210 is assumed in all samples. The concentration of Po-210 is measured using the method of Eakins and Morrison (1978). Slight modifications have been made to the above protocol that include:

11.4.1.1 The samples are volatilized at higher temperatures (>625°C).

11.4.1.2 The concentration of ascorbic acid would be increased if metal concentrations are high in samples because of their interference with the plating of polonium.

11.4.1.3 H_2O_2 is added to the oxidizing solutions to destroy additional organic matter that also appeared to be carried through the analytical procedure.

11.4.1.4 Samples are plated in 1.5 normal HCl onto 1/2 in^2 silver disks. The samples are then counted between 8 and 36 hours on a low-level alpha spectroscopy system. The system consists of gold foil surface barrier detectors placed in stand alone nucleus alpha spectrometers.

11.4.1.5 The analytical method used for radium determination includes addition of a yield tracer, followed by removal of uranium and thorium by precipitation and/or ion exchange. The Ra is subsequently co-precipitated with $BaSO_4$ and mounted for alpha spectrometry.

11.5 Data Reporting and Handling:

11.5.1 Data are obtained from three main sources: (1) diatom data; (2) dating information; and (3) water chemistry and other lake and watershed information. In addition, diatom water chemistry calibration data sets which are in the PIRLA data base (Charles et al. 1989) will be also utilized. The existing PIRLA data base is used to store and manage the EMAP-SW diatom data. Data entering into the PIRLA data base from the original count sheets is checked for correct entry by data input personnel. These counts are then sent to the original counter in order to ensure accurate input. This double-checking ensures accurate data input. The creation of a powerful and flexible data base management system (DBMS) requires a great deal of time and effort. Charles et al. (1989) describe the PIRLA data base, which is running at Queen's. The PIRLA DBMS uses the Scientific Information Retrieval system (SIR) and provides output of system files for direct statistical analysis by SAS, SPSS, BMDP, CCA, WACALIb, and other packages.

11.5.2 Attachment A shows the form used for field notes and sediment coring.

11.5.3 Attachment B shows the form used for sediment distribution and processing.

11.5.4 Attachment C shows the sediment request form.

11.5.5 Attachment D shows the custody form for EMAP-SW sediment samples.

11.5.6 Attachment E shows the form for recording sediment weight.

12. DATA ANALYSIS AND CALCULATIONS

12.1 One of the most effective means of studying diatom populations with respect to lakewater quality and/or biotic integrity is to calibrate diatom assemblages deposited in the top 1-cm sediment slice (representing the last few years of sediment accumulation) from a set of lakes with known lakewater chemical and physical characteristics (Dixit et al. 1992).

12.2 Multivariate direct-gradient analysis techniques, such as canonical correspondence analysis (CCA) (ter Braak 1986), will be applied to examine which environmental variables correlate most closely with the distributions of diatom taxa. Simultaneous ordination of both taxa and samples that can be related directly to environmental variables will be generated. Forward selection and Monte Carlo permutation tests (ter Braak 1988) will be used to test the significance of selected environmental variables (e.g. Dixit et al. 1991, 1992; Hall and Smol 1992).

12.3 The optima and tolerances of diatom taxa will be utilized to identify indicator taxa for significant environmental variables in any particular region. Region specific indicator assemblages will be also identified.

12.4 Using the top sediment diatoms, inference models will be developed to infer environmental variables. Weighted-averaging calibration and regression (WACALIB) (Line and Birks 1990) approach will be used for this. This approach is based on a species-packing model along environmental gradients, assuming a unimodal distribution of the abundance of individual species along the environmental gradients of interest. In the regression step, the optimum and tolerance parameters for each taxon are estimated from present-day (i.e. top sediment) distributions. In the calibration step, the optima and/or tolerance values of each taxon are used to estimate an inferred value of a sample based on its species composition.

12.5 Region specific indices will be developed for trophic state, acidity, and salinity, etc.

12.6 Diatom data will be used to provide information on such indicator metrics as similarity coefficients, species richness, percent sensitive species, early warning indicator taxa, and so on.

12.7 Using species richness, diversity, DCA sample scores, and multiple inferred environmental variables, an index of biotic integrity/disturbance will be developed.

12.8 Diatom assemblages from the bottom samples, representing pre-European settlement conditions, will be analyzed for all the lakes sampled. Pre-settlement lake condition will be established both qualitatively and quantitatively. A qualitative estimate of reference condition will be made simply by examining indicator taxa, indicator assemblages, and species richness and diversity. Quantitative estimates will be made by applying various weighted-averaging calibration models and indices developed from the top sediment study. Pre-settlement lake conditions will be compared with current conditions, and changes in lakewater quality will be assessed. The relative importance of the causes of changes in the lakes will be identified where possible (using indicator taxa and assemblages, and by placing bottom samples in our CCA runs as passive samples and examining the trajectory in relation to environmental arrows), and an assessment will be made of the potential sensitivity and responsiveness of lakes to future anthropogenic activity.

12.9 For top and bottom samples cumulative frequency diagrams will be drawn for several environmental characteristics and will identify what percentage of study lakes have declined in water quality, or have remained in steady state, or have improved.

13. METHOD PERFORMANCE

13.1 As with past EPA funded PIRLA-II and EMAP-SW research, all efforts will be made to keep abreast with ecologically valid, up-to-date methods of diatom models. Ordination techniques for ecological analyses have been designed (ter Braak 1987) and described within the context of other ordination techniques (Jongman et al. 1987). In addition, there are now a number of computer intensive methods (e.g. Monte Carlo simulation, bootstrapping, etc.) which will be utilized to develop reliable error estimates for inferences (Birks et al. 1990a,b). Time to time experts will be consulted to review experimental design and assist with data analysis as a prudent measure to ensure appropriate state-of-the-art analyses. QA of the environmental data (e.g. chemistry, map information) must also be rigorous.

13.2 Sediment dating:

13.2.1 Duplicate analyses are performed regularly. For example, an estimate of the precision for a recent work, where the average coefficient of variation for 11 sets of duplicates is computed, shows the average coefficient of variation was 3.2%. This is roughly equal to the uncertainty introduced by the counting statistics. Usually 100s to a few thousands counts of polonium are collected. For 1000 counts, the uncertainty is 3%.

13.2.2 When calculating absolute accuracy, it is important to point out that some uncertainty may be associated with the use of a standard solution which has an uncertainty of about 5%. However this error does not contribute to uncertainty in the analysis of sediment accumulation rates or dates because the relative values are used in these calculations rather than the absolute values.

13.2.3 Accuracy of the analyses: In each batch of samples two standard reference materials are analyzed for Pb-210; certified by the NBS (National Bureau Standards) and CANMET (Canada Mineral Energy and Technology) in roundrobin interlaboratory comparisons. This allows to evaluate the accuracy of each set of samples. For example, the value (0.62 + 0.045) obtained for one standard (CANMET CLV-1) recently was close to the accepted value of 0.66 + 0.023, indicating that the results are consistent with those of other laboratories.

13.2.4 Lower limit of detection:

13.2.4.1 The lower limit of detection is set by process and detector backgrounds. In all samples that have been analyzed to date, the Pb-210 activity in sediments is at least 10 times the lower detection limit.

13.2.4.2 With each set of samples, process backgrounds are analyzed, and detector backgrounds are measured at least once a month. A figure showing the long-term stability in our backgrounds is presented below. The process backgrounds are very rarely greater than the counter background. To calculate the lower limit of detection the background for a two day count is calculated and the definition of LLD is set as three times the background standard deviation. The average values of counting efficiency and radiochemical yield are used. The LLD for various detectors used in the lab range from 0.001 to 0.005 Bq/g. Pb-210 concentrations in sediments are usually 1.0 to 0.02 Bq/g.

14. POLLUTION PREVENTION

The microwave sediment digestion technique uses only about one-quarter of the usual amount of acid used in traditional digestion methods, and there is no venting of fumes to the atmosphere. Air flow is monitored in fume hoods.

15. WASTE MANAGEMENT

Labs performing sediment digestion and dating follow government approved protocols for handling waste. Detail guidelines can be obtained from labs.

16. REFERENCES

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Attachment A: Form for Field Notes and Sediment Coring

Well-organized, accurate, and complete field notes are essential for paleolimnological studies, and an important QA consideration. These notes will provide valuable information that will be required in interpreting the results. All field notes should include, at an absolute minimum, the following information:

Lake Name:	Date Core taken:
Lake location, State:	County:
Township:	State number:
Latitude:	Longitude:

Depth of water in which core was taken:

Location of coring site:
(attach lake morphometric map, if available, or other map showing site location, indicate how location was determined (landmark used for triangulation))

Coring crew:

Weather:

Length of core: cm Core number:

Sediment intervals:

Sediment-water interface (sharpness, disturbance, slope, presence of organisms, evidence of bioturbation etc.):

Texture, color, and large objects (e.g. leaves); indicate changes with depth:

Notes on special problems:

Attachment B: Sediment Distribution and Processing Form

The modified K.B. gravity corer used in the surface water EMAP will obtain 32 cm^3 of sediment in each 1 cm section of the core.

Total Wet Weight of the Sample:__________g

Sediment Sample Distribution

Diatoms (1 cm^3)

Pollen (2 cm^3)

Sediment dating (0.50 g dry sediment, dried at 90°C, for dating of bottom samples.

Archive remaining wet sample in cold room.

Attachment C: Sediment Request Form

Lake Name: EPA Region:

Core #: EMAP Code #:

Coring Depth:

Date of Coring:

P.I. Making Request: Date:

Depth:	Condition of sediment	
	Amount	
Interval (cm)	Wet	Dry
(g or ml)		

Attachment D: Custody Form for EMAP-SW Sediment Samples

After adding information to this form return a copy to the originator.

EPA Region:
Lake:
EMAP Code #:
Core #:
Date Cored:

Subsamples being transmitted:
Total Number Subsamples:

Missing Subsamples:

From:
To:

Sediment condition (wet, dry, ash) container type comments, special instructions

Originator:

Sig:	Date:
Sig:	Date Rec:

Originator:

Sig:	Date:
Sig:	Date Rec:

Originator:

Sig:	Date:
Sig:	Date Rec:

Originator:

Sig:	Date:
Sig:	Date Rec:

Attachment E: Sediment Weight Record

Academy of Natural Sciences
Division of Environmental Research
Patrick Center for Environmental Research
Phycology Section

SEDIMENT WEIGHT RECORD: EMAP- SURFACE WATERS (ANSP-EM-94-____-WT)

DATE SUBSAMPLES: _____/_____/199___ BY: ___________

Tracking/ Sample Number	Lake ID	Depth Interval (cm)		Crucible Number	Crucible Weight (g)	Wet Weight Sediment & Crucible (g)	Dry Weight Sediment & Crucible (g)
		top	bottom				
EM							
EM							
EM							
EM							
EM							
EM							
EM							
EM							
EM							
EM							
EM							

TIME SAMPLES PUT INTO DRYING OVEN: _____ ___/___/199___ REMOVED: _____ ___/___/199___

*************** End of Diatom Methods ***************

Academy of Natural Sciences
Division of Environmental Research
Patrick Center for Environmental Research
Phycology Section

SEDIMENT WEIGHT RECORD: EMAP- SURFACE WATERS (ANSP-EM-94-___-WT)

DATE SUBSAMPLES: ____/____/199___ BY: _________

Tracking/ Sample Number	Lake ID	Depth Interval (cm)		Crucible Number	Crucible Weight (g)	Wet Weight Sediment & Crucible (g)
		top	bottom			
EM						
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TIME SAMPLES PUT INTO DRYING OVEN: ____ ___/___/199__ REMOVED: ____ ___/___/199___