DRAFT UPDATE IVA
Cover Sheet

THIS PACKET CONTAINS NEW AND REVISED MATERIAL BEING CONSIDERED FOR INCLUSION IN:
TEST METHODS FOR EVALUATING SOLID WASTE PHYSICAL/CHEMICAL METHODS (SW-846) THIRD EDITION

Contents:

1. Cover sheet. (What you are currently reading)

2. Instructions. Read this section! It explains how Draft Update IVA relates to the rest of your SW-846.

3. Draft Update IVA Table of Contents. The Table of Contents (dated January 1998) lists all of the methods (Third Edition, Updates I, II, IIA, IIB, III, and Draft Update IVA) in the order in which they will appear in the manual when Update IVA is finalized.

4. Revised Chapter Two: Choosing the Right Method

5. Revised Chapter Three and new/revised methods for inorganic analyses.

6. Revised Chapter Four and new/revised methods for organic analyses.

7. Revised Chapter Five and a new method for miscellaneous analyses.

INSTRUCTIONS

SW-846, a methods manual, is a "living" document that changes when new data and advances in analytical techniques are incorporated into the manual as new or revised methods. To date, the Agency has formally issued proposed and promulgated Updates I, II, IIA, IIB, and III. This package contains Draft Update IVA. For specific and important information regarding this update, please read the section below entitled "About Draft Update IVA."

These instructions describe how to get your basic manual up-to-date and what to do with your Draft Update IVA package. Additional updates will be released by the Agency in the future. New instructions, to supersede these, will be included with each of those new update releases. In general, final updates should always be incorporated into SW-846 in chronological order (e.g. Update I should be incorporated before Update II).

The following definitions are provided to you as a guide:

New subscribers are defined as individuals who have recently (6-8 weeks) placed an order with the GPO and have received new copies of the 4 (four) volume set of the Third Edition, a copy of Final Update I, a copy of Final Update II/IIA, a copy of Final Update IIB, a copy of Update III, and a copy of Draft Update IVA.

Previous subscribers are defined as individuals that have received copies of the Third Edition and other SW-846 Updates (including proposed Updates) in the past and have just received their Draft Update IVA package in the mail.

BACKGROUND INFORMATION

A number of SW-846 update packages have been released to the public since the original Third Edition was released. The dates and labels on these packages can be confusing. The following is a brief summary of what new subscribers and previous subscribers should check upon receipt of the Draft Update IVA package:

NEW SUBSCRIBERS - If you are a new subscriber, you should perform the following tasks before addressing your new Draft Update IVA:

- Place the original Third Edition of SW-846 (September 1986) in the properly labeled four 3-ring notebooks according to the instructions in Update III.
- Incorporate Final Update I (July 1992) into the manual according to the instructions in Update III.
- Incorporate Final Updates II (September 1994) and IIA (August 1993) into the manual according to the instructions in Update III.
- Incorporate Final Update IIB (January 1995) into the manual according to the instructions in Update III.
- Incorporate Final Update III (December 1996) into the manual according to the instructions in Update III.

PREVIOUS SUBSCRIBERS - If you are a previous subscriber, it is important to establish exactly what is currently contained in your manual before addressing Draft Update IVA. If your manual is properly updated, the ONLY white pages in the document should be dated September 1986 (Third Edition), July 1992 (Final Update I), August 1993 (Final Update IIA), September 1994 (Final Update II), January 1995 (Final Update IIB), and December 1996 (Final Update III). Remove (and recycle or archive) any white pages from your manual that have any other dates. There may also be colored pages (e.g., pink pages for Proposed Update III) inserted in the manual. Remove all yellow, blue, green, or pink pages from the manual. These colored pages represent versions of methods and chapters that are not final. (Some individuals may chose to keep their copies of colored versions in separate binders.)

UPDATE HISTORY OF SW-846

The table below can be used as an aid to understanding the update history of SW-846, Third Edition. Finalized updates are printed in bold and underlined. An individual or organization that has held an SW-846 GPO subscription for several years may have received copies of any or all of the updates.

A BRIEF HISTORY OF THE SW-846, THIRD EDITION AND ITS UPDATES			
Package	Date Listed on Methods	Color of Paper	Status of Package
Third Edition	September 1986	White	**Finalized (Promulgated)**
Proposed Update I	December 1987	Green	Obsolete
Final Update I (Released by accident)	November 1990	White	Obsolete! Never formally finalized.
Proposed Update II (Released by accident)	November 1990	Blue	Obsolete! Never formally proposed.
Final Update I	July 1992	White	**Finalized (Promulgated)**
Proposed Update II	November 1992	Yellow	Obsolete
Proposed Update IIA* (Available from EPA by request only.)	October 1992	White	Obsolete
Final Update IIA* (Included with Final Update II.)	August 1993	White	**Finalized (Promulgated)**
Final Update II	September 1994	White	**Finalized (Promulgated)**
Final Update IIB**	January 1995	White	**Finalized (Promulgated)**
Proposed Update III	January 1995	Pink	Proposed
Final Update III	December 1996	White	**Finalized (Promulgated)**
Draft Update IVA	January 1998	Salmon	Draft

* Contains only Method 4010.

** Contains only a revised Table of Contents, a revised Chapter Six, and revised Methods 9040B and 9045C

ABOUT DRAFT UPDATE IVA

Draft Update IVA has been issued by the EPA's Office of Solid Waste and contains methods which are being considered for inclusion someday in the SW-846 methods manual. The Draft Update IVA package includes 15 revised methods, four revised chapters, a revised Table of Contents, and 13 new methods. In addition, Draft Update IVA involves the removal or integration of 44 other methods. Please see the section below entitled "Removal or Integration of Some Methods in SW-846."

In order to distinguish this draft update from other SW-846 updates, it has been printed on salmon-colored paper. (Final updates are printed on white paper.) The date "January 1998" is found in the lower right-hand corner of each page in the Draft Update IVA package. This date should be used to definitively distinguish the Draft Update IVA versions of SW-846 methods from previous and future versions.

A notice announcing the availability of Draft Update IVA has been published in the *Federal Register* and invites public comment on its content. EPA has published the notice and made this draft update available for informational purposes only, and is not at this time formally proposing to revise SW-846 by adding Update IVA to it or to incorporate the update in the RCRA regulations for required uses of SW-846 methods. EPA is only making the Agency-reviewed methods of Update IVA available to the public early, for guidance purposes.

The aforementioned notice fully explains the Agency's plans regarding this update, and EPA encourages the public to read the notice. As explained in the notice, several regulations under subtitle C of RCRA currently require that certain SW-846 methods be employed. However, any reliable analytical method may be used to meet other requirements in 40 CFR parts 260 through 270. The methods of Update IVA fall in the category of "any reliable method." The methods may currently be used in all applications for which the use of SW-846 methods is not mandatory. The methods of Draft Update IVA, however, cannot be used for compliance with required uses of SW-846 methods. The Agency also recommends that the regulated community obtain permission from the appropriate regulating entity, if required under state or local regulations, before using these methods for non-mandatory applications.

A copy of the *Federal Register* announcing the availability of Draft Update IVA (and copies of other *Federal Register* documents) can be accessed from EPA's web site at the following two Internet locations:
http://www.epa.gov/epahome/rules.html
http://www.epa.gov/fedrgstr

REQUEST FOR PUBLIC COMMENTS ON DRAFT UPDATE IVA

The EPA is requesting public comment on the methods in Update IVA (e.g., requesting suggestions or recommendations regarding the content of the methods). Comments on the suggested changes to the Draft Update IVA methods package, and other topics addressed in the Update IVA *Federal Register* notice, must be submitted within the time period specified in the "Dates" section of the notice. SW-846 methods not contained in this package are not open to comment.

The Agency is interested in comments on the content of all sections or parts of new methods to SW-846 found in Draft Update IVA. Regarding the revised methods and chapters, the Draft Update IVA *Federal Register* notice contains a table identifying those parts of each document which are open for comment. EPA is interested in comments from the public on only the identified parts because other parts of the methods have not been significantly revised from the promulgated version currently in SW-846. Please see the Draft Update IVA *Federal Register* for this information before developing and submitting comments on revised methods found in Draft Update IVA.

HOW TO SUBMIT COMMENTS

As explained in the "Addresses" section of the Draft Update IVA *Federal Register* Notice, commenters must send an original and two copies of their comments referencing docket number F-98-4TMA-FFFFF to: RCRA Information Center (RIC), Office of Solid Waste (5305G), U.S. Environmental Protection Agency Headquarters (EPA, HQ), 401 M Street, S.W., Washington, DC 20460. Courier deliveries of comments should be submitted to the RIC at the address listed below.

Comments may also be submitted electronically through the Internet to the following address: RCRA-docket@epamail.epa.gov. Comments in electronic format should be identified by the docket number F-98-4TMA-FFFFF. Submit electronic comments as an ASCII (TEXT) file and avoid the use of special characters and any form of encryption. EPA's Office of Solid Waste (OSW) also accepts comments and data on diskettes in WordPerfect 6.1 file format. On the disk label, specify the commenter's name and the word processing software and version/edition.

Commenters should not submit electronically any confidential business information (CBI). An original and two copies of the CBI must be submitted under separate cover to: Regina Magbie, RCRA CBI Document Control Officer, Office of Solid Waste (5305W), U.S. EPA, 401 M Street, S.W., Washington, DC 20460.

Public comments and supporting materials are available for viewing in the RIC, located at Crystal Gateway One, 1235 Jefferson Davis Highway, First Floor, Arlington, Virginia. The RIC is open from 9 a.m. to 4 p.m., Monday through Friday, except for Federal holidays. To review docket materials or make photocopies, the public must make an appointment by calling 703-603-9230.

REMOVAL OR INTEGRATION OF SOME METHODS IN SW-846

The Agency is also requesting comment on the removal of one obsolete headspace method and the removal of 43 atomic absorption methods. The individual methods are being deleted as part of Draft Update IVA because their inclusion is redundant given that their procedures and target analytes have been fully integrated into revised Method 7000B and new Method 7010, the general methods for the techniques. The table to follow is a list of the methods being considered for deletion from the manual.

METHODS BEING CONSIDERED FOR REMOVAL FROM SW-846	
Method No.	Method Title
3810[a]	Headspace
7020[b]	Aluminum (Atomic Absorption, Direct Aspiration)
7040[b]	Antimony (Atomic Absorption, Direct Aspiration)
7041[c]	Antimony (Atomic Absorption, Furnace Technique)
7060A[c]	Arsenic (Atomic Absorption, Furnace Technique)
7080A[b]	Barium (Atomic Absorption, Direct Aspiration)
7081[c]	Barium (Atomic Absorption, Furnace Technique)
7090[b]	Beryllium (Atomic Absorption, Direct Aspiration)
7091[c]	Beryllium (Atomic Absorption, Furnace Technique)
7130[b]	Cadmium (Atomic Absorption, Direct Aspiration)
7131A[c]	Cadmium (Atomic Absorption, Furnace Technique)
7140[b]	Calcium (Atomic Absorption, Direct Aspiration)
7190[b]	Chromium (Atomic Absorption, Direct Aspiration)
7191[c]	Chromium (Atomic Absorption, Furnace Technique)
7200[b]	Cobalt (Atomic Absorption, Direct Aspiration)
7201[c]	Cobalt (Atomic Absorption, Furnace Technique)
7210[b]	Copper (Atomic Absorption, Direct Aspiration)
7211[c]	Copper (Atomic Absorption, Furnace Technique)
7380[b]	Iron (Atomic Absorption, Direct Aspiration)
7381[c]	Iron (Atomic Absorption, Furnace Technique)

7420[b]	Lead (Atomic Absorption, Direct Aspiration)
7421[c]	Lead (Atomic Absorption, Furnace Technique)
7430[b]	Lithium (Atomic Absorption, Direct Aspiration)
7450[b]	Magnesium (Atomic Absorption, Direct Aspiration)
7460[b]	Manganese (Atomic Absorption, Direct Aspiration)
7461[c]	Manganese (Atomic Absorption, Furnace Technique)
7480[b]	Molybdenum (Atomic Absorption, Direct Aspiration)
7481[c]	Molybdenum (Atomic Absorption, Furnace Technique)
7520[b]	Nickel (Atomic Absorption, Direct Aspiration)
7521[c]	Nickel (Atomic Absorption, Furnace Method)
7550[b]	Osmium (Atomic Absorption, Direct Aspiration)
7610[b]	Potassium (Atomic Absorption, Direct Aspiration)
7740[c]	Selenium (Atomic Absorption, Furnace Technique)
7760A[b]	Silver (Atomic Absorption, Direct Aspiration)
7761[c]	Silver (Atomic Absorption, Furnace Technique)
7770[b]	Sodium (Atomic Absorption, Direct Aspiration)
7780[b]	Strontium (Atomic Absorption, Direct Aspiration)
7840[b]	Thallium (Atomic Absorption, Direct Aspiration)
7841[c]	Thallium (Atomic Absorption, Furnace Technique)
7870[b]	Tin (Atomic Absorption, Direct Aspiration)
7910[b]	Vanadium (Atomic Absorption, Direct Aspiration)
7911[c]	Vanadium (Atomic Absorption, Furnace Technique)
7950[b]	Zinc (Atomic Absorption, Direct Aspiration)
7951[c]	Zinc (Atomic Absorption, Furnace Technique)

[a] - Replaced by Method 5021
[b] - Integrated into Method 7000B
[c] - Integrated into Method 7010

In addition, Chapter Eleven is being considered for deletion from SW-846. If Chapter Eleven is removed, the manual will simply refer to the most current version of the ground-water monitoring guidance published by the Office of Solid Waste. (See the Draft Update IVA *Federal Register* notice for more information regarding the removal of Chapter Eleven.)

HANDLING OF DRAFT UPDATE IVA

Since these are only draft methods and chapters, the material in Draft Update IVA does not change anything in the official version of the manual (i.e., SW-846, Third Edition, as updated by Final Updates I, II, IIA, IIB, and III). The user should not remove any white pages from the manual at this time. Regarding the placement and storage of this draft update, the Agency recommends one of the following:

1. The subscriber may place the salmon sheets in the manual (without removing the white pages of methods) in the order that they appear in the Draft Update IVA Table of Contents. Due to the volume of material, the subscriber can split the material in any volume (e.g., Volume IIB) into two parts and place one part into an extra binder supplied by the subscriber.

2. Instead of inserting the draft methods into the manual with the final methods, the subscriber may instead place the entire Draft Update IVA package into a separate binder supplied by the subscriber.

IN SUMMARY

To summarize these instructions, please note the following important points:

- This package contains Draft Update IVA. The USEPA is issuing these methods for possible future inclusion in SW-846. The draft methods are reliable methods, approved for use in all applications for which the use of SW-846 methods is not mandatory. The methods of Draft Update IVA cannot be used for compliance with required uses of SW-846 methods.

- The public may submit comments to the EPA regarding these methods in paper and/or electronic format. SW-846 methods not contained in this package are not open to comment.

- Do not remove any white pages from your copy of SW-846 and replace them with Draft Update IVA pages.

- If you have properly inserted all other updates, Draft Update IVA will not fit in the existing four 3-ring binder note books provided with the manual. You may insert (without replacing any white pages) Draft Update IVA into the manual and split some of a volume into a separate binder that you supply. You may also keep Draft Update IVA in its own separate binder that you supply.

ASSISTANCE

After reading these instructions, if you need help due to difficulties understanding the status of the package or have technical questions regarding the methods, you may telephone the **Methods Information Communication Exchange** (MICE) at **703-821-4690** or send an E-mail to: mice@lan828.ehsg.saic.com.

If you have questions concerning your SW-846 **U.S. Government Printing Office** (GPO) subscription, you should telephone the GPO at **202-512-1806**. If you did not purchase your SW-846 from the GPO, the GPO will not be able to help you.

SW-846 AVAILABILITY ON CD-ROM

A CD-ROM version (Version 2.0) of *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods* (SW-846) has been developed by EPA in cooperation with the National Technical Information Service (NTIS). On a single disc, it includes all text and figures found in the final version of SW-846 as updated by Updates I, II, IIA, IIB, and III. (It does not include Draft Update IVA.) It can be used for word searching (e.g, analytes, keywords); and to cut and paste or export text and diagrams to update or develop laboratory standard operating procedures (SOPs). To order by phone, call NTIS at (800) 553-6847 and request order number PB97-501928FCD for a single user copy, PB97-502512FCD for a 5-user LAN copy, or PB97-502520FCD for unlimited users. To receive information by fax from NTIS about this CD-ROM, call (703) 487-4140 and enter publication number code 8698.

A CD-ROM version of Draft Update IV on CD-ROM is planned for the future. To place an early order by phone, call NTIS at (800) 553-6847 and request order number PB97-501936 (single user), PB97-502538FCD (5-user LAN), or PB97-502546FCD (unlimited users).

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NOTE: A suffix of "A" in the method number indicates revision one (the method has been revised once). A suffix of "B" in the method number indicates revision two (the method has been revised twice). A suffix of "C" in the method number indicates revision three (the method has been revised three times). **In order to properly document the method used for analysis, the entire method number including the suffix letter designation (e.g., A, B, or C) must be identified by the analyst.** A method reference found within the RCRA regulations and the text of SW-846 methods and chapters refers to the latest promulgated revision of the method, even though the method number does not include the appropriate letter suffix.

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NOTE: A suffix of "A" in the method number indicates revision one (the method has been revised once). A suffix of "B" in the method number indicates revision two (the method has been revised twice). A suffix of "C" in the method number indicates revision three (the method has been revised three times). **In order to properly document the method used for analysis, the entire method number including the suffix letter designation (e.g., A, B, or C) must be identified by the analyst.** A method reference found within the RCRA regulations and the text of SW-846 methods and chapters refers to the latest promulgated revision of the method, even though the method number does not include the appropriate letter suffix.

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CHAPTER TWO

CHOOSING THE CORRECT PROCEDURE

SW-846 analytical methods are written as quantitative trace analytical methods to demonstrate that a waste does not contain analytes of concern that cause it to be managed as a hazardous waste. As such, these methods typically contain relatively stringent quality control (QC) criteria appropriate to trace analyses. However, if a particular application does not require data of this quality, less stringent QC criteria may be used. The purpose of this chapter is to aid the analyst in choosing the appropriate methods for sample analyses, based upon the sample matrix and the analytes to be determined. The ultimate responsibility for producing reliable analytical results lies with the entity subject to the regulation. Therefore, members of the regulated community are advised to refer to this chapter and to consult with knowledgeable laboratory personnel when choosing the most appropriate suite of analytical methods. In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements.

Section 2.1 provides guidance regarding the analytical flexibility inherent to SW-846 methods and the precedence of various QC criteria. Section 2.2 reviews the information required to choose the correct combination of methods for an analytical procedure. Section 2.3 provides useful information on implementing the method selection guidance for organic analyses. Section 2.4 provides guidance on characteristic analyses and Section 2.5 provides guidance on the determination of analytes in ground water.

2.1 GUIDANCE REGARDING FLEXIBILITY INHERENT TO SW-846 METHODS AND THE PRECEDENCE OF SW-846 QUALITY CONTROL CRITERIA

The specific products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency for use in the method. Glassware, reagents, supplies, equipment and settings other than those listed in this manual may be employed, provided that method performance appropriate for the intended RCRA application has been documented. Such performance includes consideration of precision, accuracy (or bias), recovery, representativeness, comparability, and sensitivity (detection, quantitation, or reporting limits) relative to the data quality objectives for the intended use of the analytical results. In response to this inherent flexibility, if an alternative analytical procedure is employed, then EPA expects the laboratory to demonstrate and document that the procedure is capable of providing appropriate performance for its intended application. This demonstration must not be performed after the fact, but as part of the laboratory's initial demonstration of proficiency with the method. The documentation should be in writing, maintained in the laboratory, and available for inspection upon request by authorized representatives of the appropriate regulatory authorities. The documentation should include the performance data as well as a detailed description of the procedural steps as performed (i.e., a written standard operating procedure).

Given this allowance for flexibility, EPA wishes to emphasize that this manual also contains procedures for "method-defined parameters," where the analytical result is wholly dependant on the process used to make the measurement. Examples include the use of the toxicity characteristic leaching procedure (TCLP) to prepare a leachate, and the flash point, pH, paint filter liquids, and corrosivity tests. In these instances, changes to the specific methods may change the end result

and incorrectly identify a waste as nonhazardous. Therefore, when the measurement of such method-defined parameters is required by regulation, those methods are not subject to the flexibility afforded in other methods.

Analysts and data users are advised that even for those analytes that are not method-defined, different procedures may produce some difference in results. Common examples include the differences in recoveries of phenolic compounds extracted from water by separatory funnel (Method 3510) and continuous liquid-liquid (Method 3520) extraction techniques, differences in recoveries of many compounds between Soxhlet (Method 3540) and ultrasonic (Method 3550) extraction techniques, and differences resulting from the choice of acid digestion of metals (Method 3050) or microwave digestion (Method 3051). Where practical, the Agency has included guidance in the individual methods regarding known potential problems, and analysts are advised to review this information carefully in choosing or modifying analytical procedures. Chapter One describes a variety of QC procedures that may be used to evaluate the quality of the analytical results. Additional QC procedures may be described in the individual methods. The results of these QC procedures should be used by the analyst to evaluate if the choice of the analytical procedures and/or any modifications are appropriate to generate data of the quality necessary to satisfy the data quality needs of the intended application.

The performance data included in the SW-846 methods are not intended to be used as absolute QC acceptance criteria for method performance. The data are intended to be guidance, by providing typical method performance in typical matrices, to assist the analyst in selection of the appropriate method for the intended application. In addition, it is the responsibility of the laboratory to establish actual operating parameters and in-house QC acceptance criteria, based on its own laboratory SOPs and in-house QC program, to demonstrate appropriate performance of the methods used in that laboratory for the RCRA analytical applications for which they are intended.

The regulated community is further advised that the methods here or from other sources need only be used for those specific analytes of concern that are subject to regulation or other monitoring requirements. The fact that a method provides a long list of analytes does not mean that each of those analytes is subject to any or all regulations, or that all of those analytes must be analyzed each time the method is employed, or that all of the analytes can be analyzed using a single sample preparation procedure. It is EPA's intention that the target analyte list for any procedure includes those analytes necessary to meet the data quality objectives of the project, i.e., those analytes subject to monitoring requirements and set out in a RCRA permit (or other applicable regulation), plus those analytes used in the methods for QC purposes, such as surrogates, internal standards, system performance check compounds, etc. Additional analytes, not included on the analyte list of a particular method(s) but needed for a specific project, may be analyzed by that particular method(s), if appropriate performance can be demonstrated for the analytes of concern in the matrices of concern at the levels of concern.

2.1.1 Trace Analysis vs. Macroanalysis

Through the choice of sample size and concentration procedures, the methods presented in SW-846 were designed to address the problem of "trace" analyses (<1000 ppm), and have been developed for an optimized working range. These methods are also applicable to "minor" (1000 ppm - 10,000 ppm) and "major" (>10,000 ppm) analyses, as well, through use of appropriate sample preparation techniques that result in analyte concentrations within that optimized range. Such sample preparation techniques include:

1) adjustment of size of sample prepared for analysis (for homogeneous samples),
2) adjustment of injection volumes,
3) dilution or concentration of sample,
4) elimination of concentration steps prescribed for "trace" analyses, and
5) direct injection (of samples to be analyzed for volatile constituents).

The performance data presented in each of these methods were generated from "trace" analyses, and may not be applicable to "minor" and "major" analyses. Generally, extraction efficiency improves as concentration increases.

CAUTION: Great care should be taken when performing trace analyses after the analysis of concentrated samples, given the possibility of contamination.

2.1.2 Choice of Apparatus and Preparation of Reagents

Since many types and sizes of glassware and supplies are commercially available, and since it is possible to prepare reagents and standards in many different ways, the apparatus, reagents, and volumes specified in these methods may be replaced by any similar types as long as this substitution does not affect the overall quality of the analyses.

2.1.3 Quality Control Criteria Precedence

Chapter One contains general quality control (QC) guidance for analyses using SW-846 methods. QC guidance specific to a given analytical technique (e.g., extraction, cleanup, sample introduction, or analysis) may be found in Methods 3500, 3600, 5000, 7000, and 8000. Method-specific QC criteria may be found in Sec. 8.0 of each individual method (or in Sec. 11.0 of air sampling methods). When inconsistencies exist between the information in these locations, method-specific QC criteria take precedence over both technique-specific criteria and those criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One.

2.2 REQUIRED INFORMATION

In order to choose the correct combination of methods to comprise the appropriate analytical procedure, some basic information is required.

2.2.1 Physical State(s) of Sample

The phase characteristics of the sample must be known. There are several general categories of phases into which the sample may be categorized, including:

Aqueous
Sludge
TCLP or EP Extract
Solid
Ground Water
Oil or other Organic Liquid
Stack Sampling (VOST) Condensate
Multiphase Sample

There may be a substantial degree of overlap between the phases listed above and it may be useful to further divide these phases in certain instances. A multiphase sample may be a

combination of aqueous, organic liquid, sludge, and/or solid phases, and generally must undergo a phase separation as the first step in the analytical procedure.

2.2.2 Analytes

Analytes may be divided into various classes based on the determinative methods which are used to identify and quantify them. The most basic differentiation is between organic (e.g., carbon-containing) analytes and inorganic (e.g., metals and anions) analytes.

Table 2-1 is an alphabetical list of analytes cited within the SW-846 organic determinative methods (excludes immunoassay and other screening methods). These analytes have been evaluated by those methods. The methods may also be applicable to other analytes that are similar to those listed. Tables 2-2 through 2-32 list the analytes for each organic determinative method.

Table 2-33 indicates which methods are applicable to inorganic analytes.

2.2.3 Detection Limits

Some regulations may require a specific sensitivity or detection limit for an analysis, as in the determination of analytes for the Toxicity Characteristic (TC). Drinking water detection limits, for those specific organic and metallic analytes covered by the National Primary Drinking Water Regulations, are desired in the analysis of ground water.

2.2.4 Analytical Objective

Knowledge of the analytical objective will be useful in the choice of sample preparation procedures and in the selection of a determinative method. This is especially true when the sample has more than one phase. Knowledge of the analytical objective may not be possible or desirable at all management levels, but that information should be transmitted to the analytical laboratory management to ensure that the correct techniques are used during the analytical effort.

2.2.5 Detection and Monitoring

The strategy for detection of compounds in environmental or process samples may be contrasted with the strategy for collecting monitoring data. Detection samples define initial conditions. When there is little information available about the composition of the sample source, e.g., a well or process stream, mass spectral identification of organic analytes leads to fewer false positive results. Thus, the most practical form of detection for organic analytes is often mass spectral identification. However, where the sensitivity requirements exceed those that can be achieved using mass spectral method (e.g., GC/MS or HPLC/MS), it may be necessary to employ a more sensitive detection method (e.g., electron capture). In these instances, the risk of false positive results may be minimized by confirming the results through a second analysis with a dissimilar detector or chromatographic column. Thus, the choice of technique for organic analytes may be governed by the detection limit requirements and potential interferants.

Similarly, the choice of technique for metals is governed by the detection limit requirements and potential interferants.

In contrast, monitoring samples are analyzed to confirm existing and on-going conditions, tracking the presence or absence of known constituents in an environmental or process matrix. In well-defined matrices and under stable analytical conditions, less compound-specific detection modes may be used, as the risk of false positive results is less.

2.2.6 Sample Containers, Preservations, and Holding Times

Appropriate sample containers, sample preservation techniques, and sample holding times for aqueous matrices are listed in Table 2-34, near the end of this chapter. Similar information may be found in Table 3-1 of Chapter Three (inorganic analytes) and Table 4-1 of Chapter Four (organic analytes). Samples must be extracted and analyzed within the specified holding times for the results to be considered reflective of total concentrations. Analytical data generated outside of the specified holding times must be considered to be minimum values only. Such data may be used to demonstrate that a waste is hazardous where it shows the concentration of a constituent to be above the regulatory threshold but cannot be used to demonstrate that a waste is not hazardous.

2.3 IMPLEMENTING THE GUIDANCE

The choice of the appropriate sequence of methods depends on the information required and on the experience of the analyst. Figure 2-1 summarizes the organic analysis options available. Appropriate selection is confirmed by the quality control results. The use of the recommended procedures, whether they are approved or mandatory, does not release the analyst from demonstrating the correct execution of the method.

2.3.1 Extraction and Sample Preparation Procedures for Organic Analytes

Methods for preparing samples for organic analytes are shown in Table 2-35. Method 3500 and associated methods should be consulted for further details on preparing the sample for analysis.

2.3.1.1 Aqueous Samples

Methods 3510 and 3520 may be used for extraction of the semivolatile organic compounds from aqueous samples. The choice of a preparative method depends on the sample. Method 3510, a separatory funnel liquid-liquid extraction technique, is appropriate for samples which will not form a persistent emulsion interface between the sample and the extraction solvent. The formation of an emulsion that cannot be broken up by mechanical techniques will prevent proper extraction of the sample. Method 3520, a continuous liquid-liquid extraction technique, may be used for any aqueous sample and will minimize emulsion formation.

Method 3535 is solid-phase extraction technique that has been tested for organochlorine pesticides and phthalate esters and may be applicable to other semivolatile and extractable compounds as well. The aqueous sample is passed through a solid sorbent material which traps the analytes. They are then eluted from the solid-phase sorbent with a small volume of organic solvent. This technique may be used to minimize the volumes of organic solvents that are employed, but may not be appropriate for aqueous samples with high suspended solids contents.

2.3.1.1.1 Basic or Neutral Extraction of Semivolatile Analytes

The solvent extract obtained by performing Method 3510, 3520, or 3535 at a neutral or basic pH will contain the neutral organic compounds and the organic bases of interest. Refer to Table 1 in the extraction methods (3510 and/or 3520) for guidance on the requirements for pH adjustment prior to extraction and analysis.

2.3.1.1.2 Acidic Extraction of Phenols and Acid Analytes

The solvent extract obtained by performing Method 3510, 3520, or 3535 at a pH less than or equal to 2 will contain the phenols and acid extractable organics of interest.

2.3.1.2 Solid Samples

Soxhlet extraction (Methods 3540 and 3541), ultrasonic extraction (Method 3550), and accelerated solvent extraction (Method 3545) may be used with solid samples. Consolidated samples should be ground finely enough to pass through a 1 mm sieve. In limited applications, waste dilution (Methods 3580 and 3585) may be used if the entire sample is soluble in the specified solvent.

Methods 3540, 3541, 3545, and 3550 are neutral-pH extraction techniques and therefore, depending on the analysis requirements, acid-base partition cleanup (Method 3650) may be necessary. Method 3650 will only be needed if chromatographic interferences are severe enough to prevent detection of the analytes of interest. This separation will be most important if a GC method is chosen for analysis of the sample. If GC/MS is used, the ion selectivity of the technique may compensate for chromatographic interferences.

There are two extraction procedures for solid samples that employ supercritical fluid extraction (SFE). Method 3560 is a technique for the extraction of petroleum hydrocarbons from various solid matrices using carbon dioxide at elevated temperature and pressure. Method 3561 may be used to extract polynuclear aromatic hydrocarbons (PAHs) from solid matrices using supercritical carbon dioxide.

2.3.1.3 Oils and Organic Liquids

Method 3580, waste dilution, may be used to prepare oils and organic liquid samples for analysis of semivolatile and extractable organic analytes by GC or GC/MS. Method 3585 may be employed for the preparation of these matrices for volatiles analysis by GC or GC/MS. To avoid overloading the analytical detection system, care must be exercised to ensure that proper dilutions are made. Methods 3580 and 3585 give guidance on performing waste dilutions.

To remove interferences for semivolatiles and extractables, Method 3611 (Alumina cleanup) may be performed on an oil sample directly, without prior sample preparation.

Method 3650 is the only other preparative procedure for oils and other organic liquids. This procedure is a back extraction into an aqueous phase. It is generally introduced as a cleanup procedure for extracts rather than as a preparative procedure. Oils generally have

a high concentration of semivolatile compounds and, therefore, preparation by Method 3650 should be done on a relatively small aliquot of the sample. Generally, extraction of 1 mL of oil will be sufficient to obtain a saturated aqueous phase and avoid emulsions.

2.3.1.4 Sludge Samples

Determining the appropriate methods for analysis of sludges is complicated because of the lack of precise definitions of sludges with respect to the relative percent of liquid and solid components. There is no set ratio of liquid to solid which enables the analyst to determine which of the three extraction methods cited is the most appropriate. Sludges may be classified into three categories: liquid sludges, solid sludges, and emulsions, but with appreciable overlap.

If the sample is an organic sludge (solid material and organic liquid, as opposed to an aqueous sludge), the sample should be handled as a multiphase sample.

2.3.1.4.1 Liquid Sludges

Use of Method 3510 or Method 3520 may be applicable to sludges that behave like and have the consistency of aqueous liquids. Ultrasonic extraction (Method 3550) and Soxhlet (Method 3540) procedures will, most likely, be ineffective because of the overwhelming presence of the liquid aqueous phase.

2.3.1.4.2 Solid Sludges

Soxhlet extraction (Methods 3540 and 3541), accelerated solvent (Method 3545) extraction, and ultrasonic extraction (Method 3550) will be more effective when applied to sludge samples that resemble solids. Samples may be dried or centrifuged to form solid materials for subsequent determination of semivolatile compounds.

Using Method 3650, Acid-Base Partition Cleanup, on the extract may be necessary, depending on whether chromatographic interferences prevent determination of the analytes of interest.

2.3.1.4.3 Emulsions

Attempts should be made to break up and separate the phases of an emulsion. Several techniques are effective in breaking emulsions or separating the phases of emulsions, including:

1. Freezing/thawing: Certain emulsions will separate if exposed to temperatures below 0°C.

2. Salting out: Addition of a salt to make the aqueous phase of an emulsion too polar to support a less polar phase promotes separation.

3. Centrifugation: Centrifugal force may separate emulsion components by density.

4. Addition of water or ethanol: Emulsion polymers may be destabilized when a preponderance of the aqueous phase is added.

5. Forced filtering through glass wool: Many emulsions can be broken by forcing the emulsion through a pad of Pyrex glass wool in a drying column using a slight amount of air pressure (using a rubber bulb usually provides sufficient pressure).

If techniques for breaking emulsions fail, use Method 3520. If the emulsion can be broken, the different phases (aqueous, solid, or organic liquid) may then be analyzed separately.

2.3.1.5 Multiphase Samples

Choice of the procedure for separating multiphase samples is highly dependent on the objective of the analysis. With a sample in which some of the phases tend to separate rapidly, the percent weight or volume of each phase should be calculated and each phase should be individually analyzed for the required analytes.

An alternate approach is to obtain a homogeneous sample and attempt a single analysis on the combination of phases. This approach will give no information on the abundance of the analytes in the individual phases other than what can be implied by solubility.

A third alternative is to select phases of interest and to analyze only those selected phases. This tactic must be consistent with the sampling/analysis objectives or it will yield insufficient information for the time and resources expended. The phases selected should be compared with Figure 2-1 and Table 2-35 for further guidance.

2.3.2 Cleanup Procedures

Each category in Table 2-36, Cleanup Methods for Organic Analyte Extracts, corresponds to one of the possible determinative methods available in the manual. Cleanups employed are determined by the analytes of interest within the extract. However, the necessity of performing cleanup may also depend upon the matrix from which the extract was developed. Cleanup of a sample may be done exactly as instructed in the cleanup method for some of the analytes. There are some instances when cleanup using one of the methods may only proceed after the procedure is modified to optimize recovery and separation. Several cleanup techniques may be possible for each analyte category. The information provided is not meant to imply that any or all of these methods must be used for the analysis to be acceptable. Extracts with components which interfere with spectral or chromatographic determinations are expected to be subjected to cleanup procedures.

The analyst's discretion must determine the necessity for cleanup procedures, as there are no clear cut criteria for indicating their use. Method 3600 and associated methods should be consulted for further details on extract cleanup.

2.3.3 Determinative Procedures

The determinative methods for organic analytes have been divided into three categories, as shown in Table 2-37: gas chromatography/mass spectrometry (GC/MS); specific detection methods, i.e., gas chromatography (GC) with specific non-MS detectors; and high performance liquid chromatography (HPLC). This division is intended to help an analyst choose which determinative method will apply. Under each analyte column, SW-846 method numbers have been indicated, if appropriate, for the determination of the analyte. A blank has been left if no chromatographic determinative method is available.

Generally, the MS procedures are more specific but less sensitive than the appropriate gas chromatographic/specific detection method.

Method 8000 gives a general description of the techniques of gas chromatography and high performance liquid chromatography. Method 8000 should be consulted prior to application of any of the gas chromatographic methods.

Method 8081 (organochlorine pesticides), Method 8082 (polychlorinated biphenyls), Method 8141 (organophosphorus pesticides), and Method 8151 (chlorinated herbicides), are preferred over GC/MS because of the combination of selectivity and sensitivity of the flame photometric, nitrogen-phosphorus, and electron capture detectors.

Method 8260 is a GC/MS method for volatile analytes, which employs a capillary column. A variety of sample introduction techniques may be used with Method 8260, including Methods 5021, 5030, 5031, 5035, and 3585. A GC with a selective detector is also useful for the determination of volatile organic compounds in a monitoring scenario, as described in Sec. 2.2.5.

Method 8270 is a GC/MS method for semivolatile analytes, which employs a capillary column.

Table 2-37 lists several GC and HPLC methods that apply to only a small number of analytes. Methods 8031 and 8033 are GC methods for acrolein, acrylonitrile, and acetonitrile. Methods 8315 and 8316 are HPLC methods for these three analytes. Method 8316 also addresses acrylamide, which may be analyzed by Method 8032.

HPLC methods have been developed for other types of analytes, most notably carbamates (Method 8318); azo dyes, phenoxy acid herbicides, carbamates, and organophosphorus pesticides (Method 8321); PAHs (Method 8310); explosives (Methods 8330, 8331, and 8332); and some volatile organics (Methods 8315 and 8316).

Method 8430 utilizes a Fourier Transform Infrared Spectrometer (FT-IR) coupled to a gas chromatograph to determine bis(2-chloroethyl) ether and its hydrolysis products. The sample is introduced by direct aqueous injection. Method 8440 may be employed for the determination of total recoverable petroleum hydrocarbons (TRPH) in solid samples by infrared (IR) spectrophotometry. The samples may be extracted with supercritical carbon dioxide, using Method 3560.

2.4 CHARACTERISTICS

Figure 2-2 outlines a sequence for determining if a waste exhibits one or more of the characteristics of a hazardous waste.

2.4.1 EP and TCLP extracts

The leachate obtained from using either the EP (Figure 2-3A) or the TCLP (Figure 2-3B) is an aqueous sample, and therefore, requires further solvent extraction prior to the analysis of semivolatile compounds.

The TCLP leachate is solvent extracted with methylene chloride at a pH > 11 and at a pH <2 by either Method 3510 or 3520. Method 3510 should be used unless the formation of emulsions between the sample and the solvent prevent proper extraction. If this problem is encountered, Method 3520 should be employed.

The solvent extract obtained by performing either Method 3510 or 3520 at a basic or neutral pH will contain the base/neutral compounds of interest. Refer to the specific determinative method for guidance on the pH requirements for extraction prior to analysis. Method 5031 (Azeotropic Distillation) may be used as an effective preparative method for pyridine.

Due to the high concentration of acetate in the TCLP extract, it is recommended that purge-and-trap be used to introduce the volatile sample into the gas chromatograph.

2.5 GROUND WATER

Appropriate analysis schemes for the determination of analytes in ground water are presented in Figures 2-4A, 2-4B, and 2-4C. Quantitation limits for the inorganic analytes should correspond to the drinking water limits which are available.

2.5.1 Special Techniques for Inorganic Analytes

All atomic absorption analyses should employ appropriate background correction systems whenever spectral interferences could be present. Several background correction techniques are employed in modern atomic absorption spectrometers. Matrix modification can complement background correction in some cases. Since no approach to interference correction is completely effective in all cases, the analyst should attempt to verify the adequacy of correction. If the interferant is known (e.g., high concentrations of iron in the determination of selenium), accurate analyses of synthetic solutions of the interferant (with and without analyte) could establish the efficacy of the background correction. If the nature of the interferant is not established, good agreement of analytical results using two substantially different wavelengths could substantiate the adequacy of the background correction.

To reduce matrix interferences, all graphite furnace atomic absorption (GFAA) analyses should be performed using techniques which maximize an isothermal environment within the furnace cell. Data indicate that two such techniques, L'vov platform and the Delayed Atomization Cuvette (DAC), are equivalent in this respect, and produce high quality results.

All furnace atomic absorption analysis should be carried out using the best matrix modifier for the analysis. Some examples of modifiers are listed below. (See also the appropriate methods.)

Element(s)	Modifier(s)
As and Se	Nickel nitrate, palladium
Pb	Phosphoric acid, ammonium phosphate, palladium
Cd	Ammonium phosphate, palladium
Sb	Ammonium nitrate, palladium
Tl	Platinum, palladium

ICP. AA, and GFAA calibration standards must match the acid composition and strength of the acids contained in the samples. Acid strengths of the calibration standards should be stated in the raw data. When using a method which permits the use of internal standardization, and the internal standardization option is being used, matrix matching is not required.

2.6 ADDITIONAL GUIDANCE REGARDING INORGANIC ANALYSES

Methods for preparing different sample matrices for inorganic analytes are shown in Table 2-38. Guidance regarding the use of leaching and digestive methods for inorganic analysis is provided in Table 2-39.

2.7 REFERENCES

1. Barcelona, M.J. "TOC Determinations in Ground Water"; Ground Water 1984, 22(1), 18-24.

2. Riggin, R.; et al. Development and Evaluation of Methods for Total Organic Halide and Purgeable Organic Halide in Wastewater; U.S. Environmental Protection Agency. Office of Research and Development. Environmental Monitoring and Support Laboratory. ORD Publication Offices of Center for Environmental Research Information: Cincinnati, OH, 1984; EPA-600/4-84-008.

3. McKee, G.; et al. Determination of Inorganic Anions in Water by Ion Chromatography; (Technical addition to Methods for Chemical Analysis of Water and Wastewater, EPA 600/4-79-020), U.S. Environmental Protection Agency. Environmental Monitoring and Support Laboratory. ORD Publication Offices of Center for Environmental Research Information: Cincinnati, OH, 1984; EPA-600/4-84-017.

TABLE 2-1
DETERMINATIVE METHODS FOR ORGANIC ANALYTES

Analyte	Applicable Method(s)
Acenaphthene	8100, 8270, 8275, 8310, 8410
Acenaphthylene	8100, 8270, 8275, 8310, 8410
Acetaldehyde	8315
Acetone	8015, 8260, 8315
Acetonitrile	8015, 8033, 8260
Acetophenone	8270
2-Acetylaminofluorene	8270
1-Acetyl-2-thiourea	8270
Acifluorfen	8151
Acrolein (Propenal)	8015, 8260, 8315, 8316
Acrylamide	8032, 8316
Acrylonitrile	8015, 8031, 8260, 8316
Alachlor	8081
Aldicarb (Temik)	8318, 8321
Aldicarb sulfone	8318, 8321
Aldicarb sulfoxide	8321
Aldrin	8081, 8270
Allyl alcohol	8015, 8260
Allyl chloride	8021, 8260
2-Aminoanthraquinone	8270
Aminoazobenzene	8270
4-Aminobiphenyl	8270
Aminocarb	8321
2-Amino-4,6-dinitrotoluene (2-Am-DNT)	8330
4-Amino-2,6-dinitrotoluene (4-Am-DNT)	8330
3-Amino-9-ethylcarbazole	8270
Anilazine	8270
Aniline	8131, 8270
o-Anisidine	8270
Anthracene	8100, 8270, 8275, 8310, 8410
Aramite	8270
Aroclor-1016 (PCB-1016)	8082, 8270
Aroclor-1221 (PCB-1221)	8082, 8270
Aroclor-1232 (PCB-1232)	8082, 8270
Aroclor-1242 (PCB-1242)	8082, 8270
Aroclor-1248 (PCB-1248)	8082, 8270
Aroclor-1254 (PCB-1254)	8082, 8270
Aroclor-1260 (PCB-1260)	8082, 8270
Aspon	8141
Asulam	8321
Atrazine	8141
Azinphos-ethyl	8141
Azinphos-methyl	8141, 8270
Barban	8270, 8321
Baygon (Propoxur)	8318, 8321
Bendiocarb	8321

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
Benefin	8091
Benomyl	8321
Bentazon	8151
Benzal chloride	8121
Benzaldehyde	8315
Benz(a)anthracene	8100, 8270, 8275, 8310, 8410
Benzene	8021, 8260
Benzenethiol (Thiophenol)	8270
Benzidine	8270, 8325
Benzo(b)fluoranthene	8100, 8270, 8275, 8310
Benzo(j)fluoranthene	8100
Benzo(k)fluoranthene	8100, 8270, 8275, 8310
Benzoic acid	8270, 8410
Benzo(g,h,i)perylene	8100, 8270, 8275, 8310
Benzo(a)pyrene	8100, 8270, 8275, 8310, 8410
p-Benzoquinone	8270
Benzotrichloride	8121
Benzoylprop ethyl	8325
Benzyl alcohol	8270
Benzyl chloride	8021, 8121, 8260
α-BHC (α-Hexachlorocyclohexane)	8081, 8121, 8270
β-BHC (β-Hexachlorocyclohexane)	8081, 8121, 8270
δ-BHC (δ-Hexachlorocyclohexane)	8081, 8121, 8270
γ-BHC (Lindane, γ-Hexachlorocyclohexane)	8081, 8121, 8270
Bis(2-chloroethoxy)methane	8111, 8270, 8410
Bis(2-chloroethyl) ether	8111, 8270, 8410, 8430
Bis(2-chloroethyl)sulfide	8260
Bis(2-chloroisopropyl) ether	8021, 8111, 8270, 8410
Bis(2-n-butoxyethyl) phthalate	8061
Bis(2-ethoxyethyl) phthalate	8061
Bis(2-ethylhexyl) phthalate	8061, 8270, 8410
Bis(2-methoxyethyl) phthalate	8061
Bis(4-methyl-2-pentyl)-phthalate	8061
Bolstar (Sulprofos)	8141
Bromacil	8321
Bromoacetone	8021, 8260
4-Bromoaniline	8131
Bromobenzene	8021, 8260
Bromochloromethane	8021, 8260
2-Bromo-6-chloro-4-nitroaniline	8131
Bromodichloromethane	8021, 8260
2-Bromo-4,6-dinitroaniline	8131
Bromoform	8021, 8260
Bromomethane	8021, 8260
4-Bromophenyl phenyl ether	8111, 8270, 8275, 8410
Bromoxynil	8270

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
Butanal	8315
1-Butanol (n-Butyl alcohol)	8015
n-Butanol	8260
2-Butanone (Methyl ethyl ketone, MEK)	8015, 8260
Butralin	8091
n-Butyl alcohol (1-Butanol)	8015
t-Butyl alcohol	8015
n-Butylbenzene	8021, 8260
sec-Butylbenzene	8021, 8260
tert-Butylbenzene	8021, 8260
Butyl benzyl phthalate	8061, 8270, 8410
2-sec-Butyl-4,6-dinitrophenol (DNBP, Dinoseb)	8041, 8151, 8270, 8321
Caffeine	8321
Captafol	8081, 8270
Captan	8270
Carbaryl (Sevin)	8270, 8318, 8321, 8325
Carbendazim	8321
Carbofuran (Furaden)	8270, 8318, 8321
Carbon disulfide	8260
Carbon tetrachloride	8021, 8260
Carbophenothion	8141, 8270
Chloral hydrate	8260
Chloramben	8151
Chlordane (NOS)	8270
α-Chlordane	8081
γ-Chlordane	8081
Chlorfenvinphos	8141, 8270
Chloroacetonitrile	8260
2-Chloroaniline	8131
3-Chloroaniline	8131
4-Chloroaniline	8131, 8270, 8410
Chlorobenzene	8021, 8260
Chlorobenzilate	8081, 8270
2-Chlorobiphenyl	8082, 8275
2-Chloro-1,3-butadiene (Chloroprene)	8021, 8260
1-Chlorobutane	8260
Chlorodibromomethane (Dibromochloromethane)	8021, 8260
2-Chloro-4,6-dinitroaniline	8131
1-Chloro-2,4-dinitrobenzene	8091
1-Chloro-3,4-dinitrobenzene	8091
Chloroethane	8021, 8260
2-Chloroethanol	8021, 8260, 8430
2-(2-Chloroethoxy)ethanol	8430
2-Chloroethyl vinyl ether	8021, 8260
Chloroform	8021, 8260
1-Chlorohexane	8260

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
Chloromethane	8021, 8260
5-Chloro-2-methylaniline	8270
Chloromethyl methyl ether	8021
2-Chloro-5-methylphenol	8041
4-Chloro-2-methylphenol	8041
4-Chloro-3-methylphenol	8041, 8270, 8410
3-(Chloromethyl)pyridine hydrochloride	8270
1-Chloronaphthalene	8270, 8275
2-Chloronaphthalene	8121, 8270, 8410
Chloroneb	8081
2-Chloro-4-nitroaniline	8131
4-Chloro-2-nitroaniline	8131
1-Chloro-2-nitrobenzene	8091
1-Chloro-4-nitrobenzene	8091
2-Chloro-6-nitrotoluene	8091
4-Chloro-2-nitrotoluene	8091
4-Chloro-3-nitrotoluene	8091
2-Chlorophenol	8041, 8270, 8410
3-Chlorophenol	8041
4-Chlorophenol	8041, 8410
4-Chloro-1,2-phenylenediamine	8270
4-Chloro-1,3-phenylenediamine	8270
4-Chlorophenyl phenyl ether	8111, 8270, 8410
2-Chlorophenyl 4-nitrophenyl ether	8111
3-Chlorophenyl 4-nitrophenyl ether	8111
4-Chlorophenyl 4-nitrophenyl ether	8111
o-Chlorophenyl thiourea	8325
Chloroprene (2-Chloro-1,3-butadiene)	8021, 8260
3-Chloropropionitrile	8260
Chloropropham	8321
Chloropropylate	8081
Chlorothalonil	8081
2-Chlorotoluene	8021, 8260
4-Chlorotoluene	8021, 8260
Chloroxuron	8321
Chlorpyrifos	8141
Chlorpyrifos methyl	8141
Chrysene	8100, 8270, 8275, 8310, 8410
Coumaphos	8141, 8270
Coumarin Dyes	8321
p-Cresidine	8270
o-Cresol (2-Methylphenol)	8041, 8270, 8410
m-Cresol (3-Methylphenol)	8041, 8270
p-Cresol (4-Methylphenol)	8041, 8270, 8275, 8410
Crotonaldehyde	8015, 8260, 8315
Crotoxyphos	8141, 8270

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
Cyclohexanone	8315
2-Cyclohexyl-4,6-dinitrophenol	8041, 8270
2,4-D	8151, 8321
Dalapon	8151, 8321
2,4-DB	8151, 8321
DBCP (1,2-Dibromo-3-chloropropane)	8011, 8021, 8081, 8260, 8270
2,4-D, butoxyethanol ester	8321
DCM (Dichloromethane, Methylene chloride)	8021, 8260
DCPA	8081
DCPA diacid	8151
4,4'-DDD	8081, 8270
4,4'-DDE	8081, 8270
4,4'-DDT	8081, 8270
DDVP (Dichlorvos, Dichlorovos)	8141, 8270, 8321
2,2',3,3'4,4'5,5',6,6'-Decachlorobiphenyl	8275
Decanal	8315
Demeton-O, and Demeton-S	8141, 8270
2,4-D, ethylhexyl ester	8321
Diallate	8081, 8270
Diamyl phthalate	8061
2,4-Diaminotoluene	8270
Diazinon	8141
Dibenz(a,h)acridine	8100
Dibenz(a,j)acridine	8100, 8270
Dibenz(a,h)anthracene	8100, 8270, 8275, 8310
7H-Dibenzo(c,g)carbazole	8100
Dibenzofuran	8270, 8275, 8410
Dibenzo(a,e)pyrene	8100, 8270
Dibenzo(a,h)pyrene	8100
Dibenzo(a,i)pyrene	8100
Dibenzothiophene	8275
Dibromochloromethane (Chlorodibromomethane)	8021, 8260
1,2-Dibromo-3-chloropropane (DBCP)	8011, 8260, 8270
1,2-Dibromoethane (EDB, Ethylene dibromide)	8011, 8021, 8260
Dibromofluoromethane	8260
Dibromomethane	8021, 8260
2,6-Dibromo-4-nitroaniline	8131
2,4-Dibromophenyl 4-nitrophenyl ether	8111
Di-n-butyl phthalate	8061, 8270, 8410
Dicamba	8151, 8321
Dichlone	8081, 8270
3,4-Dichloroaniline	8131
1,2-Dichlorobenzene	8021, 8121, 8260, 8270, 8410
1,3-Dichlorobenzene	8021, 8121, 8260, 8270, 8410
1,4-Dichlorobenzene	8021, 8121, 8260, 8270, 8410
3,3'-Dichlorobenzidine	8270,8325

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
3,5-Dichlorobenzoic acid	8151
2,3-Dichlorobiphenyl	8082, 8275
3,3'-Dichlorobiphenyl	8275
cis-1,4-Dichloro-2-butene	8260
trans-1,4-Dichloro-2-butene	8260
Dichlorodifluoromethane	8021, 8260
1,1-Dichloroethane	8021, 8260
1,2-Dichloroethane	8021, 8260
1,1-Dichloroethene (Vinylidene chloride)	8021, 8260
cis-1,2-Dichloroethene	8021, 8260
trans-1,2-Dichloroethene	8021, 8260
Dichlorofenthion	8141
Dichloromethane (DCM, Methylene chloride)	8021, 8260
2,6-Dichloro-4-nitroaniline	8131
2,3-Dichloronitrobenzene	8091
2,4-Dichloronitrobenzene	8091
3,5-Dichloronitrobenzene	8091
3,4-Dichloronitrobenzene	8091
2,5-Dichloronitrobenzene	8091
2,3-Dichlorophenol	8041
2,4-Dichlorophenol	8041, 8270, 8410
2,5-Dichlorophenol	8041
2,6-Dichlorophenol	8041, 8270
3,4-Dichlorophenol	8041
3,5-Dichlorophenol	8041
2,4-Dichlorophenol 3-methyl-4-nitrophenyl ether	8111
2,6-Dichlorophenyl 4-nitrophenyl ether	8111
3,5-Dichlorophenyl 4-nitrophenyl ether	8111
2,5-Dichlorophenyl 4-nitrophenyl ether	8111
2,4-Dichlorophenyl 4-nitrophenyl ether	8111
2,3-Dichlorophenyl 4-nitrophenyl ether	8111
3,4-Dichlorophenyl 4-nitrophenyl ether	8111
Dichloroprop (Dichlorprop)	8151, 8321
1,2-Dichloropropane	8021, 8260
1,3-Dichloropropane	8021, 8260
2,2-Dichloropropane	8021, 8260
1,3-Dichloro-2-propanol	8021, 8260
1,1-Dichloropropene	8021, 8260
cis-1,3-Dichloropropene	8021, 8260
trans-1,3-Dichloropropene	8021, 8260
Dichlorovos (DDVP, Dichlorvos)	8141, 8270, 8321
Dichlorprop (Dichloroprop)	8151, 8321
Dichlorvos (DDVP, Dichlorovos)	8141, 8270, 8321
Dicrotophos	8141, 8270
Dicofol	8081
Dicyclohexyl phthalate	8061

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
Dieldrin	8081, 8270
1,2,3,4-Diepoxybutane	8260
Diesel range organics (DRO)	8015, 8440
Diethylene glycol	8430
Diethyl ether	8015, 8260
Diethyl phthalate	8061, 8270, 8410
Diethylstilbestrol	8270
Diethyl sulfate	8270
Dihexyl phthalate	8061
Diisobutyl phthalate	8061
Dimethoate	8141, 8270, 8321
3,3'-Dimethoxybenzidine	8270, 8325
Dimethylaminoazobenzene	8270
2,5-Dimethylbenzaldehyde	8315
7,12-Dimethylbenz(a)anthracene	8270
3,3'-Dimethylbenzidine	8270, 8325
α,α-Dimethylphenethylamine	8270
2,3-Dimethylphenol	8041
2,4-Dimethylphenol	8041, 8270
2,5-Dimethylphenol	8041
2,6-Dimethylphenol	8041
3,4-Dimethylphenol	8041
Dimethyl phthalate	8061, 8270, 8410
Dinitramine	8091
2,4-Dinitroaniline	8131
1,2-Dinitrobenzene	8091, 8270
1,3-Dinitrobenzene (1,3-DNB)	8091, 8270, 8330
1,4-Dinitrobenzene	8091, 8270
4,6-Dinitro-2-methylphenol	8270, 8410
2,4-Dinitrophenol	8041, 8270, 8410
2,5-Dinitrophenol	8041
2,4-Dinitrotoluene (2,4-DNT)	8091, 8270, 8330, 8410
2,6-Dinitrotoluene (2,6-DNT)	8091, 8270, 8330, 8410
Dinocap	8270
Dinonyl phthalate	8061
Dinoseb (2-sec-Butyl-4,6-dinitrophenol, DNBP)	8041, 8151, 8270, 8321
Di-n-octyl phthalate	8061, 8270, 8410
Dioxacarb	8318
1,4-Dioxane	8015, 8260
Dioxathion	8141
Di-n-propyl phthalate	8410
Diphenylamine	8270
5,5-Diphenylhydantoin	8270
1,2-Diphenylhydrazine	8270
Disperse Blue 3	8321
Disperse Blue 14	8321

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
Disperse Brown 1	8321
Disperse Orange 3	8321
Disperse Orange 30	8321
Disperse Red 1	8321
Disperse Red 5	8321
Disperse Red 13	8321
Disperse Red 60	8321
Disperse Yellow 5	8321
Disulfoton	8141, 8270, 8321
Diuron	8321, 8325
1,3-DNB (1,3-Dinitrobenzene)	8091, 8270, 8330
DNBP (2-sec-Butyl-4,6-dinitrophenol, Dinoseb)	8151, 8270, 8321
2,4-DNT (2,4-Dinitrotoluene)	8091, 8270, 8275, 8330, 8410
2,6-DNT (2,6-Dinitrotoluene)	8091, 8270, 8330, 8410
EDB (1,2-Dibromoethane, Ethylene dibromide)	8011, 8021, 8260
Endosulfan I	8081, 8270
Endosulfan II	8081, 8270
Endosulfan sulfate	8081, 8270
Endrin	8081, 8270
Endrin aldehyde	8081, 8270
Endrin ketone	8081, 8270
Epichlorohydrin	8021, 8260
EPN	8141, 8270
Ethanol	8015, 8260
Ethion	8141, 8270
Ethoprop	8141
Ethyl acetate	8015, 8260
Ethylbenzene	8021, 8260
Ethyl carbamate	8270
Ethyl cyanide (Propionitrile)	8015, 8260
Ethylene dibromide (EDB, 1,2-Dibromoethane)	8011, 8021, 8260
Ethylene glycol	8015, 8430
Ethylene oxide	8015, 8260
Ethyl methacrylate	8260
Ethyl methanesulfonate	8270
Etridiazole	8081
Famphur	8141, 8270, 8321
Fenitrothion	8141
Fensulfothion	8141, 8270, 8321
Fenthion	8141, 8270
Fenuron	8321
Fluchloralin	8270
Fluometuron	8321
Fluoranthene	8100, 8270, 8275, 8310, 8410
Fluorene	8100, 8270, 8275, 8310, 8410
Fluorescent Brightener 61	8321

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
Fluorescent Brightener 236	8321
Fonophos	8141
Formaldehyde	8315
Furaden (Carbofuran)	8270, 8318, 8321
Gasoline range organics (GRO)	8015
Halowax-1000	8081
Halowax-1001	8081
Halowax-1013	8081
Halowax-1014	8081
Halowax-1051	8081
Halowax-1099	8081
Heptachlor	8081, 8270
2,2',3,3',4,4',5-Heptachlorobiphenyl	8082, 8275
2,2',3,4,4',5,5'-Heptachlorobiphenyl	8082, 8275
2,2',3,4,4',5',6-Heptachlorobiphenyl	8082
2,2',3,4',5,5',6-Heptachlorobiphenyl	8082, 8275
Heptachlor epoxide	8081, 8270
Heptanal	8315
Hexachlorobenzene	8081, 8121, 8270, 8275, 8410
2,2',3,3,4,4'-Hexachlorobiphenyl	8275
2,2',3,4,4',5'-Hexachlorobiphenyl	8082, 8275
2,2',3,4,5,5'-Hexachlorobiphenyl	8082
2,2',3,5,5',6-Hexachlorobiphenyl	8082
2,2',4,4',5,5'-Hexachlorobiphenyl	8082
Hexachlorobutadiene	8021, 8121, 8260, 8270, 8410
α-Hexachlorocyclohexane (α-BHC)	8081, 8121, 8270
β-Hexachlorocyclohexane (β-BHC)	8081, 8121, 8270
δ-Hexachlorocyclohexane (δ-BHC)	8081, 8121, 8270
γ-Hexachlorocyclohexane (γ-BHC, Lindane)	8081, 8121, 8270
Hexachlorocyclopentadiene	8081, 8121, 8270, 8410
Hexachloroethane	8121, 8260, 8270, 8410
Hexachlorophene	8270
Hexachloropropene	8270
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	8330
Hexamethylphosphoramide (HMPA)	8141, 8270
Hexanal	8315
2-Hexanone	8260
Hexyl 2-ethylhexyl phthalate	8061
HMPA (Hexamethyl phosphoramide)	8141, 8270
HMX (Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)	8330
1,2,3,4,6,7,8-HpCDD	8280, 8290
HpCDD, total	8280, 8290
1,2,3,4,6,7,8-HpCDF	8280, 8290
1,2,3,4,7,8,9-HpCDF	8280, 8290
HpCDF, total	8280, 8290
1,2,3,4,7,8-HxCDD	8280, 8290

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
1,2,3,6,7,8-HxCDD	8280, 8290
1,2,3,7,8,9-HxCDD	8280, 8290
HxCDD, total	8280, 8290
1,2,3,4,7,8-HxCDF	8280, 8290
1,2,3,6,7,8-HxCDF	8280, 8290
1,2,3,7,8,9-HxCDF	8280, 8290
2,3,4,6,7,8-HxCDF	8280, 8290
HxCDF	8280, 8290
Hydroquinone	8270
3-Hydroxycarbofuran	8318, 8321
5-Hydroxydicamba	8151
2-Hydroxypropionitrile	8260
Indeno(1,2,3-cd)pyrene	8100, 8270, 8275, 8310
Iodomethane (Methyl iodide)	8260
Isobutyl alcohol (2-Methyl-1-propanol)	8015, 8260
Isodrin	8081, 8270
Isophorone	8270, 8410
Isopropalin	8091
Isopropyl alcohol (2-Propanol)	8015, 8260
Isopropylbenzene	8021, 8260
p-Isopropyltoluene	8021, 8260
Isosafrole	8270
Isovaleraldehyde	8315
Kepone	8270
Lannate (Methomyl)	8318, 8321
Leptophos	8141, 8270
Lindane (γ-Hexachlorocyclohexane, γ-BHC)	8081, 8121, 8270
Linuron (Lorox)	8321, 8325
Lorox (Linuron)	8321, 8325
Malathion	8141, 8270
Maleic anhydride	8270
Malononitrile	8260
MCPA	8151, 8321
MCPP	8151, 8321
Merphos	8141, 8321
Mestranol	8270
Mesurol (Methiocarb)	8318, 8321
Methacrylonitrile	8260
Methanol	8015, 8260
Methapyrilene	8270
Methiocarb (Mesurol)	8318, 8321
Methomyl (Lannate)	8318, 8321
Methoxychlor	8081, 8270
Methyl acrylate	8260
2-Methyl-1-propanol (Isobutyl alcohol)	8015, 8260
Methyl-t-butyl ether	8260

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
3-Methylcholanthrene	8100, 8270
2-Methyl-4,6-dinitrophenol	8041
4,4'-Methylenebis(2-chloroaniline)	8270
4,4'-Methylenebis(N,N-dimethylaniline)	8270
Methyl ethyl ketone (MEK, 2-Butanone)	8015, 8260
Methylene chloride (Dichloromethane, DCM)	8021, 8260
Methyl iodide (Iodomethane)	8260
Methyl isobutyl ketone (MIBK, 4-Methyl-2-pentanone)	8015, 8260
Methyl methacrylate	8260
Methyl methanesulfonate	8270
2-Methylnaphthalene	8270, 8410
Methyl parathion (Parathion, methyl)	8270, 8141, 8321
4-Methyl-2-pentanone (MIBK, Methyl isobutyl ketone)	8015, 8260
2-Methylphenol (o-Cresol)	8041, 8270, 8410
3-Methylphenol (m-Cresol)	8041, 8270
4-Methylphenol (p-Cresol)	8041, 8270, 8410
2-Methylpyridine (2-Picoline)	8015, 8260, 8270
Methyl-2,4,6-trinitrophenylnitramine (Tetryl)	8330
Mevinphos	8141, 8270
Mexacarbate	8270, 8321
MIBK (Methyl isobutyl ketone, 4-Methyl-2-pentanone)	8015, 8260
Mirex	8081, 8270
Monocrotophos	8141, 8270, 8321
Monuron	8321, 8325
Naled	8141, 8270, 8321
Naphthalene	8021, 8100, 8260, 8270, 8275, 8310, 8410
NB (Nitrobenzene)	8091, 8260, 8270, 8330, 8410
1,2-Naphthoquinone	8091
1,4-Naphthoquinone	8270, 8091
1-Naphthylamine	8270
2-Naphthylamine	8270
Neburon	8321
Nicotine	8270
5-Nitroacenaphthene	8270
2-Nitroaniline	8131, 8270, 8410
3-Nitroaniline	8131, 8270, 8410
4-Nitroaniline	8131, 8270, 8410
5-Nitro-o-anisidine	8270
Nitrobenzene (NB)	8091, 8260, 8270, 8330, 8410
4-Nitrobiphenyl	8270
Nitrofen	8081, 8270
Nitroglycerin	8332
2-Nitrophenol	8041, 8270, 8410
3-Nitrophenol	8041
4-Nitrophenol	8041, 8151, 8270, 8410
4-Nitrophenyl phenyl ether	8111

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
2-Nitropropane	8260
Nitroquinoline-1-oxide	8270
N-Nitrosodi-n-butylamine	8015, 8260, 8270
N-Nitrosodiethylamine	8270
N-Nitrosodimethylamine	8070, 8270, 8410
N-Nitrosodi-n-butylamine (N-Nitrosodibutylamine)	8015, 8260, 8270
N-Nitrosodiphenylamine	8070, 8270, 8410
N-Nitrosodi-n-propylamine	8070, 8270, 8410
N-Nitrosomethylethylamine	8270
N-Nitrosomorpholine	8270
N-Nitrosopiperidine	8270
N-Nitrosopyrrolidine	8270
2-Nitrotoluene (o-Nitrotoluene, 2-NT)	8091, 8330
3-Nitrotoluene (m-Nitrotoluene, 3-NT)	8091, 8330
4-Nitrotoluene (p-Nitrotoluene, 4-NT)	8091, 8330
o-Nitrotoluene (2-Nitrotoluene, 2-NT)	8091, 8330
m-Nitrotoluene (3-Nitrotoluene, 3-NT)	8091, 8330
p-Nitrotoluene (4-Nitrotoluene, 4-NT)	8091, 8330
5-Nitro-o-toluidine	8270
trans-Nonachlor	8081
2,2'3,3'4,4'5,5'6-Nonachlorobiphenyl	8082, 8275
Nonanal	8315
2-NT (2-Nitrotoluene, o-Nitrotoluene)	8091, 8330
3-NT (3-Nitrotoluene, m-Nitrotoluene)	8091, 8330
4-NT (4-Nitrotoluene, p-Nitrotoluene)	8091, 8330
OCDD	8280, 8290
OCDF	8280, 8290
2,2',3,3',4,4'5,5'-Octachlorobiphenyl	8275
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	8330
Octamethyl pyrophosphoramide	8270
Octanal	8315
Oxamyl	8321
4,4'-Oxydianiline	8270
Paraldehyde	8015, 8260
Parathion	8270
Parathion, ethyl	8141
PCB-1016 (Aroclor-1016)	8082, 8270
PCB-1221 (Aroclor-1221)	8082, 8270
PCB-1232 (Aroclor-1232)	8082, 8270
PCB-1242 (Aroclor-1242)	8082, 8270
PCB-1248 (Aroclor-1248)	8082, 8270
PCB-1254 (Aroclor-1254)	8082, 8270
PCB-1260 (Aroclor-1260)	8082, 8270
PCNB	8081
1,2,3,7,8-PeCDD	8280, 8290
PeCDD, total	8280, 8290

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
1,2,3,7,8-PeCDF	8280, 8290
2,3,4,7,8-PeCDF	8280, 8290
PeCDF, total	8280, 8290
Pendimethaline (Penoxalin)	8091
Penoxalin (Pendimethaline)	8091
Pentachlorobenzene	8121, 8270
2,2',3,4,5'-Pentachlorobiphenyl	8082
2,2',4,5,5'-Pentachlorobiphenyl	8082, 8275
2,3,3',4',6-Pentachlorobiphenyl	8082
2,3',4,4',5-Pentachlorobiphenyl	8275
Pentachloroethane	8260
Pentachloronitrobenzene	8091, 8270
Pentachlorophenol	8041, 8151, 8270, 8410
Pentafluorobenzene	8260
Pentanal (Valeraldehyde)	8315
2-Pentanone	8015, 8260
Permethrin (*cis* and *trans*)	8081
Perthane	8081
Phenacetin	8270
Phenanthrene	8100, 8270, 8275, 8310, 8410
Phenobarbital	8270
Phenol	8041, 8270, 8410
1,4-Phenylenediamine	8270
Phorate	8141, 8270, 8321
Phosalone	8270
Phosmet	8141, 8270
Phosphamidon	8141, 8270
Phthalic anhydride	8270
Picloram	8151
2-Picoline (2-Methylpyridine)	8015, 8260, 8270
Piperonyl sulfoxide	8270
Profluralin	8091
Promecarb	8318
Pronamide	8270
Propachlor	8081, 8321
Propanal (Propionaldehyde)	8315, 8321
1-Propanol	8015, 8260
2-Propanol (Isopropyl alcohol)	8015, 8260
Propargyl alcohol	8260
Propenal (Acrolein)	8260, 8315
Propham	8321
ß-Propiolactone	8260
Propionaldehyde (Propanal)	8315
Propionitrile (Ethyl cyanide)	8015, 8260
Propoxur (Baygon)	8318, 8321
n-Propylamine	8260

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
n-Propylbenzene	8021, 8260
Propylthiouracil	8270
Prothiophos (Tokuthion)	8141
Pyrene	8100, 8270, 8275, 8310, 8410
Pyridine	8015, 8260
RDX (Hexahydro-1,3,5-trinitro-1,3,5-triazine)	8330
Resorcinol	8270
Ronnel	8141
Rotenone	8325
Safrole	8270
Sevin (Carbaryl)	8270, 8318, 8321, 8325
Siduron	8321, 8325
Simazine	8141
Silvex (2,4,5-TP)	8151, 8321
Solvent Red 3	8321
Solvent Red 23	8321
Stirophos (Tetrachlorvinphos)	8141, 8270
Strobane	8081
Strychnine	8270, 8321
Styrene	8021, 8260
Sulfallate	8270
Sulfotepp	8141
Sulprofos (Bolstar)	8141
2,4,5-T	8151, 8321
2,4,5-T, butoxyethanol ester	8321
2,4,5-T, butyl ester	8321
2,3,7,8-TCDD	8280, 8290
TCDD, total	8280, 8290
2,3,7,8-TCDF	8280, 8290
TCDF, total	8280, 8290
Tebuthiuron	8321
Temik (Aldicarb)	8318, 8321
Terbufos	8141, 8270
1,2,3,4-Tetrachlorobenzene	8121
1,2,3,5-Tetrachlorobenzene	8121
1,2,4,5-Tetrachlorobenzene	8121, 8270
2,2',3,5'-Tetrachlorobiphenyl	8082, 8275
2,2',4,5'-Tetrachlorobiphenyl	8275
2,2',5,5'-Tetrachlorobiphenyl	8082, 8275
2,3',4,4'-Tetrachlorobiphenyl	8082, 8275
1,1,1,2-Tetrachloroethane	8021, 8260
1,1,2,2-Tetrachloroethane	8021, 8260
Tetrachloroethene	8021, 8260
2,3,4,5-Tetrachlorophenol	8041
2,3,4,6-Tetrachlorophenol	8041, 8270
2,3,5,6-Tetrachlorophenol	8041

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
2,3,4,5-Tetrachloronitrobenzene	8091
2,3,5,6-Tetrachloronitrobenzene	8091
Tetrachlorvinphos (Stirophos)	8141, 8270
Tetraethyl dithiopyrophosphate	8270
Tetraethyl pyrophosphate (TEPP)	8141, 8270
Tetrazene	8331
Tetryl (Methyl-2,4,6-trinitrophenylnitramine)	8330
Thiofanox	8321
Thionazin (Zinophos)	8141, 8270
Thiophenol (Benzenethiol)	8270
1,3,5-TNB (1,3,5-Trinitrobenzene)	8270, 8330
2,4,6-TNT (2,4,6-Trinitrobenzene)	8330
TOCP (Tri-o-cresylphosphate)	8141
Tokuthion (Prothiophos)	8141
m-Tolualdehyde	8315
o-Tolualdehyde	8315
p-Tolualdehyde	8315
Toluene	8021, 8260
Toluene diisocyanate	8270
o-Toluidine	8015, 8260, 8270
Toxaphene	8081, 8270
2,4,5-TP (Silvex)	8151, 8321
2,4,6-Trichloroaniline	8131
2,4,5-Trichloroaniline	8131
1,2,3-Trichlorobenzene	8021, 8121, 8260
1,2,4-Trichlorobenzene	8021, 8121, 8260, 8270, 8275, 8410
2,2',5-Trichlorobiphenyl	8082, 8275
2,3',5-Trichlorobiphenyl	8275
2,4',5-Trichlorobiphenyl	8082, 8275
1,3,5-Trichlorobenzene	8121
1,1,1-Trichloroethane	8021, 8260
1,1,2-Trichloroethane	8021, 8260
Trichloroethene	8021, 8260
Trichlorofluoromethane	8021, 8260
Trichlorfon	8141, 8321
Trichloronate	8141
1,2,3-Trichloro-4-nitrobenzene	8091
1,2,4-Trichloro-5-nitrobenzene	8091
2,4,6-Trichloronitrobenzene	8091
2,3,4-Trichlorophenol	8041
2,3,5-Trichlorophenol	8041
2,3,6-Trichlorophenol	8041
2,4,5-Trichlorophenol	8041, 8270, 8410
2,4,6-Trichlorophenol	8041, 8270, 8410
2,4,6-Trichlorophenyl 4-nitrophenyl ether	8111
2,3,6-Trichlorophenyl 4-nitrophenyl ether	8111

TABLE 2-1. (Continued)

Analyte	Applicable Method(s)
2,3,5-Trichlorophenyl 4-nitrophenyl ether	8111
2,4,5-Trichlorophenyl 4-nitrophenyl ether	8111
3,4,5-Trichlorophenyl 4-nitrophenyl ether	8111
2,3,4-Trichlorophenyl 4-nitrophenyl ether	8111
1,2,3-Trichloropropane	8021, 8260
O,O,O-Triethyl phosphorothioate	8270
Trifluralin	8091, 8081, 8270
2,4,5-Trimethylaniline	8270
1,2,4-Trimethylbenzene	8021, 8260
1,3,5-Trimethylbenzene	8021, 8260
Trimethyl phosphate	8270
1,3,5-Trinitrobenzene (1,3,5-TNB)	8270, 8330
2,4,6-Trinitrobenzene (2,4,6-TNT)	8330
Tris-BP (Tris-(2,3-dibromopropyl) phosphate)	8270, 8321
Tri-o-cresylphosphate (TOCP)	8141
Tri-p-tolyl phosphate	8270
Tris-(2,3-dibromopropyl) phosphate (Tris-BP)	8270, 8321
Valeraldehyde (Pentanal)	8315
Vinyl acetate	8260
Vinyl chloride	8021, 8260
Vinylidene chloride (1,1-Dichloroethene)	8021, 8260
o-Xylene	8021, 8260
m-Xylene	8021, 8260
p-Xylene	8021, 8260
Zinophos (Thionazin)	8141, 8270

TABLE 2-2
METHOD 8011 (MICROEXTRACTION AND GAS CHROMATOGRAPHY)

1,2-Dibromo-3-chloropropane (DBCP)
1,2-Dibromoethane (EDB)

TABLE 2-3
METHOD 8015 (GC/FID) - NONHALOGENATED VOLATILES

Acetone	Isobutyl alcohol
Acetonitrile	Isopropyl alcohol
Acrolein	Methanol
Acrylonitrile	Methyl ethyl ketone (MEK)
Allyl alcohol	Methyl isobutyl ketone (MIBK)
1-Butanol (n-Butyl alcohol)	N-Nitroso-di-n-butylamine
t-Butyl alcohol	Paraldehyde
Crotonaldehyde	2-Pentanone
Diethyl ether	2-Picoline
1,4-Dioxane	1-Propanol
Ethanol	Propionitrile
Ethyl acetate	Pyridine
Ethylene glycol	o-Toluidine
Ethylene oxide	Gasoline range organics (GRO)
	Diesel range organics (DRO)

TABLE 2-4
METHOD 8021 (GC, PHOTOIONIZATION AND ELECTROLYTIC CONDUCTIVITY DETECTORS) - AROMATIC AND HALOGENATED VOLATILES

Allyl chloride	cis-1,2-Dichloroethene
Benzene	trans-1,2-Dichloroethene
Benzyl chloride	1,2-Dichloropropane
Bis(2-chloroisopropyl) ether	1,3-Dichloropropane
	2,2-Dichloropropane
Bromoacetone	1,3-Dichloro-2-propanol
Bromobenzene	1,1-Dichloropropene
Bromochloromethane	cis-1,3-Dichloropropene
Bromodichloromethane	trans-1,3-Dichloropropene
Bromoform	Epichlorhydrin
Bromomethane	Ethylbenzene
n-Butylbenzene	Hexachlorobutadiene
sec-Butylbenzene	Isopropylbenzene
tert-Butylbenzene	p-Isopropyltoluene
Carbon tetrachloride	Methylene chloride
Chlorobenzene	Naphthalene
Chlorodibromomethane	n-Propylbenzene
Chloroethane	Styrene
2-Chloroethanol	1,1,1,2-Tetrachloroethane
2-Chloroethyl vinyl ether	1,1,2,2-Tetrachloroethane
Chloroform	Tetrachloroethene
Chloromethyl methyl ether	Toluene
Chloroprene	1,2,3-Trichlorobenzene
Chloromethane	1,2,4-Trichlorobenzene
2-Chlorotoluene	1,1,1-Trichloroethane
4-Chlorotoluene	1,1,2-Trichloroethane
1,2-Dibromo-3-chloropropane	Trichloroethene
1,2-Dibromoethane	Trichlorofluoromethane
Dibromomethane	1,2,3-Trichloropropane
1,2-Dichlorobenzene	1,2,4-Trimethylbenzene
1,3-Dichlorobenzene	1,3,5-Trimethylbenzene
1,4-Dichlorobenzene	Vinyl chloride
Dichlorodifluoromethane	o-Xylene
1,1-Dichloroethane	m-Xylene
1,2-Dichloroethane	p-Xylene
1,1-Dichloroethene	

TABLE 2-5
METHODS 8031 AND 8032 (GC) AND 8033 (GC WITH NITROGEN-PHOSPHORUS DETECTION)

Method 8031: Acrylonitrile
Method 8032: Acrylamide
Method 8033: Acetonitrile

TABLE 2-6
METHOD 8041 (GC) - PHENOLS

2-Chloro-5-methylphenol	2,4-Dinitrophenol
4-Chloro-2-methylphenol	2,5-Dinitrophenol
4-Chloro-3-methylphenol	Dinoseb
2-Chlorophenol	2-Methyl-4,6-dinitrophenol
3-Chlorophenol	2-Methylphenol (o-Cresol)
4-Chlorophenol	3-Methylphenol (m-Cresol)
2-Cyclohexyl-4,6-dinitro-phenol	4-Methylphenol (p-Cresol)
	2-Nitrophenol
2,3-Dichlorophenol	3-Nitrophenol
2,4-Dichlorophenol	4-Nitrophenol
2,5-Dichlorophenol	Pentachlorophenol
2,6-Dichlorophenol	Phenol
3,4-Dichlorophenol	2,3,4,5-Tetrachlorophenol
3,5-Dichlorophenol	2,3,4,6-Tetrachlorophenol
2,3-Dimethylphenol	2,3,5,6-Tetrachlorophenol
2,4-Dimethylphenol	2,3,4-Trichlorophenol
2,5-Dimethylphenol	2,3,5-Trichlorophenol
2,6-Dimethylphenol	2,3,6-Trichlorophenol
3,4-Dimethylphenol	2,4,5-Trichlorophenol
	2,4,6-Trichlorophenol

TABLE 2-7
METHOD 8061 (GC/ECD) - PHTHALATE ESTERS

Bis(2-n-butoxyethyl) phthalate	Dicyclohexyl phthalate
Bis(2-ethoxyethyl) phthalate	Dihexyl phthalate
Bis(2-ethylhexyl) phthalate	Diisobutyl phthalate
Bis(2-methoxyethyl) phthalate	Di-n-butyl phthalate
Bis(4-methyl-2-pentyl)-phthalate	Diethyl phthalate
	Dinonyl phthalate
Butyl benzyl phthalate	Dimethyl phthalate
Diamyl phthalate	Di-n-octyl phthalate
	Hexyl 2-ethylhexyl phthalate

TABLE 2-8
METHOD 8070 (GC) - NITROSAMINES

N-Nitrosodimethylamine
N-Nitrosodiphenylamine
N-Nitrosodi-n-propylamine

TABLE 2-9
METHOD 8081 (GC) - ORGANOCHLORINE PESTICIDES AND PCBs

Alachlor
Aldrin
α-BHC
β-BHC
δ-BHC
γ-BHC (Lindane)
Captafol
Chlorobenzilate
α-Chlordane
γ-Chlordane
Chlordane (NOS)
Chloroneb
Chloropropylate
Chlorothalonil
DBCP
DCPA
4,4'-DDD
4,4'-DDE
4,4'-DDT
Diallate
Dichlone
Dicofol
Dieldrin
Endosulfan I
Endosulfan II
Endosulfan sulfate
Endrin
Endrin aldehyde
Endrin ketone
Etridiazole
Halowax-1000
Halowax-1001
Halowax-1013
Halowax-1014
Halowax-1051
Halowax-1099
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
Hexachlorocyclopentadiene
Isodrin
Methoxychlor
Mirex
Nitrofen
trans-Nonachlor
PCNB
Permethrin (*cis* and *trans*)
Perthane
Propachlor
Strobane
Toxaphene
Trifluralin

TABLE 2-10
METHOD 8082 (GC) - POLYCHLORINATED BIPHENYLS

Aroclor 1016	2,2',3,4,5'-Pentachlorobiphenyl
Aroclor 1221	2,2',4,5,5'-Pentachlorobiphenyl
Aroclor 1232	2,3,3',4',6-Pentachlorobiphenyl
Aroclor 1242	2,2',3,4,4',5'-Hexachlorobiphenyl
Aroclor 1248	2,2',3,4,5,5'-Hexachlorobiphenyl
Aroclor 1254	2,2',3,5,5',6-Hexachlorobiphenyl
Aroclor 1260	2,2',4,4',5,5'-Hexachlorobiphenyl
2-Chlorobiphenyl	2,2',3,3',4,4',5-Heptachlorobiphenyl
2,3-Dichlorobiphenyl	2,2',3,4,4',5,5'-Heptachlorobiphenyl
2,2',5-Trichlorobiphenyl	2,2',3,4,4',5',6-Heptachloro-biphenyl
2,4',5-Trichlorobiphenyl	2,2',3,4',5,5',6-Heptachlorobiphenyl
2,2',3,5'-Tetrachlorobiphenyl	2,2',3,3',4,4',5,5',6-Nonachloro-biphenyl
2,2',5,5'-Tetrachlorobiphenyl	
2,3',4,4'-Tetrachlorobiphenyl	

TABLE 2-11
METHOD 8091 (GC) - NITROAROMATICS AND CYCLIC KETONES

Benefin	2,4-Dinitrotoluene
Butralin	2,6-Dinitrotoluene
1-Chloro-2,4-dinitrobenzene	Isopropalin
1-Chloro-3,4-dinitrobenzene	1,2-Naphthoquinone
1-Chloro-2-nitrobenzene	1,4-Naphthoquinone
1-Chloro-4-nitrobenzene	Nitrobenzene
2-Chloro-6-nitrotoluene	2-Nitrotoluene
4-Chloro-2-nitrotoluene	3-Nitrotoluene
4-Chloro-3-nitrotoluene	4-Nitrotoluene
2,3-Dichloronitrobenzene	Penoxalin [Pendimethalin]
2,4-Dichloronitrobenzene	Pentachloronitrobenzene
3,5-Dichloronitrobenzene	Profluralin
3,4-Dichloronitrobenzene	2,3,4,5-Tetrachloronitrobenzene
2,5-Dichloronitrobenzene	2,3,5,6-Tetrachloronitrobenzene
Dinitramine	1,2,3-Trichloro-4-nitrobenzene
1,2-Dinitrobenzene	1,2,4-Trichloro-5-nitrobenzene
1,3-Dinitrobenzene	2,4,6-Trichloronitrobenzene
1,4-Dinitrobenzene	Trifluralin

TABLE 2-12
METHOD 8100 - POLYNUCLEAR AROMATIC HYDROCARBONS

Acenaphthene	Dibenz(a,h)anthracene
Acenaphthylene	7H-Dibenzo(c,g)carbazole
Anthracene	Dibenzo(a,e)pyrene
Benz(a)anthracene	Dibenzo(a,h)pyrene
Benzo(b)fluoranthene	Dibenzo(a,i)pyrene
Benzo(j)fluoranthene	Fluoranthene
Benzo(k)fluoranthene	Fluorene
Benzo(g,h,i)perylene	Indeno(1,2,3-cd)pyrene
Benzo(a)pyrene	3-Methylcholanthrene
Chrysene	Naphthalene
Dibenz(a,h)acridine	Phenanthrene
Dibenz(a,j)acridine	Pyrene

TABLE 2-13
METHOD 8111 (GC) - HALOETHERS

Bis(2-chloroethoxy)methane	2,3-Dichlorophenyl 4-nitrophenyl ether
Bis(2-chloroethyl) ether	3,4-Dichlorophenyl 4-nitrophenyl ether
Bis(2-chloroisopropyl) ether	4-Nitrophenyl phenyl ether
4-Bromophenyl phenyl ether	2,4,6-Trichlorophenyl 4-nitrophenyl ether
4-Chlorophenyl phenyl ether	2,3,6-Trichlorophenyl 4-nitrophenyl ether
2-Chlorophenyl 4-nitrophenyl ether	2,3,5-Trichlorophenyl 4-nitrophenyl ether
3-Chlorophenyl 4-nitrophenyl ether	2,4,5-Trichlorophenyl 4-nitrophenyl ether
4-Chlorophenyl 4-nitrophenyl ether	3,4,5-Trichlorophenyl 4-nitrophenyl ether
2,4-Dibromophenyl 4-nitrophenyl ether	2,3,4-Trichlorophenyl 4-nitrophenyl ether
2,4-Dichlorophenyl 3-methyl-4-nitrophenyl ether	
2,6-Dichlorophenyl 4-nitrophenyl ether	
3,5-Dichlorophenyl 4-nitrophenyl ether	
2,5-Dichlorophenyl 4-nitrophenyl ether	
2,4-Dichlorophenyl 4-nitrophenyl ether	

TABLE 2-14
METHOD 8121 (GC) - CHLORINATED HYDROCARBONS

Benzal chloride	δ-Hexachlorocyclohexane [δ-BHC]
Benzotrichloride	γ-Hexachlorocyclohexane [γ-BHC]
Benzyl chloride	Hexachlorocyclopentadiene
2-Chloronaphthalene	Hexachloroethane
1,2-Dichlorobenzene	Pentachlorobenzene
1,3-Dichlorobenzene	1,2,3,4-Tetrachlorobenzene
1,4-Dichlorobenzene	1,2,3,5-Tetrachlorobenzene
Hexachlorobenzene	1,2,4,5-Tetrachlorobenzene
Hexachlorobutadiene	1,2,3-Trichlorobenzene
α-Hexachlorocyclohexane [α-BHC]	1,2,4-Trichlorobenzene
β-Hexachlorocyclohexane [β-BHC]	1,3,5-Trichlorobenzene

TABLE 2-15
METHOD 8131 (GC) - ANILINE AND SELECTED DERIVATIVES

Aniline	2,6-Dibromo-4-nitroaniline
4-Bromoaniline	3,4-Dichloroaniline
2-Bromo-6-chloro-4-nitroanilne	2,6-Dichloro-4-nitroaniline
2-Bromo-4,6-dintroaniline	2,4-Dinitroaniline
2-Chloroaniline	2-Nitroaniline
3-Chloroaniline	3-Nitroaniline
4-Chloroaniline	4-Nitroaniline
2-Chloro-4,6-dinitroaniline	2,4,6-Trichloroaniline
2-Chloro-4-nitroaniline	2,4,5-Trichloroaniline
4-Chloro-2-nitroaniline	

TABLE 2-16
METHOD 8141 (GC) - ORGANOPHOSPHORUS COMPOUNDS

Aspon	Fenthion
Atrazine	Fonophos
Azinphos-ethyl	Hexamethyl phosphoramide (HMPA)
Azinphos-methyl	Leptophos
Bolstar (Sulprofos)	Malathion
Carbophenothion	Merphos
Chlorfenvinphos	Mevinphos
Chlorpyrifos	Monocrotophos
Chlorpyrifos methyl	Naled
Coumaphos	Parathion, ethyl
Crotoxyphos	Parathion, methyl
Demeton-O, and -S	Phorate
Diazinon	Phosmet
Dichlorofenthion	Phosphamidon
Dichlorvos (DDVP)	Ronnel
Dicrotophos	Simazine
Dimethoate	Stirophos (Tetrachlorvinphos)
Dioxathion	Sulfotepp
Disulfoton	Tetraethyl pyrophosphate (TEPP)
EPN	Terbufos
Ethion	Thionazin (Zinophos)
Ethoprop	Tokuthion (Prothiophos)
Famphur	Trichlorfon
Fenitrothion	Trichloronate
Fensulfothion	Tri-o-cresyl phosphate (TOCP)

TABLE 2-17
METHOD 8151 (GC USING METHYLATION OR PENTAFLUOROBENZYLATION DERIVATIZATON) - CHLORINATED HERBICIDES

Acifluorfen	Dicamba	MCPP
Bentazon	3,5-Dichlorobenzoic acid	4-Nitrophenol
Chloramben	Dichloroprop	Pentachlorophenol
2,4-D	Dinoseb	Picloram
Dalapon	5-Hydroxydicamba	2,4,5-TP (Silvex)
2,4-DB	MCPA	2,4,5-T
DCPA diacid		

TABLE 2-18
METHOD 8260 (GC/MS)- VOLATILE ORGANIC COMPOUNDS

Acetone
Acetonitrile
Acrolein (Propenal)
Acrylonitrile
Allyl alcohol
Allyl chloride
Benzene
Benzyl chloride
Bis(2-chloroethyl)-sulfide
Bromoacetone
Bromobenzene
Bromochloromethane
Bromodichloromethane
Bromoform
Bromomethane
n-Butanol
2-Butanone (MEK)
t-Butyl alcohol
n-Butylbenzene
sec-Butylbenzene
tert-Butylbenzene
Carbon disulfide
Carbon tetrachloride
Chloral hydrate
Chloroacetonitrile
Chlorobenzene
1-Chlorobutane
Chlorodibromomethane
Chloroethane
2-Chloroethanol
2-Chloroethyl vinyl ether
Chloroform
1-Chlorohexane
Chloromethane
Chloroprene
3-Chloropropionitrile
2-Chlorotoluene
4-Chlorotoluene
Crotonaldehyde
1,2-Dibromo-3-chloropropane
1,2-Dibromoethane
Dibromofluoromethane
Dibromomethane
1,2-Dichlorobenzene
1,3-Dichlorobenzene
1,4-Dichlorobenzene
cis-1,4-Dichloro-2-butene
trans-1,4-Dichloro-2-butene
Dichlorodifluoromethane
1,1-Dichloroethane
1,2-Dichloroethane
1,1-Dichloroethene
cis-1,2-Dichloroethene
trans-1,2-Dichloro-ethene
1,2-Dichloropropane
1,3-Dichloropropane
2,2-Dichloropropane
1,3-Dichloro-2-propanol
1,1-Dichloropropene
cis-1,3-Dichloropropene
trans-1,3-Dichloro-propene
1,2,3,4-Diepoxybutane
Diethyl ether
1,4-Dioxane
Epichlorohydrin
Ethanol
Ethyl acetate
Ethylbenzene
Ethylene oxide
Ethyl methacrylate
Hexachlorobutadiene
Hexachloroethane
2-Hexanone
2-Hydroxypropionitrile
Iodomethane
Isobutyl alcohol
Isopropylbenzene
p-Isopropyltoluene
Malononitrile
Methacrylonitrile
Methanol
Methyl-t-butyl ether
Methylene chloride
Methyl acrylate
Methyl methacrylate
4-Methyl-2-pentanone (MIBK)
Naphthalene
Nitrobenzene
2-Nitropropane
N-Nitroso-di-n-butylamine
Paraldehyde
Pentachloroethane
Pentafluorobenzene
2-Pentanone
2-Picoline
1-Propanol
2-Propanol
Propargyl alcohol
ß-Propiolactone
Propionitrile (Ethyl cyanide)
n-Propylamine
n-Propylbenzene
Pyridine
Styrene
1,1,1,2-Tetrachloro-ethane
1,1,2,2-Tetrachloro-ethane
Tetrachloroethene
Toluene
o-Toluidine
1,2,3-Trichlorobenzene
1,2,4-Trichlorobenzene
1,1,1-Trichloroethane
1,1,2-Trichloroethane
Trichloroethene
Trichlorofluoromethane
1,2,3-Trichloropropane
1,2,4-Trimethylbenzene
1,3,5-Trimethylbenzene
Vinyl acetate
Vinyl chloride
o-Xylene
m-Xylene
p-Xylene

TABLE 2-19
METHOD 8270 (GC/MS) - SEMIVOLATILE ORGANIC COMPOUNDS

Acenaphthene
Acenaphthylene
Acetophenone
2-Acetylaminofluorene
1-Acetyl-2-thiourea
Aldrin
2-Aminoanthraquinone
Aminoazobenzene
4-Aminobiphenyl
3-Amino-9-ethyl-carbazole
Anilazine
Aniline
o-Anisidine
Anthracene
Aramite
Aroclor-1016
Aroclor-1221
Aroclor-1232
Aroclor-1242
Aroclor-1248
Aroclor-1254
Aroclor-1260
Azinphos-methyl
Barban
Benz(a)anthracene
Benzidine
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Benzoic acid
Benzo(g,h,i)perylene
Benzo(a)pyrene
p-Benzoquinone
Benzyl alcohol
α-BHC
β-BHC
δ-BHC
γ-BHC (Lindane)
Bis(2-chloroethoxy)-methane
Bis(2-chloroethyl) ether
Bis(2-chloroisopropyl) ether
Bis(2-ethylhexyl) phthalate
4-Bromophenyl phenyl ether
Bromoxynil
Butyl benzyl phthalate
Captafol
Captan
Carbaryl
Carbofuran
Carbophenothion
Chlordane (NOS)
Chlorfenvinphos
4-Chloroaniline
Chlorobenzilate
5-Chloro-2-methyl-aniline
4-Chloro-3-methylphenol
3-(Chloromethyl)-pyridine hydro-chloride
1-Chloronaphthalene
2-Chloronaphthalene
2-Chlorophenol
4-Chloro-1,2-phenylene-diamine
4-Chloro-1,3-phenylene-diamine
4-Chlorophenyl phenyl ether
Chrysene
Coumaphos
p-Cresidine
Crotoxyphos
2-Cyclohexyl-4,6-dinitrophenol
4,4'-DDD
4,4'-DDE
4,4'-DDT
Demeton-O
Demeton-S
Diallate (cis or trans)
2,4-Diaminotoluene
Dibenz(a,j)acridine
Dibenz(a,h)anthracene
Dibenzofuran
Dibenzo(a,e)pyrene
1,2-Dibromo-3-chloropropane
Di-n-butyl phthalate
Dichlone
1,2-Dichlorobenzene
1,3-Dichlorobenzene
1,4-Dichlorobenzene
3,3'-Dichlorobenzidine
2,4-Dichlorophenol
2,6-Dichlorophenol
Dichlorovos
Dicrotophos
Dieldrin
Diethyl phthalate
Diethylstilbestrol
Diethyl sulfate
Dimethoate
3,3'-Dimethoxybenzidine
Dimethylaminoazobenzene
7,12-Dimethylbenz(a)-anthracene
3,3'-Dimethylbenzidine
α,α-Dimethylphenethyl-amine
2,4-Dimethylphenol
Dimethyl phthalate
1,2-Dinitrobenzene
1,3-Dinitrobenzene
1,4-Dinitrobenzene
4,6-Dinitro-2-methyl-phenol
2,4-Dinitrophenol
2,4-Dinitrotoluene
2,6-Dinitrotoluene
Dinocap
Dinoseb
Diphenylamine
5,5-Diphenylhydantoin
1,2-Diphenylhydrazine
Di-n-octyl phthalate
Disulfoton
Endosulfan I
Endosulfan II
Endosulfan sulfate
Endrin
Endrin aldehyde
Endrin ketone
EPN
Ethion
Ethyl carbamate
Ethyl methanesulfonate
Famphur
Fensulfothion

TABLE 2-19 (CONTINUED)

Fenthion
Fluchloralin
Fluoranthene
Fluorene
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
Hexachlorobutadiene
Hexachlorocyclo-
pentadiene
Hexachloroethane
Hexachlorophene
Hexachloropropene
Hexamethylphosphoramide
Hydroquinone
Indeno(1,2,3-cd)pyrene
Isodrin
Isophorone
Isosafrole
Kepone
Leptophos
Malathion
Maleic anhydride
Mestranol
Methapyrilene
Methoxychlor
3-Methylcholanthrene
4,4'-Methylenebis-
(2-chloroaniline)
4,4'-Methylenebis-
(N,N-dimethylaniline)
Methyl methanesulfonate
2-Methylnaphthalene
Methyl parathion
2-Methylphenol
3-Methylphenol
4-Methylphenol
Mevinphos
Mexacarbate
Mirex
Monocrotophos
Naled
Naphthalene
1,4-Naphthoquinone
1-Naphthylamine
2-Naphthylamine
Nicotine
5-Nitroacenaphthene
2-Nitroaniline
3-Nitroaniline
4-Nitroaniline
5-Nitro-o-anisidine
Nitrobenzene
4-Nitrobiphenyl
Nitrofen
2-Nitrophenol
4-Nitrophenol
Nitroquinoline-1-oxide
N-Nitrosodi-n-
butylamine
N-Nitrosodiethylamine
N-Nitrosodimethylamine
N-Nitrosodiphenylamine
N-Nitrosodi-n-propyl-
amine
N-Nitrosomethylethyl-
amine
N-Nitrosomorpholine
N-Nitrosopiperidine
N-Nitrosopyrrolidine
5-Nitro-o-toluidine
Octamethyl pyrophos-
phoramide
4,4'-Oxydianiline
Parathion
Pentachlorobenzene
Pentachloronitrobenzene
Pentachlorophenol
Phenacetin
Phenanthrene
Phenobarbital
Phenol
1,4-Phenylenediamine
Phorate
Phosalone
Phosmet
Phosphamidion
Phthalic anhydride
2-Picoline (2-Methylpyridine)
Piperonyl sulfoxide
Pronamide
Propylthiouracil
Pyrene
Resorcinol
Safrole
Strychnine
Sulfallate
Terbufos
1,2,4,5-Tetrachloro
benzene
2,3,4,6-Tetrachloro-
phenol
Tetrachlorvinphos
Tetraethyl dithio-
pyrophosphate
Tetraethyl
pyrophosphate
Thionazine
Thiophenol
(Benzenethiol)
Toluene diisocyanate
o-Toluidine
Toxaphene
1,2,4-Trichlorobenzene
2,4,5-Trichlorophenol
2,4,6-Trichlorophenol
O,O,O-Triethyl
phosphorothioate
Trifluralin
2,4,5-Trimethylaniline
Trimethyl phosphate
1,3,5-Trinitrobenzene
Tris(2,3-dibromopropyl)
phosphate
Tri-p-tolyl phosphate

TABLE 2-20
METHOD 8275 (TE/GC/MS) - SEMIVOLATILE ORGANIC COMPOUNDS

Acenaphthene	Pyrene	2,3',4,4',5-Penta-chlorobiphenyl
Acenaphthylene	1,2,4-Trichlorobenzene	2,2',3,4,4',5'-Hexachlorobiphenyl
Anthracene	2-Chlorobiphenyl	2,2',3,3',4,4'-Hexachlorobiphenyl
Benz(a)anthracene	3,3'-Dichlorobiphenyl	2,2',3,4',5,5',6-Heptachlorobiphenyl
Benzo(a)pyrene	2,2',5-Trichloro-biphenyl	2,2',3,4,4',5,5'-Heptachlorobiphenyl
Benzo(b)fluoranthene	2,3',5-Trichloro-biphenyl	2,2',3,3',4,4',5-Heptachlorobiphenyl
Benzo(g,h,i)perylene	2,4',5-Trichloro-biphenyl	2,2',3,3',4,4',5,5'-Octachlorobiphenyl
Benzo(k)fluoranthene	2,2',5,5'-Tetrachloro-biphenyl	2,2',3,3'4,4',5,5',6-Nonachlorobiphenyl
4-Bromophenyl phenyl ether	2,2'4,5'-Tetrachloro-biphenyl	2,2',3,3'4,4',5,5',6,6'-Decachlorobiphenyl
1-Chloronaphthalene	2,2'3,5'-Tetrachloro-biphenyl	
Chrysene	2,3',4,4'-Tetrachloro-biphenyl	
Dibenzofuran	2,2',4,5,5'-Penta-chlorobiphenyl	
Dibenz(a,h)anthracene		
Dibenzothiophene		
Fluoranthene		
Fluorene		
Hexachlorobenzene		
Indeno(1,2,3-cd)pyrene		
Naphthalene		
Phenanthrene		

TABLE 2-21
METHODS 8280 (HRGC/LRMS) AND 8290 (HRGC/HRMS) -
POLYCHLORINATED DIBENZO-*p*-DIOXINS (PCDDs)
AND POLYCHLORINATED DIBENZOFURANS (PCDFs)

2,3,7,8-TCDD	HpCDD, total	1,2,3,7,8,9-HxCDF
TCDD, total	OCDD	2,3,4,6,7,8-HxCDF
1,2,3,7,8-PeCDD	2,3,7,8-TCDF	HxCDF, total
PeCDD, total	TCDF, total	1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8-HxCDD	1,2,3,7,8-PeCDF	1,2,3,4,7,8,9-HpCDF
1,2,3,6,7,8-HxCDD	2,3,4,7,8-PeCDF	HpCDF, total
1,2,3,7,8,9-HxCDD	PeCDF, total	OCDF
HxCDD, total	1,2,3,4,7,8-HxCDF	
1,2,3,4,6,7,8-HpCDD	1,2,3,6,7,8-HxCDF	

TABLE 2-22
METHOD 8310 (HPLC) - POLYNUCLEAR AROMATIC HYDROCARBONS

Acenaphthene	Chrysene
Acenaphthylene	Dibenzo(a,h)anthracene
Anthracene	Fluoranthene
Benzo(a)anthracene	Fluorene
Benzo(a)pyrene	Indeno(1,2,3-cd)pyrene
Benzo(b)fluoranthene	Naphthalene
Benzo(g,h,i)perylene	Phenanthrene
Benzo(k)fluoranthene	Pyrene

TABLE 2-23
METHOD 8315 - CARBONYL COMPOUNDS

Acetaldehyde	Hexanal (Hexaldehyde)
Acetone	Isovaleraldehyde
Acrolein	Nonanal
Benzaldehyde	Octanal
Butanal (Butyraldehyde)	Pentanal (Valeraldehyde)
Crotonaldehyde	Propanal
Cyclohexanone	(Propionaldehyde)
Decanal	m-Tolualdehyde
2,5-Dimethylbenzaldehyde	o-Tolualdehyde
Formaldehyde	p-Tolualdehyde
Heptanal	

TABLE 2-24
METHOD 8316 (HPLC)

Acrylamide
Acrylonitrile
Acrolein

TABLE 2-25
METHOD 8318 (HPLC) - N-METHYLCARBAMATES

Aldicarb (Temik)
Aldicarb sulfone
Carbaryl (Sevin)
Carbofuran (Furadan)
Dioxacarb
3-Hydroxycarbofuran
Methiocarb (Mesurol)
Methomyl (Lannate)
Promecarb
Propoxur (Baygon)

TABLE 2-26. METHOD 8321 (HPLC/TS/MS) - NONVOLATILE ORGANIC COMPOUNDS

Azo Dyes
Disperse Red 1
Disperse Red 5
Disperse Red 13
Disperse Yellow 5
Disperse Orange 3
Disperse Orange 30
Disperse Brown 1
Solvent Red 3
Solvent Red 23

Chlorinated Phenoxyacid Compounds
2,4-D
2,4-D, butoxyethanol ester
2,4-D, ethylhexyl ester
2,4-DB
Dalapon
Dicamba
Dichlorprop
Dinoseb
MCPA
MCPP
Silvex (2,4,5-TP)
2,4,5-T
2,4,5-T, butyl ester
2,4,5-T, butoxyethanol ester

Alkaloids
Strychnine
Caffeine

Organophosphorus Compounds
Asulam
Fensulfothion
Dichlorvos
Dimethoate
Disulfoton
Parathion methyl
Merphos
Methomyl
Monocrotophos
Famphur
Naled
Phorate
Trichlorfon
Thiofanox
Tris(2,3-dibromopropyl) phosphate (Tris-BP)

Anthraquinone Dyes

Disperse Blue 3
Disperse Blue 14
Disperse Red 60
Coumarin Dyes

Fluorescent Brighteners

Fluorescent Brightener 61
Fluorescent Brightener 236

Carbamates

Aldicarb
Aldicarb sulfone
Aldicarb sulfoxide
Aminocarb
Barban
Benomyl
Bromacil
Bendiocarb
Carbaryl
Carbendazim
Carbofuran
3-Hydroxycarbofuran
Chloroxuron
Chloropropham
Diuron
Fenuron
Fluometuron
Linuron
Methiocarb
Methomyl
Mexacarbate
Monuron
Neburon
Oxamyl
Propachlor
Propham
Propoxur
Siduron
Tebuthiuron

TABLE 2-27
METHOD 8325 (HPLC/PB/MS) - NONVOLATILE ORGANIC COMPOUNDS

Benzidine	3,3'-Dimethylbenzidine
Benzoylprop ethyl	Diuron
Carbaryl	Linuron (Lorox)
o-Chlorophenyl thiourea	Monuron
3,3'-Dichlorobenzidine	Rotenone
3,3'-Dimethoxybenzidine	Siduron

TABLE 2-28
METHOD 8330 (HPLC) - NITROAROMATICS AND NITRAMINES

4-Amino-2,6-dinitrotoluene (4-Am-DNT)	Nitrobenzene (NB)
2-Amino-4,6-dinitrotoluene (2-Am-DNT)	2-Nitrotoluene (2-NT)
1,3-Dinitrobenzene (1,3-DNB)	3-Nitrotoluene (3-NT)
2,4-Dinitrotoluene (2,4-DNT)	4-Nitrotoluene (4-NT)
2,6-Dinitrotoluene (2,6-DNT)	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	1,3,5-Trinitrobenzene (1,3,5-TNB)
Methyl-2,4,6-trinitrophenyl-nitramine (Tetryl)	2,4,6-Trinitrotoluene (2,4,6-TNT)

TABLE 2-29
METHOD 8331 (REVERSE PHASE HPLC)

Tetrazene

TABLE 2-30
METHOD 8332 (HPLC)

Nitroglycerine

TABLE 2-31
METHOD 8410 - SEMIVOLATILE ORGANIC COMPOUNDS

Acenaphthene	2,6-Dinitrotoluene
Acenaphthylene	Di-n-octyl phthalate
Anthracene	Di-n-propyl phthalate
Benzo(a)anthracene	Fluoranthene
Benzo(a)pyrene	Fluorene
Benzoic acid	Hexachlorobenzene
Bis(2-chloroethoxy)methane	1,3-Hexachlorobutadiene
Bis(2-chloroethyl) ether	Hexachlorocyclopentadiene
Bis(2-chloroisopropyl) ether	Hexachloroethane
Bis(2-ethylhexyl) phthalate	Isophorone
4-Bromophenyl phenyl ether	2-Methylnaphthalene
Butyl benzyl phthalate	2-Methylphenol
4-Chloroaniline	4-Methylphenol
4-Chloro-3-methylphenol	Naphthalene
2-Chloronaphthalene	2-Nitroaniline
2-Chlorophenol	3-Nitroaniline
4-Chlorophenol	4-Nitroaniline
4-Chlorophenyl phenyl ether	Nitrobenzene
Chrysene	2-Nitrophenol
Dibenzofuran	4-Nitrophenol
Di-n-butyl phthalate	N-Nitrosodimethylamine
1,2-Dichlorobenzene	N-Nitrosodiphenylamine
1,3-Dichlorobenzene	N-Nitroso-di-n-propylamine
1,4-Dichlorobenzene	Pentachlorophenol
2,4-Dichlorophenol	Phenanthrene
Diethyl phthalate	Phenol
Dimethyl phthalate	Pyrene
4,6-Dinitro-2-methylphenol	1,2,4-Trichlorobenzene
2,4-Dinitrophenol	2,4,5-Trichlorophenol
2,4-Dinitrotoluene	2,4,6-Trichlorophenol

TABLE 2-32
METHOD 8430 (GC/FT-IR) - BIS(2-CHLOROETHYL) ETHER
AND ITS HYDROLYSIS PRODUCTS

Bis(2-chloroethyl) ether
2-Chloroethanol
2-(2-Chloroethoxy)ethanol
Diethylene glycol
Ethylene glycol

TABLE 2-33. DETERMINATIVE METHODS FOR INORGANIC ANALYTES

Analyte	Applicable Method(s)
Aluminum	6010, 6020, 6800, 7000, 7010
Antimony	6010, 6020, 6200, 6800, 7000, 7062
Arsenic	6010, 6020, 6200, 7010, 7061, 7062, 7063
Barium	6010, 6020, 6200, 6800, 7000, 7010
Beryllium	6010, 6020, 7000, 7010
Boron	6800
Bromide	6500, 9056, 9211
Cadmium	6010, 6020, 6200, 6800, 7000, 7010
Calcium	6010, 6020, 6200, 6800, 7000
Chloride	6500, 9056, 9057, 9212, 9250, 9251, 9253
Chromium	6010, 6020, 6200, 6800, 7000, 7010
Chromium, hexavalent	7195, 7196, 7197, 7198, 7199
Cobalt	6010, 6020, 6200, 7000, 7010
Copper	6010, 6020, 6200, 6800, 7000, 7010
Cyanide	9010, 9012, 9013, 9213
Fluoride	6500, 9056, 9214
Iron	6010, 6020, 6200, 6800, 7000, 7010
Lead	6010, 6020, 6200, 6800, 7000, 7010
Lithium	6010, 7000
Magnesium	6010, 6020, 6800, 7000
Manganese	6010, 6020, 6200, 7000, 7010
Mercury	4500, 6020, 6200, 6800, 7470, 7471, 7472, 7473, 7474
Molybdenum	6010, 6200, 6800, 7000, 7010
Nickel	6010, 6020,, 6200, 6800, 7000, 7010
Nitrate	6500, 9056, 9210
Nitrite	6500, 9056, 9216
Osmium	7000
Phosphate	6500, 9056
Phosphorus	6010
Phosphorus, white	7580
Potassium	6010, 6020, 6200, 6800, 7000
Rubidium	6200
Selenium	6010, 6020, 6200, 6800, 7010, 7741, 7742
Silver	6010, 6020, 6200, 6800, 7000, 7010
Sodium	6010, 6020, 7000
Strontium	6010, 6200, 6800, 7000
Sulfate	6500, 9035, 9036, 9038, 9056
Sulfide	9030, 9031, 9215
Thallium	6010, 6020, 6200, 6800, 7000, 7010
Thorium	6200
Tin	6200, 7000
Titanium	6200
Vanadium	6010, 6020, 6200, 6800, 7000, 7010
Zinc	6010, 6020, 6200, 6800, 7000, 7010
Zirconium	6200

TABLE 2-34
CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES
FOR AQUEOUS MATRICES[A]

Name	Container[1]	Preservation	Maximum holding time
Inorganic Tests:			
Chloride	P, G	None required	28 days
Cyanide, total and amenable to chlorination	P, G	Cool to 4°C; if oxidizing agents present add 5 mL 0.1N $NaAsO_2$ per L or 0.06 g of ascorbic acid per L; adjust pH>12 with 50% NaOH. See Method 9010 for other interferences.	14 days
Hydrogen ion (pH)	P, G	None required	24 hours
Nitrate	P, G	Cool to 4°C	48 hours
Sulfate	P, G	Cool to 4°C	28 days
Sulfide	P, G	Cool to 4°C, add zinc acetate	7 days
Metals:			
Chromium VI	P, G	Cool to 4°C	24 hours
Mercury	P, G	HNO_3 to pH<2	28 days
Metals, except chromium VI and mercury	P, G	HNO_3 to pH<2	6 months
Organic Tests:			
Acrolein and acrylonitrile	G, PTFE-lined septum	Cool to 4°C, 0.008% $Na_2S_2O_3$[3], Adjust pH to 4-5	14 days
Benzidines	G, PTFE-lined cap	Cool to 4°C, 0.008% Na_2S_2O3[3]	7 days until extraction, 40 days after extraction
Chlorinated hydrocarbons	G, PTFE-lined cap	Cool to 4°C, 0.008% $Na_2S_2O_3$[3]	7 days until extraction, 40 days after extraction
Dioxins and Furans	G, PTFE-lined cap	Cool to 4°C, 0.008% $Na_2S_2O_3$[3]	30 days until extraction, 45 days after extraction
Haloethers	G, PTFE-lined cap	Cool to 4°C, 0.008% $Na_2S_2O_3$[3]	7 days until extraction, 40 days after extraction
Nitroaromatics and cyclic ketones	G, PTFE-lined cap	Cool to 4°C, 0.008% $Na_2S_2O_3$[3], store in dark	7 days until extraction, 40 days after extraction
Nitrosamines	G, PTFE-lined cap	Cool to 4°C, 0.008% $Na_2S_2O_3$[3], store in dark	7 days until extraction, 40 days after extraction

(continued on next page)

TABLE 2-34 (continued)

Name	Container[1]	Preservation	Maximum holding time
Oil and grease	G	Cool to 4°C, add 5 mL diluted HCl	28 days
Organic carbon, total (TOC)	P, G	Cool to 4°C, store in dark[2]	28 days
Organochlorine pesticides	G, PTFE-lined cap	Cool to 4°C	7 days until extraction, 40 days after extraction
Organophosphorus pesticides	G, PTFE-lined cap	Cool to 4°C[4]	7 days until extraction, 40 days after extraction
PCBs	G, PTFE-lined cap	Cool to 4°C	7 days until extraction, 40 days after extraction
Phenols	G, PTFE-lined cap	Cool to 4°C, 0.008% Na_2S_2O3[3]	7 days until extraction, 40 days after extraction
Phthalate esters	G, PTFE-lined cap	Cool to 4°C	7 days until extraction, 40 days after extraction
Polynuclear aromatic hydrocarbons	G, PTFE-lined cap	Cool to 4°C, 0.008% $Na_2S_2O_3$[3], store in dark	7 days until extraction, 40 days after extraction
Purgeable aromatic hydrocarbons	G, PTFE-lined septum	Cool to 4°C, 0.008% $Na_2S_2O_3$[2,3]	14 days
Purgeable Halocarbons	G, PTFE-lined septum	Cool to 4°C, 0.008% $Na_2S_2O_3$[3]	14 days
Total organic halides (TOX)	G, PTFE-lined cap	Cool to 4°C, Adjust to pH<2 with H_2SO_4	28 days
Radiological Tests:			
Alpha, beta and radium	P, G	HNO_3 to pH<2	6 months

[A] Table originally excerpted, in part, from Table II, 49 FR 28, October 26, 1984, and revised as appropriate for SW-846. See Chapter Three, Chapter Four, or the individual methods for more information.

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

[2] Adjust to pH<2 with H_2SO_4, HCl or solid $NaHSO_4$. Free chlorine must be removed prior to adjustment.

[3] Free chlorine must be removed by the appropriate addition of $Na_2S_2O_3$.

[4] Adjust samples to pH 5-8 using NaOH or H_2SO_4.

TABLE 2-35
PREPARATION METHODS FOR ORGANIC ANALYTES
(Note: Footnote text is located on the last page of the table.)

	Matrix			
Analyte Type	Aqueous[1]	Solids	Sludges and Emulsions[1,2]	Organic Liquids, Tars, Oils
Acid Extractable	3510 3520 (pH ≤ 2)	3540 3541 3545 3550	3520 (pH ≤ 2)	3650 3580[3]
Acrolein, Acrylonitrile, and Acetonitrile	5031	5031	5031	3585
Acrylamide	8032[4]			
Aniline and Selected Derivatives	3510 3520 (pH >11) 5031[11]	3540 3541 3545 3550	3520 (pH >11)	3580[3]
Aromatic Volatiles	5021 5030 5032	5021 5032 5035	5030 5032	3585
Base/Neutral Extractable	3510 3520 (pH >11)	3540 3541 3545 3550	3520 (pH >11)	3650 3580[3]
Carbamates	8318[5] 8321	8318[5] 8321	8318[5]	8318[5]
Chlorinated Herbicides	8151[6] (pH ≤ 2) 8321	8151[6] 8321	8151[6] (pH ≤ 2)	3580[3]
Chlorinated Hydrocarbons	3510 3520 (pH as received)	3540 3541 3550	3520 (pH as recieved)	3580[3]
Dyes	3510 3520	3540 3541 3545 3550		
Explosives	8330[7] 8331[8]	8330[7] 8331[8]		
Formaldehyde	8315[9]	8315[9]		
Haloethers	3510 3520	3540 3541 3545 3550		

TABLE 2-35
PREPARATION METHODS FOR ORGANIC ANALYTES
(continued)

Analyte Type	Matrix			
	Aqueous[1]	Solids	Sludges and Emulsions[1,2]	Organic Liquids, Tars, Oils
Halogenated Volatiles	5021 5030 5032	5021 5032 5035	5030	3585
Nitroaromatic and Cyclic Ketones	3510 3520 (pH 5-9)	3540 3541 3545 3550	3520 (pH 5-9)	3580[3]
Nitrosamines	3510 3520	3540 3541 3545 3550		
Non-halogenated Volatiles	5021 5031 5032	5021 5031 5032	5021 5031 5032	5032 3585
Organochlorine Pesticides	3510 3520 3535 (pH 5-9)	3540 3541 3545 3550	3520 (pH 5-9)	3580[3]
Organophosphorus Pesticides	3510 3520 (pH 5-8)	3540 3541 3545	3520 (pH 5-8)	3580[3]
Phenols	3510 3520 (pH ≤ 2)	3540 3541 3545 3550 3562	3520 (pH ≤ 2)	3650 3580[3]
Phthalate Esters	3510 3520 3535 (pH 5-7)	3540 3541 3545 3550	3520 (pH 5- 7)	3580[3]
Polychlorinated Biphenyls	3510 3520 3535 (pH 5-9)	3540 3541 3545 3562	3520 (pH 5-9)	3580[3]
PCDDs and PCDFs	8280[10] 8290[10]	8280[10] 8290[10]	8280[10] 8290[10]	8280[10] 8290[10]

TABLE 2-35
PREPARATION METHODS FOR ORGANIC ANALYTES
(continued)

Analyte Type	Matrix			
	Aqueous[1]	Solids	Sludges and Emulsions[1,2]	Organic Liquids, Tars, Oils
Polynuclear Aromatic Hydrocarbons	3510 3520 (pH as received)	3540 3541 3545 3550 3561	3520 (pH as received)	3580[3]
Volatile Organics	5021 5030 5031 5032	5021 5031 5032 5035	5021 5030 5031 5032	3585

Footnotes for Table 2-35

1 The pH at which extraction should be performed is shown in parentheses.
2 If attempts to break an emulsion are unsuccessful, these methods may be used.
3 Method 3580 is only appropriate if the sample is soluble in the specified solvent.
4 Method 8032 contains the extraction, cleanup, and determinative procedures for this analyte.
5 Method 8318 contains the extraction, cleanup, and determinative procedures for these analytes.
6 Method 8151 contains the extraction, cleanup, and determinative procedures for these analytes.
7 Method 8330 contains the extraction, cleanup, and determinative procedures for these analytes.
8 Method 8331 is for Tetrazene only, and contains the extraction, cleanup, and determinative procedures for this analyte.
9 Method 8315 contains the extraction, cleanup, and determinative procedures for this analyte.
10 Methods 8280 and 8290 contain the extraction, cleanup, and determinative procedures for these analytes.
11 Method 5031 may be used when only aniline is to be determined.

TABLE 2-36. CLEANUP METHODS FOR ORGANIC ANALYTE EXTRACTS

Analyte Type	Method
Acid Extractable	3650, 3640
Base/Neutral Extractable	3650, 3640
Carbamates	8318[1]
Chlorinated Herbicides	8151[2]
Chlorinated Hydrocarbons	3620 3640
Haloethers	3620 3640
Nitroaromatics & Cyclic Ketones	3620 3640
Nitrosamines	3610, 3620, 3640
Organochlorine Pesticides	3620 3630 3640 3660
Organophosphorus Pesticides	3620
Phenols	3630 3640 3650 8041[3]
Phthalate Esters	3610 3611 3620 3640
Polychlorinated Biphenyls	3620 3630 3640 3660 3665
Polychlorinated Dibenzo-*p*-Dioxins and Polychlorinated Dibenzofurans	8280[4] 8290[4]
Polynuclear Aromatic Hydrocarbons	3610 3611 3630 3640 3650

1 Method 8318 contains the extraction, cleanup, and determinative procedures for these analytes.
2 Method 8151 contains the extraction, cleanup, and determinative procedures for these analytes.
3 Method 8041 includes a dervatization technique followed by GC/ECD analysis, if interferences are encountered using GC/FID.
4 Methods 8280 and 8290 contain the extraction, cleanup, and determinative procedures for these analytes.

TABLE 2-37. DETERMINATIVE METHODS ORGANIC ANALYTES

Analyte Type	GC/MS Method	Specific GC Method	HPLC Method
Acid Extractable	8270		
Acrolein, Acrylonitrile, Acetonitrile	8260	8031 8033[1]	8315[2] 8316
Acrylamide	8260	8032	8316
Aniline and Selected Derivatives	8270	8131	
Aromatic Volatiles	8260	8021	
Base/Neutral Extractable	8270		8325[4]
Carbamates			8318, 8321
Chlorinated Herbicides	8270[3]	8151	8321
Chlorinated Hydrocarbons	8270	8121	
Dyes			8321
Explosives			8330, 8331, 8332
Formaldehyde			8315
Haloethers	8270	8111	
Halogenated Volatiles	8260	8011, 8021	
Nitroaromatics and Cyclic Ketones	8270	8091	8330[5]
Nitrosoamines	8270	8070	
Non-halogenated Volatiles	8260	8015	8315
Organochlorine Pesticides	8270[3]	8081	
Organophosphorus Pesticides	8270[3]	8141	8321
Phenols	8270	8041	
Petroleum Hydrocarbons		8015	
Phthalate Esters	8270	8061	
Polychlorinated Biphenyls	8270[3]	8082	
PCDDs and PCDFs	8280 8290		
Polynuclear Aromatic Hydrocarbons	8270	8100	8310
Volatile Organics	8260	8011, 8015, 8021, 8031, 8032, 8033	8315 8316

1 Of these analytes, Method 8033 is for acetonitrile only.
2 Of these analytes, Method 8315 is for acrolein only.
3 This method is an alternative confirmation method, not the method of choice.
4 Benzidines and related compounds.
5 Nitroaromatics (see "Explosives").

TABLE 2-38

PREPARATION METHODS FOR INORGANIC ANALYSES [1]

MATRIX	METHOD
Surface Water	3005, 3010, 3015, 3020
Ground Water	3005, 3010, 3015, 3020
Extracts	3010, 3015, 3020
Aqueous samples containing suspended solids	3010, 3015, 3020
Oils	3031, 3040, 3051, 3052[2]
Oil Sludges	3031, 3052[2]
Tars	3031, 3052[2]
Waxes	3031, 3040, 3052[2]
Paints	3031, 3052[2]
Paint Sludges	3031, 3052[2]
Petroleum Products	3031, 3040, 3052[2]
Sediments	3050, 3051, 3052[2], 3060[3]
Sludges	3050, 3051, 3052[2], 3060[3]
Soil Samples	3050, 3051, 3052[2], 3060[3]
Ashes	3052[2]
Biological Tissues	3052[2]

[1]It is the responsibility of the analyst to refer to each analytical method to determine applicability of the chosen method to a specific waste type and target analyte.

[2]For total decomposition analysis ONLY.

[3] For the analysis of samples for hexavalent chromium ONLY.

TABLE 2-39

USE OF LEACHING, EXTRACTION AND DIGESTION METHODS
FOR INORGANIC ANALYSIS
(Generally ordered by increasing strength)

METHOD	REAGENTS & CONDITIONS	USE
1310	dilute acetic acid (synthetic municipal solid waste leachate)	Simulate leaching of a waste in a municipal solid waste landfill
1311	dilute acetic acid (synthetic municipal solid waste leachate)	Simulate leaching of a waste in a municipal solid waste landfill
1312	dilute H_2SO_4 and HNO_3 (synthetic acid rain)	Simulate acid rain leaching of a waste
1320	dilute H_2SO_4 and HNO_3 (synthetic acid rain)	Simulate long-term acid rain leaching of a waste
3040	solvent	Dissolution of oils, oily wastes, greasses and waxes
3005	HNO_3, heat	Surface and ground waters
3020	HNO_3, heat	Aqueous samples and extracts for GFAA work only
3010	HNO_3, HCl, heat	Aqueous samples and extracts
3060A	Na_2CO_3/NaOH, heat	Soils, sludges, sediments and some industrial wastes for the analysis of hexavalent chromium only.
3015	HNO_3, HCl (optional), pressure, heat	Aqueous samples and extracts
3050	HNO_3, H_2O_2, HCl (optional), heat	Sediments, soils, and sludges
3051	HNO_3, HCl (optional), pressure, heat	Sludges, sediments, soils and oils
3031	Potassium permanganate, H_2SO_4, HNO_3, HCl, heat	Oils, oily sludges, tars, waxes, paint, paint sludge
3052	HNO_3, HF, HCl (optional) H_2O_2 (optional), heat, pressure	Siliceous, organic and other complex matrices for total sample decomposition

FIGURE 2-1
ORGANIC ANALYSIS OPTIONS FOR SOILD AND LIQUID MATRICES

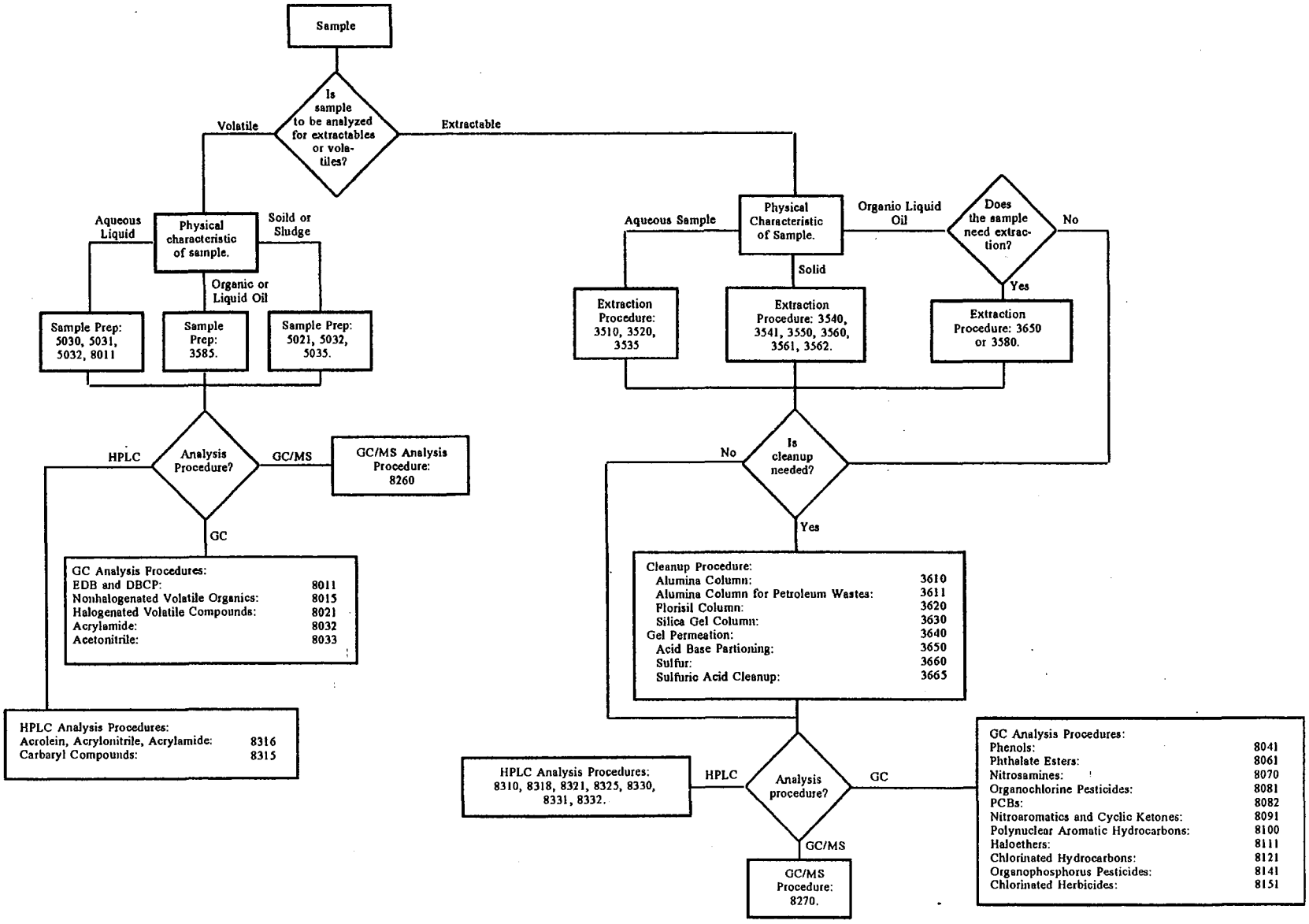


FIGURE 2-2
SCHEMATIC OF SEQUENCE TO DETERMINE
IF A WASTE IS HAZARDOUS BY CHARACTERISTIC

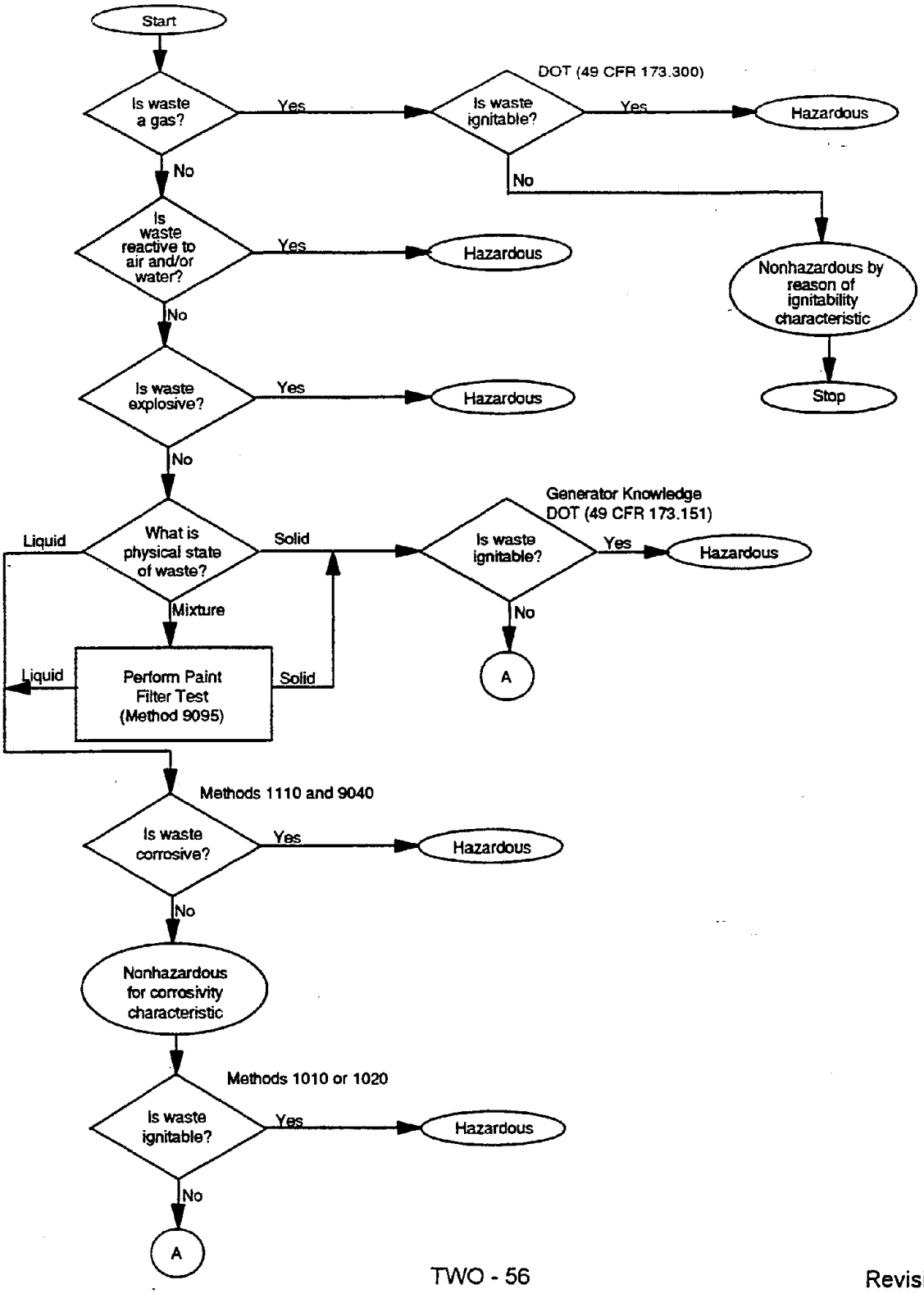


FIGURE 2-2
(Continued)

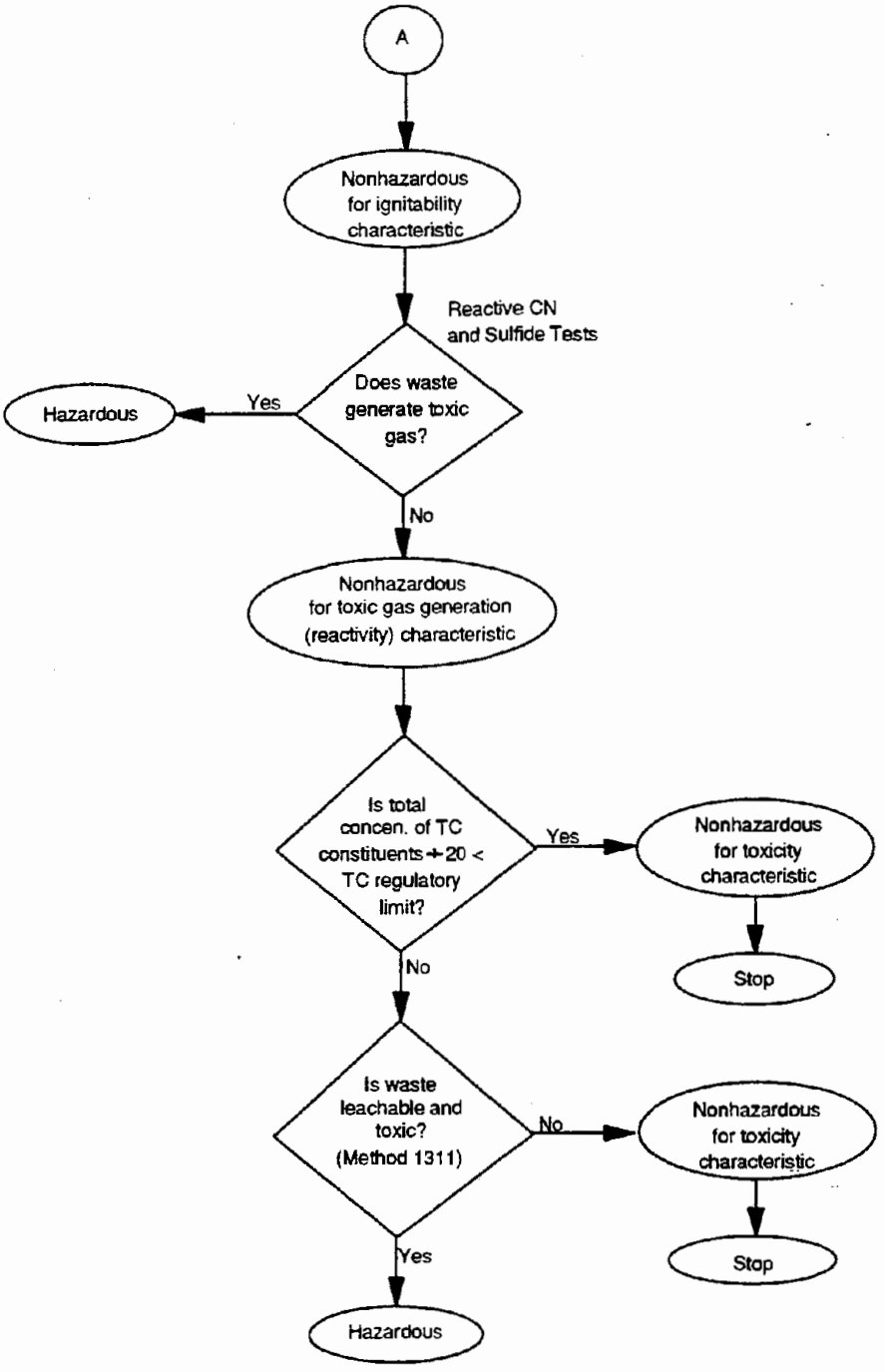


FIGURE 2-3A
RECOMMENDED SW-846 METHODS FOR ANALYSIS OF EP LEACHATES

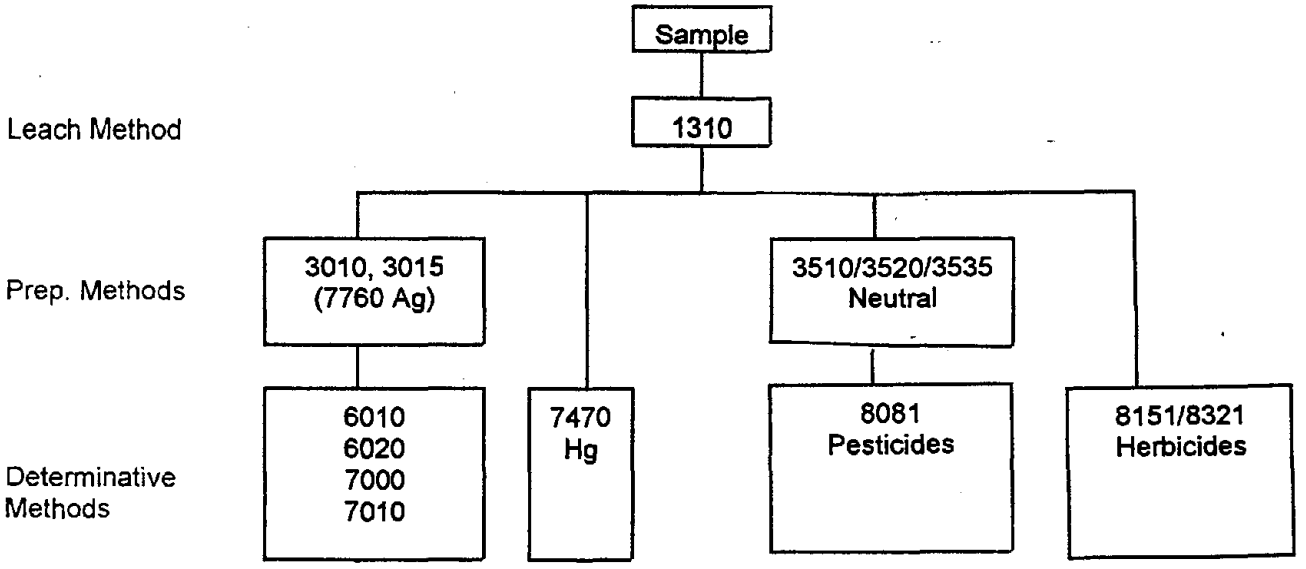


FIGURE 2-3B
RECOMMENDED SW-846 METHODS FOR ANALYSIS OF TCLP LEACHATES

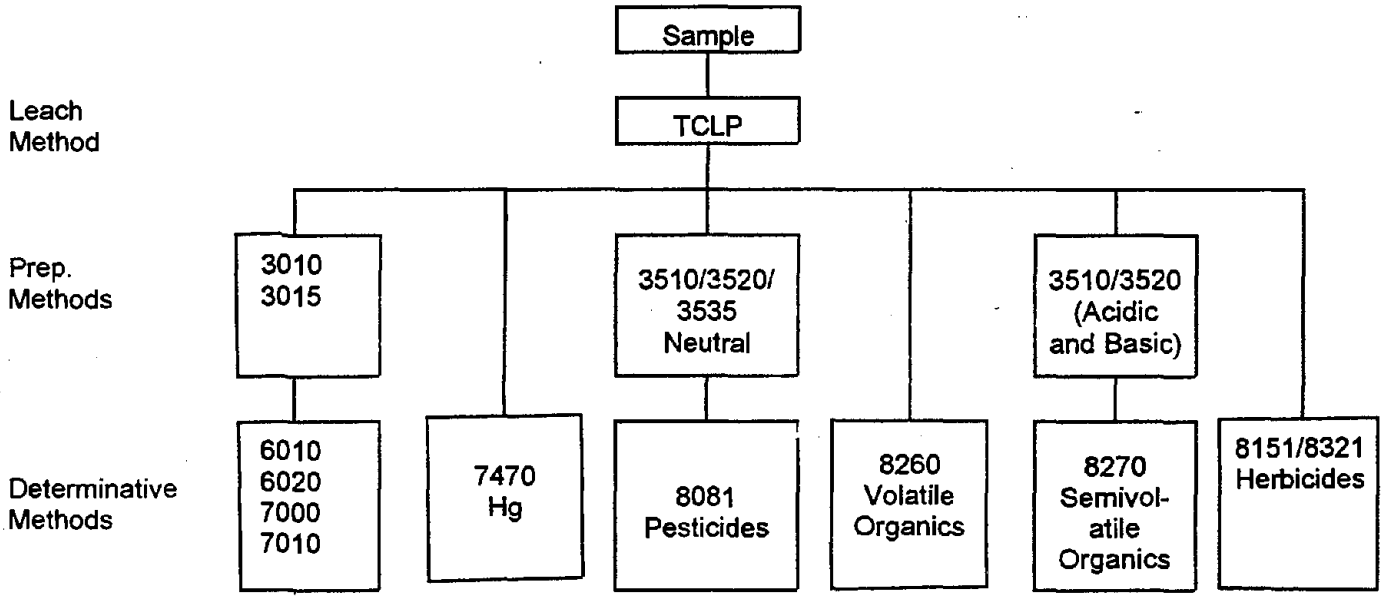
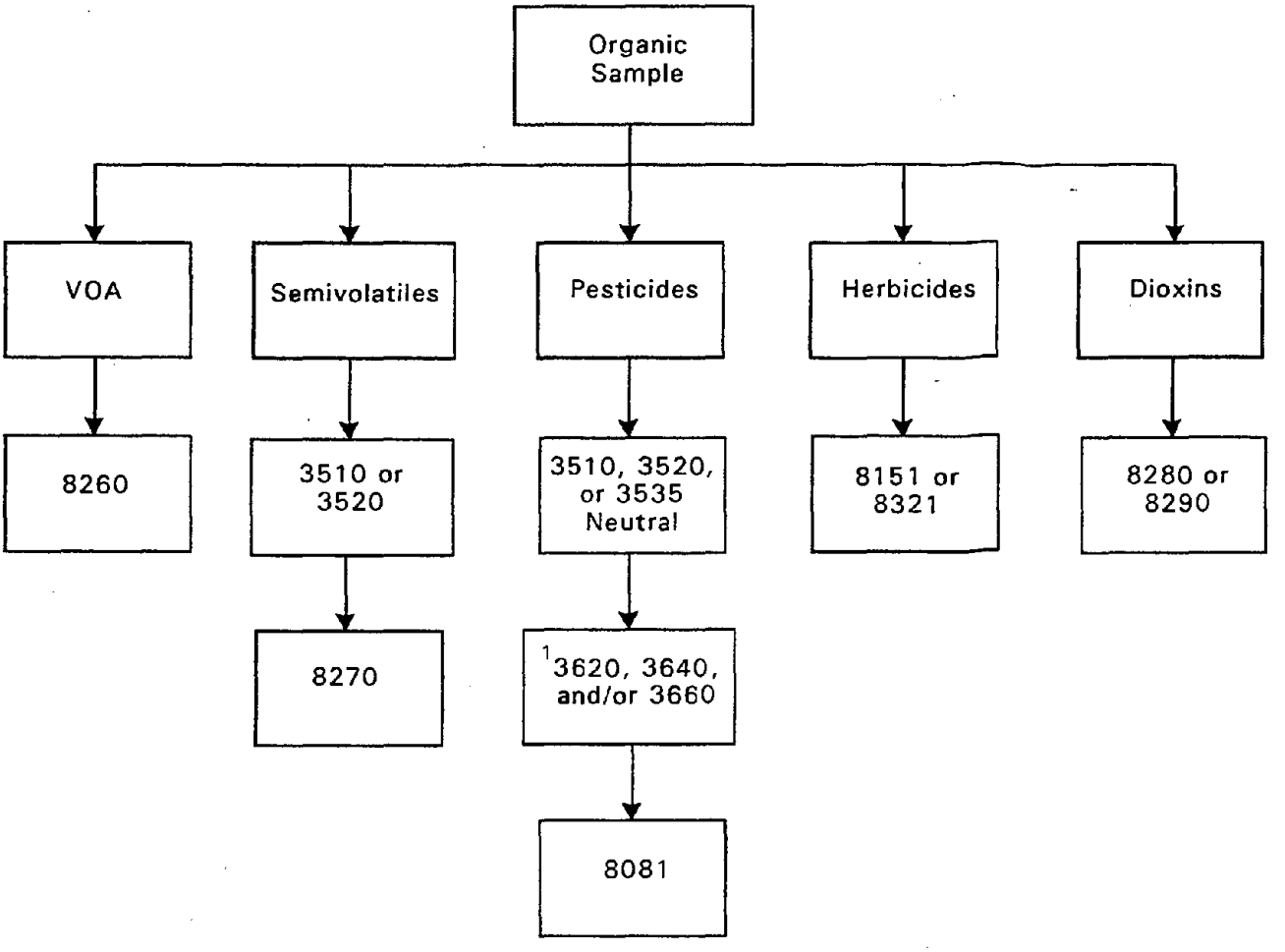
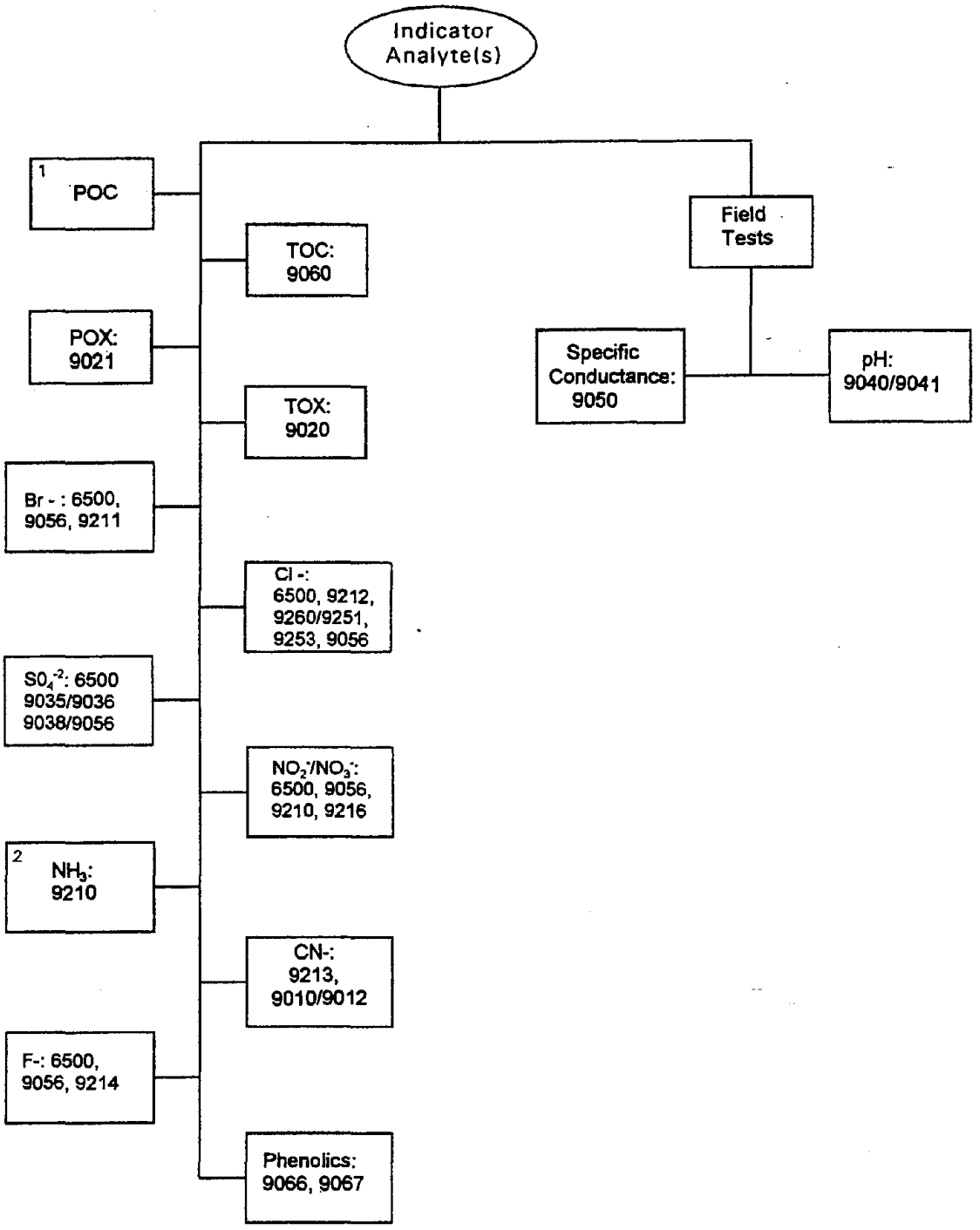


FIGURE 2-4A.
GROUND WATER ANALYSIS: ORGANIC ANALYTES



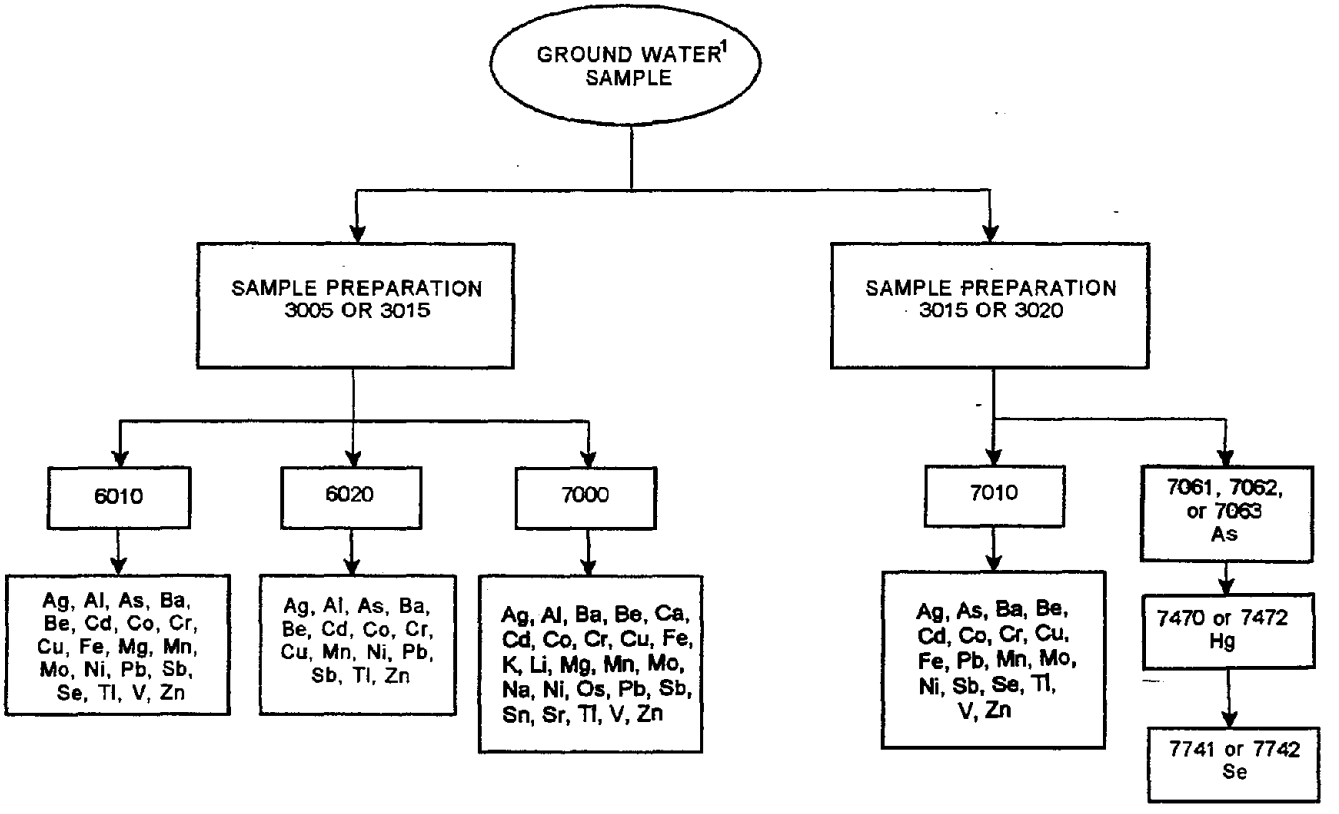
1 - Optional: Cleanup required only if interferences prevent analysis.

FIGURE 2-4B.
GROUND WATER ANALYSIS: INDICATOR ANALYTES



1 - Barcelona, 1984, (See Reference 1)
2 - Riggin, 1984, (See Reference 2)

FIGURE 2-4C.
GROUND WATER ANALYSIS: INORGANIC ANALYTES



[1] When analyzing for total dissolved metals, digestion is not necessary if the samples are filtered at the time of collection, and then acidified to the same concentration as the standards.

CHAPTER THREE

INORGANIC ANALYTES

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgements necessary to meet the data quality objectives or needs for the intended use of the data.

3.1 SAMPLING CONSIDERATIONS

3.1.1 Introduction

This manual contains procedures for the analysis of inorganic analytes in a variety of matrices. These methods are written as specific steps in the overall analysis scheme -- sample handling and preservation, sample digestion or preparation, and sample analysis for specific inorganic components. From these methods, the analyst must assemble a total analytical protocol which is appropriate for the sample to be analyzed and for the information required. This introduction discusses the options available in general terms, provides background information on the analytical techniques, and highlights some of the considerations to be made when selecting a total analysis protocol.

3.1.2 Definition of Terms

Optimum concentration range: A range, defined by limits expressed in concentration, below which scale expansion must be used and above which curve correction should be considered. This range will vary with the sensitivity of the instrument and the operating conditions employed.

Sensitivity: (a) Atomic Absorption: The concentration in milligrams of metal per liter that produces an absorption of 1%. (b) Inductively Coupled Plasma (ICP): The slope of the analytical curve, i.e., the functional relationship between emission intensity and concentration.

Method detection limit (MDL): The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The MDL is determined from analysis of a sample in a given matrix containing the analyte which has been processed through the preparative procedure.

Total recoverable metals: The concentration of metals in an unfiltered sample following treatment with hot dilute mineral acid (Method 3005).

Dissolved metals: The concentration of metals determined in a sample after the sample is filtered through a 0.45-µm filter (Method 3005).

Suspended metals: The concentration of metals determined in the portion of a sample that is retained by a 0.45-µm filter (Method 3005).

Total metals: The concentration of metals determined in a sample following digestion by Methods 3010, 3015, 3020, 3050, 3051, or 3052.

Instrument detection limit (IDL): The concentration equivalent to a signal due to the analyte which is equal to three times the standard deviation of a series of 7 replicate measurements of a reagent blank's signal at the same wavelength.

Interference check sample (ICS): A solution containing both interfering and analyte elements of known concentration that can be used to verify background and inter-element correction factors.

Initial calibration verification (ICV) standard: A certified or independently prepared solution used to verify the accuracy of the initial calibration. For ICP analysis, it must be run at each wavelength used in the analysis.

Continuing calibration verification (CCV): Used to assure calibration accuracy during each analysis run. It must be run for each analyte as described in the particular analytical method. At a minimum, it should be analyzed at the beginning of the run and after the last analytical sample. Its concentration should be at or near the mid-range levels of the calibration curve.

Calibration standards: A series of known standard solutions used by the analyst for calibration of the instrument (i.e., preparation of the analytical curve).

Linear dynamic range: The concentration range over which the analytical curve remains linear.

Method blank: A volume of reagent water processed through each sample preparation procedure.

Calibration blank: A volume of reagent water acidified with the same amounts of acids as were the standards and samples.

Laboratory control standard: A volume of reagent water spiked with known concentrations of analytes and carried through the preparation and analysis procedure as a sample. It is used to monitor loss/recovery values.

Method of standard addition (MSA): The standard-addition technique involves the use of the unknown and the unknown plus one or more known amounts of standard. See Method 7000, for detailed instructions.

Sample holding time: The storage time allowed between sample collection and sample analysis when the designated preservation and storage techniques are employed.

Check Standard: A solution containing a known concentration of analyte derived from externally prepared test materials. The check standard is obtained from a source external to the laboratory and is used to check laboratory performance.

3.1.3 Sample Handling and Preservation

Sample holding times, digestion volumes and suggested collection volumes are listed in Table 3-1. The sample volumes required depend upon the number of different digestion procedures necessary for analysis. This may be determined by the application of graphite-furnace atomic absorption spectrophotometry (GFAA), flame atomic absorption spectrophotometry (FLAA), inductively coupled argon plasma emission spectrometry (ICP), hydride-generation atomic absorption spectrometry (HGAA), inductively coupled plasma mass spectrometry (ICP-MS) or cold-vapor atomic absorption spectrometry (CVAA) techniques, each of which may require different digestion

procedures. The indicated volumes in Table 3-1 refer to that recommended for the individual digestion procedures and to that recommended for sample collection volumes. In all cases for waste testing, representative sampling must be maintained.

In the determination of trace metals, containers can introduce either positive or negative errors in the measurement of trace metals by (a) contributing contaminants through leaching or surface desorption, and (b) depleting concentrations through adsorption. Thus the collection and treatment of the sample prior to analysis require particular attention. The following cleaning treatment sequence has been determined to be adequate to minimize contamination in the sample bottle, whether borosilicate glass, linear polyethylene, polypropylene, or Teflon: detergent, tap water, 1:1 nitric acid, tap water, 1:1 hydrochloric acid, tap water, and reagent water.

NOTE: **Chromic acid should not be used to clean glassware**, especially if chromium is to be included in the analytical scheme. Commercial, non-chromate products (e.g., Nochromix) may be used in place of chromic acid if adequate cleaning is documented by an analytical quality control program. (Chromic acid should also not be used with plastic bottles.)

3.1.4 Safety

The toxicity or carcinogenicity of each reagent used in these methods has not been precisely defined. However, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in these methods. A reference file of material data-handling sheets should also be made available to all personnel involved in the chemical analysis. The following additional references to laboratory safety are available:

1. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

2. "OSHA Safety and Health Standards, General Industry," 29 CFR 1910.

3. "Proposed OSHA Safety and Health Standards, Laboratories," Occupational Safety and Health Administration, 51 FR 26660, July 24, 1986.

4. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety.

3.1.5 Sample Preparation

For all non-speciated digestion methods, great reduction in analytical variability can be achieved by use of appropriate sample preparation procedures. Generally the reduction in subsampling variance is accomplished by drying the sample, reducing its particle size, and homogeneously mixing the resulting fines.

Specifically, if the sample can not be well mixed and homogenized on an as received basis, then air or oven drying at 60°C or less, crushing, sieving, grinding, and mixing should be performed as needed to homogenize the sample until the subsampling variance is less than the data quality objectives of the analysis. While proper sample preparation generally produces great reduction in

analytical variability, be aware that in certain unusual circumstances there could be loss of volatile metals (e.g. Hg, organometallics) or irreversible chemical changes (e.g., precipitation of insoluble species, change in valence state) caused by inappropriate sample preparation procedures.

Variability inherent in the analytical determinative procedure is assessed by matrix spiking of individually digested samples. Variability due to sample heterogeneity is assessed by analyzing sample replicates. For most samples, sampling imprecision is much greater than analytical imprecision. Because of this, the greatest advances in environmental monitoring are occurring in the area of sample collection and preparation.

3.1.6 Clean Chemistry and the Analytical Blank

The significant role of the analytical blank in chemical analysis of trace metals cannot be over stressed. Sensitive instrumentation such as inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and graphite-furnace atomic absorption spectrophotometry (GFAA) requires that sample preparation be at least as sophisticated as the instruments used in analysis. The analytical blank is normally a primary source of error in trace element analysis. Trace analysis is as dependent on control of the analytical blank as it is on the accuracy and precision of the instrument making the measurement. Inability to control contamination that is external to the sample, or those contributions of the analyte coming from all other sources than the sample, is frequently the limiting factor in trace (parts per million (ppm) to parts per billion (ppb)) and ultra-trace (ppb to parts per trillion (ppt)) analysis. Analytical blank contributions occur from the following four major sources (Ref. 1-5):

- the atmosphere in which the sample preparation and analysis are conducted,
- the purity of the reagents used in sample preparation, including all reagents and the quantities added directly to the sample,
- the materials and equipment used in digestion or extraction vessels that come in contact with the sample during the sample preparation and analysis, and
- the analyst's technique and skill in preparing the samples and performing the analyses.

Only under very few circumstances can the analyst ignore the contribution of the uncertainty of the blank when calculating the uncertainty of the overall analytical result. One condition to consider is whether the concentration of the blank is insignificant compared to the analytical level. For example, when the blank value is less than 10^3-10^4 smaller than the sample measurement, the uncertainty of the blank measurement is insignificant compared with the uncertainty of the analytical measurement. This situation only occurs when the blank signal is extremely low compared to the measurement, which is rarely the case when trace and ultra-trace analyses are conducted. Typically the blank value is significant and must be subtracted from the measurement. Because the blank concentration is closer to the detection limit of the instrument, the imprecision of the blank is large compared with the blank measurement itself. This relationship causes the analytical blank to frequently become the limiting factor in the overall measurement precision.

To compute the overall standard deviation for a final measurement, several sources of error and imprecision must be combined. The standard deviation for each component of the computation of *y* must be considered when determining the overall measurement uncertainty.

$$y(\pm s_y) = a(\pm s_a) - b(\pm s_b)$$

As shown by the following equation, the standard deviation of the result, s_y is given by combining the standard deviations of the measurements (Reference 6).

$$s_y = \sqrt{s_a^2 + s_b^2 + s_c^2}$$

In this case, "*a*" represents the standard deviation of the measurement, "*b*" represents the standard deviation of the blank, that must be subtracted, and "*c*" represents the uncertainty associated with the sampling error. This example will only consider the uncertainty of the measurement "a" and the blank "*b*", as the sampling uncertainty "*c*" is beyond the scope of this discussion (presented previously and may be found in other literature, References 7, 8).

The following example illustrates a common relationship that causes the imprecision of the blank to be the limiting factor in the overall uncertainty of the analysis. Analysis of a set of samples determines the mean value to be 55.5 ± 0.3, with an analytical blank of 11 ± 5 (which is too large to be ignored). The uncertainties of these results are the standard deviations of the replicate measurements. The analytical blank becomes the dominant uncertainty in calculating the uncertainty of the final result. Here the blank subtracted mean y = 55.5 - 11 = 44.5, and the standard deviation is 5. The net result of the analysis is 44.5 ± 5. Essentially, the entire uncertainty is due to the uncertainty of the analytical blank.

It has been suggested by some environmental laboratories that having a blank concentration below the instrumental detection limit while the measurement is detectable provides a more convenient measurement. This is, however, not an appropriate approach for minimizing contribution of the blank. A blank value below the limit of detection does not remove its influence. Just because the blank is not detectable does not mean it is not influencing the measurement. An accurate measurement of the blank value with high precision provides the most accurate overall analytical estimate of the concentration.

The four primary areas that effect the analytical blank can be demonstrated using standard reference materials in analysis. Table 3-2 illustrates and isolates the main blank influencing parameters: environment, reagents, materials, and analyst skills. The skill of the analyst was kept constant as the same analyst changed the environment, reagents, and combinations of these parameters in the analysis (3). Trace elements in glass (TEG) National Institute of Standards and Technology (NIST) standard reference material was used to keep sample homogeneity constant and to permit removal of the sampling error by using sample sizes where appropriate homogeneity had previously been demonstrated.

It is important to note that the relationship of the precision and measurement remained relatively constant. This relationship yields no information about the accuracy of the data. The significance of the first two major sources of contamination, environment and reagents, can be evaluated. In the example above, the contamination in the laboratory air and in the acid used for the reagent blank altered the accuracy of the example above by over two orders of magnitude for both lead and silver. The larger influence of the two sources in this example is the laboratory environment in which the samples were prepared.

The Sample Preparation and Analysis Atmosphere

The laboratory environment in which the sample is prepared is the major source of contamination for most elements. Some rare elements may be an exception, but for the majority of elements of interest, contamination from airborne sources is the most significant of the four main sources. Table 3-3 illustrates concentrations of lead in the air.

This contamination can also be seen in the comparison of 58,000 particles per liter of air measured in a normal laboratory in Pittsburgh, Pennsylvania, and inside a clean chamber in an adjacent laboratory five meters away. Figure 1 demonstrates the dramatic difference between the two environments. Cost effective methods of creating clean chambers for sample preparation are documented along with this data in the references (1).

Any laboratory air that comes in contact with the sample may deposit some portion of its elemental content into the sample. The sample is especially vulnerable to this transfer when it is being decomposed in acid. The acid will leach particles from the air resulting in unwanted ions in solution, mixing with those of the sample.

To prevent air from contaminating the sample, the sample must be processed in a clean environment. This is much easier to accomplish than it may appear at first. These precautions are becoming state-of-the-art in many analytical and environmental laboratories. The prevention of airborne contamination is most frequently dealt with by employing a laminar flow clean bench or a clean laboratory facility. Instructions are referenced for the construction of both from component parts; both are relatively inexpensive and uncomplicated, once the concepts are understood (Ref. 1).

There are many sources of airborne contamination. Several of the sources have been described and their particle size ranges are provided in Figure 2. These diverse sources primarily provide particulates in discrete size ranges. Depending on whether the laboratory is located in an industrial, urban, or rural area, or near the sea, the distribution of these source particles will be different, as will their composition. The vertical dashed line in Figure 2 indicates the particle size cutoff, usually 0.5 µm, for the high efficiency particulate air (HEPA) filter used to prevent particulate contamination. Particles above this size cannot pass through a HEPA filter that is in good working order. These filters were developed jointly by the Massachusetts Institute of Technology and Arthur D. Little & Company, Inc., for the Manhattan Project during World War II and are in common use today (Refs.1, 11).

The definition of clean air is derived from Federal Standard 209a, which defines cleanliness levels. Table 3-4 lists these conditions. "Laminar flow" is directed coherent air movement that does not contain any turbulence.

A dramatic reduction in airborne contaminants can be expected by using HEPA filtered air in laminar flow clean hoods or entire clean laboratories. Table 3-5 demonstrates the dramatic differences in airborne contaminant concentrations in an ordinary laboratory, a clean laboratory, and a clean hood inside a clean laboratory.

Reagent Purity

For acid decomposition, leaching, and extraction, the purity of the reagents used is extremely important to the overall level of the blank. Reagents have very different purities depending on their

processing grade and purpose. Frequently, the analyst must purchase special reagents or purify lesser grade reagents prior to use in order to minimize the analytical blank.

In addition to the purity of the reagents, the quantity that must be added is also significant. When reagents are added, they bring with them elemental and molecular components that exist as contaminants. The more reagent that must be used due to reasons other than the stoichiometric reaction, the higher the blank. Reagents of high purity must either be purchased or produced in the laboratory.

In the preparation of high purity reagents, there is only one significant and practical choice for the method of purification. Sub-boiling distillation (Refs. 13, 14), different from normal distillation, uses an infrared radiation source to heat the reagent to a temperature just below the boiling point. Not allowing the reagents to boil prevents the "Brownian movement" of solution droplets produced when bubbles burst at the surface of the liquid. These aerosolized solution particles are carried everywhere in the apparatus and physically transport metal ions and contaminants that should have been left in solution. Sub-boiling distillation is a slower but very reliable method of purifying all of the common mineral acids and many organic reagents used in analytical methods. It relies exclusively on the vapor pressure of the reagent, and contaminant, and can therefore be specifically optimized for purification of the mineral acids if the object is to remove metal ions. Of all acids, nitric acid, for a variety of reasons, can be purified to excellent quality. Because large quantities of reagents are necessary for many laboratories and a continuous supply of these reagents is desirable, methods for constructing a sub-boiling distillation apparatus are provided in the references; sources of these apparatus are also provided. Purchasing sub-boiling acids from commercial sources is also an option (1). Construction or purchase of sub-boiling reagent purification equipment is cost effective depending on the quantity of reagents required.

Materials for Sample Preparation, Storage, and Analysis

For elemental analysis, specific, preferred materials are used for the construction of sample vessels and instrument components that come in contact with the sample. Over the past two decades, materials identified as being non-contaminating have become the top choices for bottles, beakers, reaction vessels, storage containers, nebulizers, and instrument components for trace and ultra-trace analysis. These materials are the same as those currently being used in many digestion vessels, bomb liners, and microwave vessels. These materials are thermally durable, chemically resistant or inert, non-contaminating, and have appropriate compression and tensile strength. Table 3-6 lists the specific types of materials of non-contaminating nature and chemical inertness to most acid reactions, in order of preference. These materials have been evaluated and tested extensively for their elemental contamination characteristics (Refs. 1-3, 15, 16).

With the exception of polyethylene, these are the most common materials used for sample preparation vessels, both atmospheric pressure vessels and closed vessel liners that come in contact with the sample. These materials are the most stable to acid reactions (with the exception of quartz and glass if hydrofluoric acid is used). Fluoropolymers are the most common and were adapted from other chemical uses for application in pressure systems. The fluoropolymers, TFM, PFA and TFE or PTFE have the highest range of use temperatures of most plastics, ranging from 270 to 300 °C. They are also chemically inert to the majority of mineral acids and combinations thereof. Sulfuric acid has a boiling point of approximately 330 °C and can damage all fluoropolymers by melting them. Quartz and glass can safely contain sulfuric acid at these high temperatures, but borosilicate glass is not appropriate for ultra-trace elemental analysis (Refs. 3, 15). Glass actually forms a gel layer that hydrates and leaches, transferring elemental components from the glass to

the sample solution. While these are minute quantities, there are many low level analyses where these contributions would be detected in the blank and the sample.

Polyethylene is suitable for storage of diluted samples after decomposition, but it does not have a thermal use temperature appropriate for decomposition. It is also not sufficiently inert to be useful as a decomposition vessel or vessel liner, similar to polycarbonate and polypropylene. The low cost of polyethylene and its relative inertness to cool, weakly acidic solutions make it an excellent storage container for trace element solutions (Ref. 1).

Analytical Technique and Synergistic Equipment

The fourth significant source of analytical blank contamination is the skill of the analyst and the appropriateness of the technique that is being performed. Analytical blank control has been explained as the combination of atmosphere, reagent, material, and the protocol being used correctly. Here the skill and awareness of the analyst and the way in which combinations of the aforementioned clean chemistry tools are applied is significant to the final result of contamination and analytical blank control. Sample preparation instrumentation may also assist in these protocols. For example, microwave sample preparation assists each of these parameters in synergistic ways, thus lowering the analytical blank, improving blank precision, and enhancing overall quality control and transferability of methods. Some instrumentation and fundamental processes involved in specific sample preparation procedures assists the analyst by incorporating useful clean chemistry concepts into instrumentation and method structure. Such instrumentation is pertinent since microwave methods now exist that provide sample preparation for leaching or total analysis of all elements simultaneously. Analyst skill involving clean chemistry is assisted by the method structure and microwave equipment as indicated below.

- If a closed or controlled atmospheric microwave vessel is prepared in a clean hood and sealed before leaving the clean environment, the sample will not be affected by atmospheric contamination during the reaction, since it has not been removed from a clean environment.

- The vessel materials described would not normally be used by many laboratories that can benefit, so the advantages of the fluoropolymers would not be realized if they were not required in most microwave reaction vessels.

- The time the sample spends in decomposition, leaching, or extraction is typically reduced from hours to minutes, thus reducing the potential leaching from the container walls

- Because most microwave systems are sealed systems, evaporation of the reagent before it reacts productively is prevented and smaller quantities of reagents are used thus preventing excess blank accumulation.

The blank is reduced in size and is more consistent due to limiting the exposure variables. An example of the these components working together has been provided in the literature where analysis under different conditions has verified these conclusions (Refs. 1, 18, 19). The example illustrates the isolation of the blank optimization areas: environment, reagents, materials, and analysis skills. The skill of the analyst is kept more constant as the instrument dictates more clean, chemically appropriate procedures.

References

(1) Kingston, S. H. M.; Walter, P. J.; Chalk, S.; Lorentzen, E.; Link, D. In *Microwave - Enhanced Chemistry: Fundmentals, Sample Preparation, and Applications*; Kingston, H. M. S., Haswell, S. J., Eds.; American Chemcial Society: Washington D.C., 1997, pp 257-279.

(2) Tolg, G.; Tschopel, P. In *Determination of Trace Elements*; Alfaassi, Z. B., Ed.; VCH: New York, 1994, pp 1-38.

(3) Murphy, T. J. In *National Bureau of Standards Special Publication 422: Accuracy in Trace Analysis: Sampling, Sample Handling, and Analysis*; National Bureau of Standards: Gaithersburg, MD, 1976, pp 509-539.

(4) Moody, J. R. "NBS Clean Laboratories for Trace Element Analysis", *Anal. Chem.* **1982,** *54*, 1358A-1376A.

(5) Adeloju, S. B.; Bond, A. M. "Influence of Laboratory Environment on the Precision and Accuracy of Trace Element Analysis", *Anal. Chem.* **1985,** *57*, 1728-1733.

(6) Skoog, D. A.; West, D. M.; Holler, F. J. *Fundamentals of Analytical Chemistry*, 6th ed.; Saunders College Publishing: Fort Worth, 1992.

(7) Taylor, J. K. *Statistical Techniques for Data Analysis*; Lewis Publishers: Chelsea, MI, 1990.

(8) Taylor, J. K. In *Principles of Environmental Sampling*; Keith, L. H., Ed.; American Chemical Society: Washington, DC, 1996, pp 77-83.

(9) Rabinowitz, M. B.; Wetherill, G. W. "Identifying Sources of Lead Contamination by Stable Isotope Techniques", *Environ. Sci. Technol.* **1972,** *6*, 705-709.

(10) Maienthal, E. J. In *U. S. National Bureau of Standards Technical Note 545*; Taylor, J. K., Ed.; U. S. Governmental Printing Office: Washington, D. C., 1970, pp 53-54.

(11) Zief, M.; Mitchell, J. W. In *Chemical Analysis*; Elving, P. J., Ed.; John Wiley & Sons: New York, 1976; Vol. 47.

(12) Miller, G. T. *Living in the Environment*; Wadsworth Inc.: Belmont, CA, 1994.

(13) Kuehner, E. C.; Alvarez, R.; Paulsen, P. J.; Murphy, T. J. "Production and Analysis of Special High-Purity Acids Purified by Sub-Boiling Distillation", *Anal. Chem.* **1972,** *44*, 2050-2056.

(14) Moody, J. R.; E.S., B. "Purified Reagents for Trace Metal Analysis", *Talanta* **1982,** *29*, 1003-1010.

(15) Moody, J. R.; Lindstrom, R. M. "Selection and Cleaning of Plastic Containers for Storage of Trace Element Samples", *Anal. Chem.* **1977,** *49*, 2264-2267.

(16) Moody, J. R. "The Sampling, Handling and Storage of Materials for Trace Analysis", *Philos. Trans. R. Soc. London* **1982,** *305*, 669-680.

(17) Kuehner, E. C.; Freeman, D. H. In *Purification of Inorganic and Organic Materials*; Zief, M., Ed.; Marcel Dekker: New York, 1969, pp 297-306.

(18) Skelly, E. M.; DiStefano, F. T. "Clean Room and Microwave Digestion Techniques: Improvement in Detection Limits for Aluminum Determination by GF-AAS", *Appl. Spectrosc.* **1988,** *42*, 1302-1306.

(19) Prevatt, F. J. "Clean Chemistry for Trace Analysis", *Environmental Testing and Analysis* **1995,** *4*, 24-27.

TABLE 3-1.

SAMPLE HOLDING TIMES, RECOMMENDED DIGESTION VOLUMES AND RECOMMENDED COLLECTION VOLUMES FOR INORGANIC DETERMINATIONS IN AQUEOUS AND SOLID SAMPLES

Measurement	Digestion Volume. (mL)[a, c]	Collection Volume (mL)[a,c]	Treatment/ Preservative Holding Time[b]
Inorganic Analytes (except hexavalent chromium and mercury):			
Aqueous			
Total	100	600	HNO_3 to pH <2 6 months
Dissolved	100	600	Filter on site; HNO_3 to pH <2 6 months
Suspended	100	600	Filter on site 6 months
Solid			
Total	2 g	200 g	6 months
Hexavalent Chromium:			
Aqueous	100	400	24 hours Store at 4°± 2°C until analyzed
Solid	2.5 g	100 g	One month to extraction, 4 days after extraction Store at 4°± 2°C until analyzed
Mercury:			
Aqueous			
Total	100	400	HNO_3 to pH <2 28 days
Dissolved	100	400	Filter; HNO_3 to pH <2 28 days
Solid			
Total	0.2 g	200 g	28 days Store at 4°± 2°C until analyzed

[a] Unless stated otherwise.
[b] Either glass or plastic containers may be used.
[c] Any sample volume reduction from the reference method's instructions must be made in the exact proportion as described in the method and representative sampling must be maintained.

TABLE 3-2

EXAMPLES OF THE ANALYTICAL BLANK INFLUENCE
ON TRACE ANALYSIS OF ELEMENTS IN GLASS (Ref. 3)

CONDITIONS	Pb (ng)	Ag (ng)
Initial analysis of TEG* standard	330±250	970±500
Analysis using sub-boiled distilled acids	260±200	----------
Analysis in class 100 hood	20±8	207±200
Analysis using sub-boiled acids in class 100 hood	2±1	3±2

* TEG - Trace Element in glass, SUMS 610 - 619, ± s.

TABLE 3-3

EXAMPLES OF LEAD CONCENTRATIONS IN AIR

SITE	LEAD CONCENTRATION ($\mu g\ m^{-3}$)
Downtown Air, St. Louis, MO	18.84 (Ref. 9)
Rural Park Air, Southeastern MO	0.77 (Ref. 10)
Laboratory Air, NIST, MD	0.4 (Ref.3)

TABLE 3-4

CLEANLINESS LEVELS IN FEDERAL STANDARD 209a (Ref. 11)

CLASS[A]	MAXIMUM CONTAMINATION IN WORK AREA (particles ft^{-3})
100	100 particles > 0.5 µm 0 particles > 5.0 µm
10,000	10,000 particles > 0.5 µm 65 particles > 5.0 µm
100,000	100,000 particles > 0.5 µm 700 particles > 5.0 µm

[A]The standard required laminar-flow equipment to attain this level of cleanliness.
Since measurement of dust particles smaller than 0.5 µm introduces substantial errors, 0.5 µm has been adopted as the criterion of measurement.

TABLE 3-5

PARTICULATE CONCENTRATIONS IN LABORATORY AIR (Ref. 10)

SITE	CONCENTRATION ($\mu g\ m^{-3}$)			
	Iron	Copper	Lead	Cadmium
Ordinary Laboratory	0.2	0.02	0.4	0.002
Clean Room	0.001	0.002	0.0002	ND
Clean Hood	0.0009	0.007	0.0003	0.0002

ND - Not Detected

TABLE 3-6

NON-CONTAMINATING MATERIALS AND SPECIFICATION FOR USE IN ULTRA-TRACE ANALYSIS AND AS DECOMPOSITION VESSELS AND SAMPLE CONTAINERS (Ref. 11)

Listed from highest to lowest preference for use in sample containment
Fluoropolymers: PFA*, TFM, TFE*, FEP*, Tefzel*
Quartz - Synthetic
Polyethylene (suitable for storgage only, not for acid digestion)
Quartz - Natural
Borosilicate Glass

* Various forms of Teflon®

FIGURE 1

COMPARISON OF PARTICLE COUNT ANALYSIS, COUNTS VS. PARTICLE SIZE, OF A CLEAN ROOM AND A STANDARD LABORATORY AT DUQUESNE UNIVERSITY IN PITTSBURGH, PA (Ref. 1)

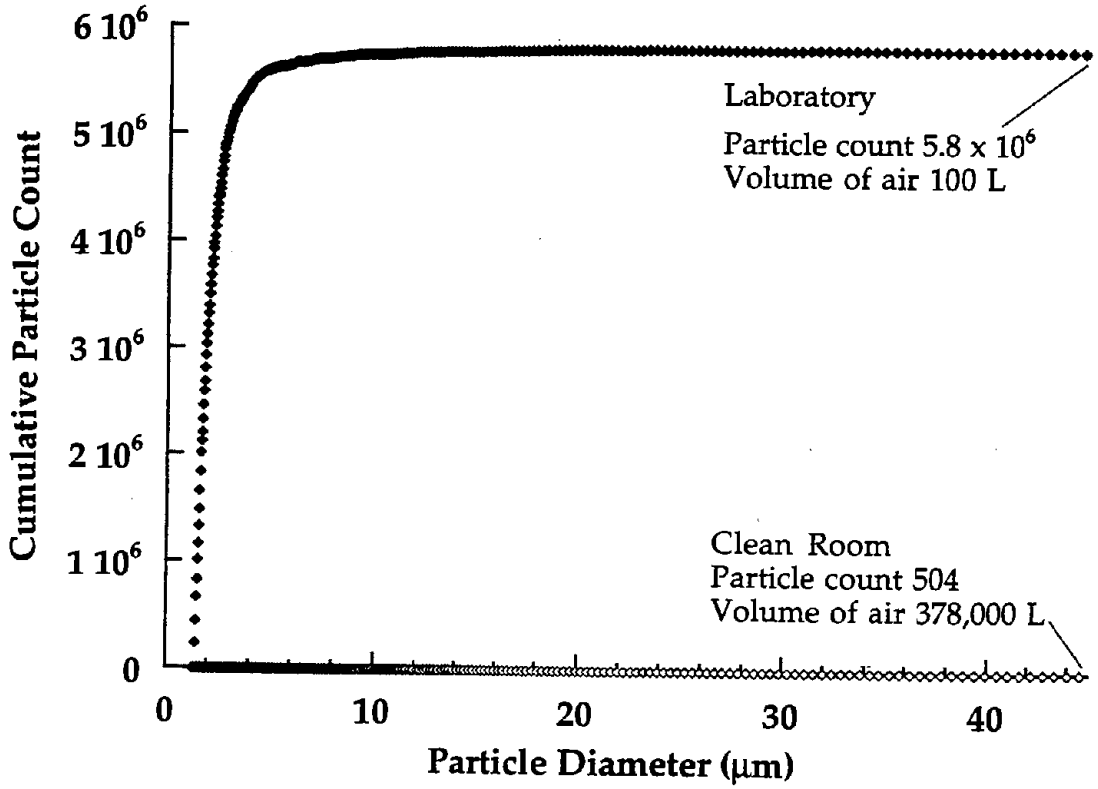
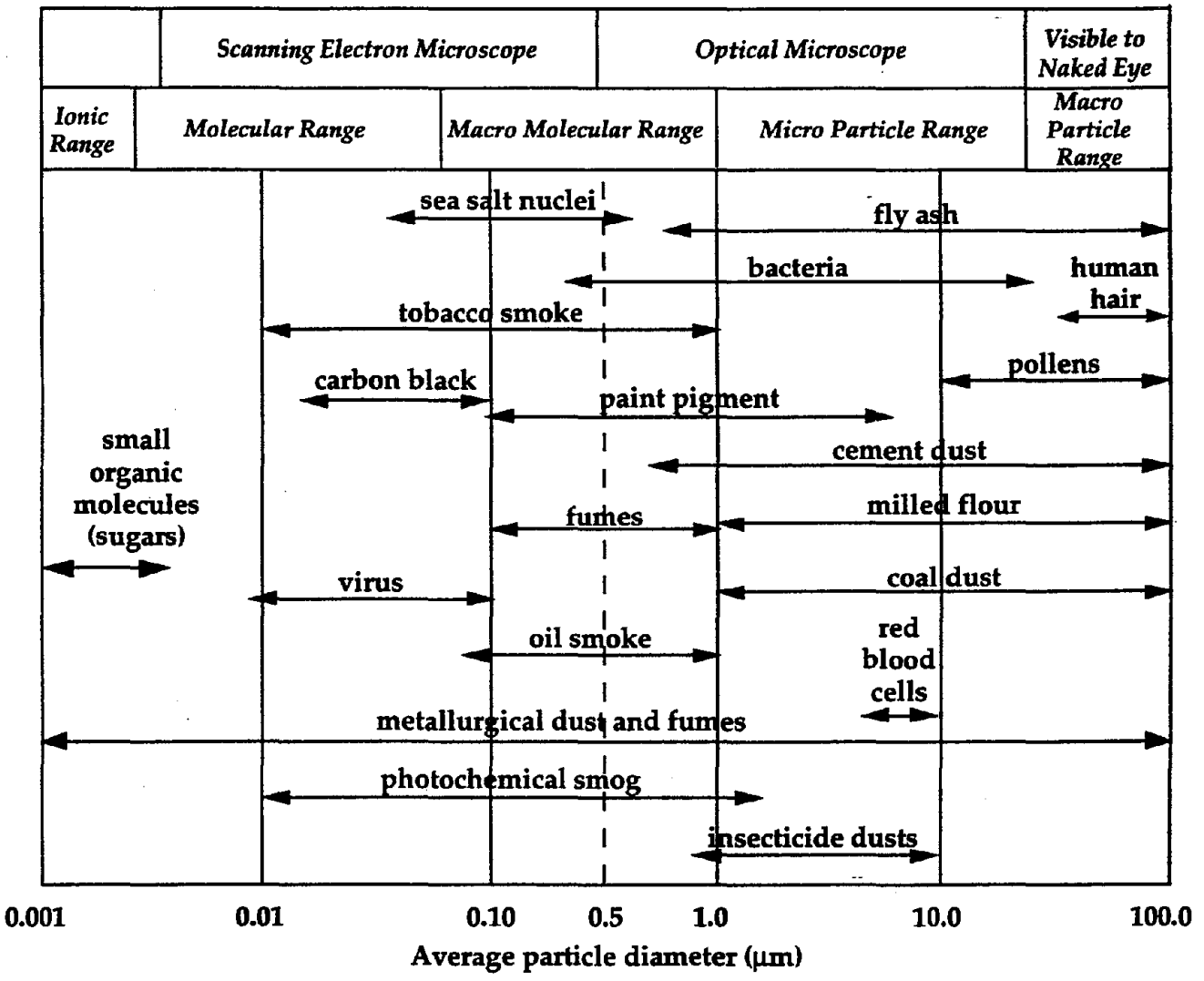


FIGURE 2

PARTICLE SIZE COMPARISON CHART FOR COMMON PARTICULATES (Refs. 1, 12)



3.2 SAMPLE DIGESTION METHODS

The methods in SW-846 for sample digestion or dissolution are as follows[1]:

Method 3005 prepares ground water and surface water samples for total recoverable and dissolved metal determinations by FLAA, ICP-AES, or ICP-MS. The unfiltered or filtered sample is heated with dilute HCl and HNO_3 prior to metal determination.

Method 3010 prepares waste samples for total recoverable metal determinations by FLAA, ICP-AES, or ICP-MS. The samples are vigorously digested with nitric acid followed by dilution with hydrochloric acid. The method is applicable to aqueous samples, leachates, and mobility-procedure extracts.

Method 3015 prepares aqueous samples, mobility-procedure extracts, and wastes that contain suspended solids for total recoverable metal determinations by FLAA, GFAA, ICP-AES, or ICP-MS. Nitric acid and hydrochloric acid are added to the sample in a Teflon digestion vessel and heated in a microwave unit prior to metals determination.

Method 3020 prepares waste samples for total recoverable metals determinations by furnace GFAA or ICP-MS. The samples are vigorously digested with nitric acid followed by dilution with nitric acid. The method is applicable to aqueous samples, leachates, and mobility-procedure extracts.

Method 3031 prepares waste oils, oil sludges, tars, waxes, paints, paint sludges and other viscous petroleum products for analysis by FLAA, GFAA, and ICP-AES. The samples are vigorously digested with nitric acid, sulfuric acid, hydrochloric acid, and potassium permanganate prior to analysis.

Method 3040 prepares oily waste samples for determination of soluble metals by FLAA, and ICP-AES methods. The samples are dissolved and diluted in organic solvent prior to analysis. The method is applicable to the organic extract in the oily waste EP procedure and other samples high in oil, grease, or wax content.

Method 3050 prepares waste samples for total recoverable metals determinations by FLAA and ICP-AES, or GFAA and ICP-MS depending on the options chosen. The samples are vigorously digested in nitric acid and hydrogen peroxide followed by dilution with either nitric or hydrochloric acid. The method is applicable to soils, sludges, and solid waste samples.

Method 3051 prepares sludges, sediments, soils and oils for total recoverable metal determinations by FLAA, GFAA, ICP-AES or ICP-MS. Nitric acid and hydrochloric acid are added to the representative sample in a fluorocarbon digestion vessel and heated in a microwave unit prior to metals determination.

Method 3052 prepares siliceous and organically based matrices including ash, biological tissue, oil, oil contaminated soil, sediment, sludge, and soil for total analysis by FLAA, CVAA, GFAA, ICP-AES, and ICP-MS. Nitric acid and hydrofluoric acid are added to a representative sample in a fluorocarbon digestion vessel and heated in a microwave unit prior to analysis.

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[1] Please note that chlorine is an interferant in ICP-MS analyses and its use should be discouraged except when absolutely necessary.

Method 3060 prepares soils, sludges, sediments and similar waste materials for hexavalent chromium determination. The samples are digested and heated to dissolve the Cr(VI) and stabilize it against reduction to Cr(III).

Prior to employing the above methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgements necessary to meet the data quality objectives or needs for the intended use of the data.

METHOD 3015A

MICROWAVE ASSISTED ACID DIGESTION OF AQUEOUS SAMPLES AND EXTRACTS

1.0 SCOPE AND APPLICATION

1.1 This microwave method is designed to perform extraction using microwave heating with nitric acid (HNO_3), or alternatively, nitric acid and hydrochloric acid (HCl). Since this method is not intended to accomplish total decomposition of the sample, the extracted analyte concentrations may not reflect the total content in the sample. This method is applicable to the microwave-assisted acid extraction/dissolution of available metals in aqueous samples, drinking water, mobility-procedure extracts, and wastes that contain suspended solids for the following elements:

Element		CASRN[a]
Aluminum	(Al)	7429-90-5*
Antimony	(Sb)	7440-36-0*
Arsenic	(As)	7440-38-2
Barium	(Ba)	7440-39-3*
Beryllium	(Be)	7440-41-7*
Boron	(B)	7440-42-8
Cadmium	(Cd)	7440-43-9
Calcium	(Ca)	7440-70-2
Chromium	(Cr)	7440-47-3*
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Iron	(Fe)	7439-89-6*
Lead	(Pb)	7439-92-1
Magnesium	(Mg)	7439-95-4*
Manganese	(Mn)	7439-96-5
Mercury	(Hg)	7439-97-6
Molybdenum	(Mo)	7439-98-7
Nickel	(Ni)	7440-02-0
Potassium	(K)	7440-09-7
Selenium	(Se)	7782-49-2
Silver	(Ag)	7440-22-4*
Sodium	(Na)	7440-23-5
Strontium	(Sr)	7440-24-6
Thallium	(Tl)	7440-28-0
Vanadium	(V)	7440-62-2*
Zinc	(Zn)	7440-66-6

[a]Chemical Abstract Service Registry Number

*Elements which typically require the addition of HCl for optimum recoveries. Other elements and matrices may be analyzed by this method if performance is demonstrated for the analyte of interest, in the matrices of interest, at the concentration levels of interest (see Sec. 9.0).

1.2 This method provides options for improving the performance for certain analytes, such as antimony, iron, aluminum, and silver by the addition of hydrochloric acid, when necessary. It is intended to provide a rapid multi-element acid extraction prior to analysis so that decisions can be made about materials and site clean-up levels, and as an estimate of metal toxicity. Digests produced by the method are suitable for analysis by inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-AES), flame atomic absorption spectrophotometry (FLAA), and graphite furnace atomic absorption spectrophotometry (GFAA). However, the addition of HCl may limit the methods of detection, or increase the difficulties of detection with some techniques.

Due to the rapid advances in microwave technology, consult the manufacturer's recommended instructions for guidance on their microwave digestion system. This method is generic and may be implemented using a wide variety of laboratory microwave equipment.

2.0 SUMMARY OF METHOD

2.1 A representative 45 mL aqueous sample is extracted in 5 mL concentrated nitric acid or, optionally, 4 mL concentrated nitric acid and 1 mL concentrated hydrochloric acid, for 20 minutes using microwave heating with a suitable laboratory microwave unit. The temperature of the acid-sample mixture is brought to 170 ± 5 °C in 10 minutes, and maintained at 170 ± 5 °C for 10 minutes to accelerate the leaching process. The sample and acid(s) are placed in a fluorocarbon polymer (such as PFA or TFM) or quartz microwave vessel or vessel liner. The vessel is sealed and heated in the microwave unit. After cooling, the vessel contents are filtered, centrifuged, or allowed to settle and then diluted to volume and analyzed by the appropriate determinative method.

3.0 DEFINITIONS

Refer to Chapter One and Chapter Three for a listing of applicable definitions.

4.0 INTERFERENCES

4.1 Digestion of samples which contain organics will create high pressures due to the evolution of gaseous digestion products. This may cause venting of the vessels with potential loss of sample components and/or analytes. When warranted by the potential reactivity of the sample, a smaller sample size may be used, and the concentration for final calculations adjusted, but the final water volume prior to addition of acid(s) is recommended to be 45 mL. This is recommended in order to retain the heat characteristics of the calibration procedure if used. Variations of the method, due to very reactive materials, are specifically addressed in Section 11.3.3. Limits of quantitation will change with sample quantity (dilution) and with instrumentation.

4.2 Many samples can be dissolved by this method. However, when the sample contains suspended solids which are made up of refractory compounds, such as silicon dioxide, titanium dioxide, alumina, and other oxides, they will not be dissolved and in some cases may sequester target analyte elements. These bound elements are considered nonmobile in the environment and are excluded from most aqueous pollutant transport mechanisms.

5.0 SAFETY

5.1 The microwave unit cavity must be corrosion resistant and well ventilated. All electronics must be well protected against corrosion for safe operation.

CAUTION: There are many safety and operational recommendations specific to the model and manufacturer of the microwave equipment used in individual laboratories. A listing of these specific suggestions is beyond the scope of this method. The analyst is advised to consult the equipment manual, the equipment manufacturer, and other appropriate literature for proper and safe operation of the microwave equipment and vessels. For further details and safety literature, references 1, 7 and 8 review methods and safety in microwave sample preparation.

5.2 The method requires microwave transparent and reagent resistant materials such as fluorocarbon polymers (examples are PFA and TFM) or quartz to contain acids and samples. For higher pressure capabilities, the vessel may be contained within layers of different microwave transparent materials for strength, durability, and safety. The internal volume of the vessel should be at least 100 mL, and the vessel must be capable of withstanding pressures of at least 30 atm (435 psi), and capable of controlled pressure relief. These specifications are to provide an appropriate, safe, and durable reaction vessel of which there are many adequate designs by many suppliers.

CAUTION: The outer layers of vessels are frequently not as acid or reagent resistant as the liner material. In order to retain the specified performance and safety requirements, these outer layers must not be chemically degraded or physically damaged. Routine examination of the vessel materials is necessary to ensure their safe use.

CAUTION: Another safety concern relates to the use of sealed containers without pressure relief devices. Temperature is the important variable controlling the reaction. Pressure is needed to attain elevated temperatures, but must be safely contained. Some digestion vessels constructed from certain fluorocarbons may crack, burst, or explode in the unit under certain pressures. Only fluorocarbon (such as PFA, TFM, and others) or quartz containers with pressure relief mechanisms or containers with fluorocarbon or quartz liners and pressure relief mechanisms are considered acceptable.

CAUTION: An aqueous sample must contain no more than 1% (V/V or g/V) oxidizable organic material. Upon oxidation, organic material, whether liquid or solid, contributes to gaseous digestion products. Pressure build-up above the pressure limit will result in venting of the closed digestion vessel.

CAUTION: Laboratories should not use domestic (kitchen) type microwave ovens for this method because of significant safety issues. When acids such as nitric and hydrochloric are used to effect sample digestion in microwave units in open or sealed vessel(s), there is the potential for acid vapors released to corrode the safety devices that prevent the microwave magnetron from shutting off when the door is opened. This can result in operator exposure to microwave energy. Use of a system with isolated and corrosion resistant instrument components and safety devices prevents this from occurring.

Users are advised not to use domestic (kitchen) type microwave ovens or sealed containers which are not equipped with controlled pressure relief mechanisms for microwave acid digestions by this method. Use of laboratory-grade microwave equipment is required to prevent safety hazards. For further details, consult references 1, 7, and 8.

6.0 EQUIPMENT AND SUPPLIES

6.1 Microwave apparatus requirements

6.1.1 The temperature performance requirements necessitate the microwave decomposition system to sense the temperature to within ± 2.5 °C and automatically adjust the microwave field output power within 2 seconds of sensing. Temperature sensors should be accurate to ± 2 °C (including the final reaction temperature of 170 ± 5 °C). Temperature feedback control provides the primary performance mechanism for the method. Due to the variability in sample matrix types and microwave digestion equipment (i.e., different vessel types and microwave oven designs), temperature feedback control is preferred for reproducible microwave heating. For further details, consult reference 7.

Alternatively, for a specific vessel type, specific set of reagent(s), and sample type, a calibration control mechanism can be developed similar to those described in previous microwave methods (See EPA Method 3051). Through calibration of the microwave power for a specific number and type of vessel, vessel load, and heat loss characteristics of a specific vessel series, the reaction temperature profile described in Section 11.3.5 can be reproduced (Reference 7). The calibration settings are specific for the number and type of vessel and microwave system being used, in addition to the specific reagent combination being used. Therefore, no specific calibration settings are provided in this method. These settings may be developed by using temperature monitoring equipment for each specific set of microwave equipment and vessel type. They may be used as previously described in EPA Methods 3052 and 3051. In this circumstance, the microwave system provides programmable power, which can be programmed to within ± 12 W of the required power. Typical systems provide 600 W - 1200 W of power. Calibration control provides backward compatibility with older laboratory microwave systems which may not be equipped for temperature monitoring or feedback control and with lower cost microwave systems for some repetitive analyses. Older vessels with lower pressure capabilities may not be compatible (References 4 - 8).

6.1.2 The accuracy of the temperature measurement system should be periodically validated at an elevated temperature. This can be done using a container of silicon oil (a high temperature oil) and an external, calibrated temperature measurement system. The oil should be adequately stirred to ensure a homogeneous temperature, and both the microwave temperature sensor and the external temperature sensor placed into the oil. After heating the oil to a constant temperature of 170 ± 5°C, the temperature should be measured using both sensors. If the measured temperatures vary by more than 1 to 2°C, the microwave temperature measurement system should be calibrated. Consult the microwave manufacturer's instructions about the specific temperature sensor calibration procedure (see EPA Method 3052).

6.1.3 A rotating turntable is employed to ensure the homogeneous distribution of microwave radiation within the unit. The speed of the turntable should be a minimum of 3 rpm. Other types of equipment that are used to assist in achieving uniformity of the microwave field may also be appropriate.

6.2 Class A or appropriate mechanical pipette, volumetric flask, or graduated cylinder, 50 or 100 mL capacity or equivalent.

6.3 Filter paper, qualitative or equivalent.

6.4 Filter funnel, glass, polypropylene, or other appropriate material.

6.5 Analytical balance, of appropriate capacity and resolution, meeting data quality objectives.

7.0 REAGENTS

7.1 All acids should be sub-boiling distilled and/or high purity where possible to minimize blank levels due to metallic contamination. Other grades may be used, provided it is first ascertained that the reagent is of sufficient purity to permit its use without decreasing the accuracy of the determination. If the purity of a reagent is questionable, the reagent should be analyzed to determine the level of impurities. The reagent blank must be less than the MDL in order to be used.

7.1.1 Concentrated nitric acid (HNO_3). The acid should be analyzed to determine levels of impurity. If the method blank is less than the MDL, the acid can be used.

7.1.2 Concentrated hydrochloric acid (HCl). The acid should be analyzed to determine levels of impurity. If the method blank is less than the MDL, the acid can be used.

7.2 Reagent Water. Reagent water shall be interference free. All references to water in the method refer to reagent water unless otherwise specified. For further details, consult Reference 2.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of SW-846. Refer to that chapter, as updated, for guidance.

8.2 All sample containers must be prewashed with acids, water, and metal-free detergents, if necessary, depending on the use history of the container (Reference 7). Plastic and glass containers are both suitable. For further information, see Chapter Three.

8.3 Aqueous waste waters must be acidified to a pH < 2 with HNO_3.

9.0 QUALITY CONTROL

9.1 All quality control data must be maintained and available for reference or inspection for a period of three years. This method is restricted to use by, or under supervision of, experienced analysts.

9.2 Duplicate samples should be processed on a routine basis. A duplicate sample is a sample brought through the whole sample preparation and analysis process. A duplicate sample should be processed with each analytical batch or every 20 samples, whichever is the greater number. A duplicate sample should be prepared for each matrix type (i.e., wastewaters, extracts, etc.).

9.3 Spiked samples or standard reference materials should be included with each group of samples processed, or every 20 samples, whichever is the greater number. A spiked sample should also be included whenever a new sample matrix is being analyzed.

9.4 Periodically, the accuracy of the temperature measurement system used to control the microwave equipment should be validated per Section 6.1.2.

9.5 (Not necessary if using temperature feedback control.) Each day that samples are extracted, the microwave-power calibration should be verified by heating 1 kg of ASTM Type II water (at 22 ± 3 °C) in a covered, microwave-transparent vessel for 2 min at the setting for 490 W and measuring the water temperature after heating per Section 10.1.5. If the power calculated (per Section 12) differs from 490 W by more than ± 10 W, the microwave settings should be recalibrated per Section 10.0.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Calibration of Microwave Equipment

NOTE: If the microwave unit uses temperature feedback control to control the performance specifications of the method, then performing the calibration procedure is not necessary.

10.1.1 Calibration is the normalization and reproduction of a microwave field strength to permit reagent and energy coupling in a predictable and reproducible manner. It balances reagent heating and heat loss from the vessels and is equipment dependent due to the heat retention and loss characteristics of the specific vessel. Available power is evaluated to permit the microwave field output in watts to be transferred from one microwave system to another.

Use of calibration to control this reaction requires balancing output power, coupled energy, and heat loss to reproduce the temperature heating profile given in section 11.3.5. The conditions for each acid mixture and each batch containing the same specified number of vessels must be determined individually. Only identical acid mixtures and vessel models and specified numbers of vessels may be used in a given batch.

10.1.2 For cavity type microwave equipment, calibration is accomplished by measuring the temperature rise in 1 kg of water exposed to microwave radiation for a fixed period of time. The analyst can relate power in watts to the partial power setting of the system. The calibration format required for laboratory microwave systems depends on the type of electronic system used by the manufacturer to provide partial microwave power. Few systems have an accurate and precise linear relationship between percent power settings and absorbed power. Where linear circuits have been utilized, the calibration curve can be determined by a three-point calibration method (see Section 10.1.4). Otherwise, the analyst must use the multiple point calibration method (see Section 10.1.3). Assistance in calibration and software guidance of calibration are available in References 7 and 8.

10.1.3 Multiple point calibration involves the measurement of absorbed power over a large range of power settings. Typically, for a 600 W unit, the following power settings are measured: 100, 99, 98, 97, 95, 90, 80, 70, 60, 50, and 40% using the procedure described in Section 10.1.5. This data is clustered about the customary working power ranges. Nonlinearity has been encountered at the upper end of the calibration. If the system's electronics are known to have nonlinear deviations in any region of proportional power control, it will be necessary to make a set of measurements that bracket the power to be used. The final calibration point should be at the partial power setting that will be used in the test. This setting should be checked periodically to evaluate the integrity of the calibration. If a significant change is detected (± 10 W), then the entire calibration should be re-evaluated.

10.1.4 The three-point calibration involves the measurement of absorbed power at three different power settings. Power is measured at 100% and 50% using the procedure described in Section 10.1.5. From this 2-point line, determine the partial power setting that corresponds to the power, in watts, specified in the procedure to reproduce the heating profile specified in Section 11.3.6. Measure the absorbed power at that partial power setting. If the measured absorbed power does not correspond to the specified power within ± 10 W, use the multiple point calibration in Section 10.1.3. This point should also be used to periodically verify the integrity of the calibration.

10.1.5 Equilibrate a large volume of water to room temperature (22 ± 3 °C). One kg of reagent water is weighed (1,000.0 ± 0.1 g) into a fluorocarbon beaker or a beaker made of some other material that does not significantly absorb microwave energy (glass absorbs microwave energy and is not recommended). The initial temperature of the water should be 22 ± 3 °C measured to ± 0.05 °C. The covered beaker is circulated continuously (in the normal sample path) through the microwave field for 2 minutes at the desired partial power setting with the system's exhaust fan on maximum (as it will be during normal operation). The beaker is removed and the water is vigorously stirred. Use a magnetic stirring bar inserted immediately after microwave irradiation (irradiating with the stir bar in the vessel could cause electrical arcing) and record the maximum temperature within the first 30 seconds to ± 0.05 °C. Use a new sample for each additional measurement. If the water is reused (after making adjustments for any loss of weight due to heating), both the water and the beaker must have returned to 22 ± 3 °C. Three measurements at each power setting should be made.

The absorbed power is determined by the following relationship:

Eq. 1 $$P = \frac{(K)(C_p)(m)(\Delta T)}{t}$$

Where:

P = the apparent power absorbed by the sample in watts (W) (joule sec^{-1})

K = the conversion factor for thermochemical calories sec^{-1} to watts (K= 4.184)

C_p = the heat capacity, thermal capacity, or specific heat (cal g^{-1} $°C^{-1}$) of water

m = the mass of the water sample in grams (g)

ΔT = the final temperature minus the initial temperature (°C)

t = the time in seconds (s)

Using the experimental conditions of 2 minutes (120 sec) and 1 kg (1000 g) of distilled water (heat capacity at 25 °C is 0.9997 cal g^{-1} $°C^{-1}$), the calibration equation simplifies to:

Eq. 2 $$P = (\Delta T)(34.86)$$

NOTE: Stable line voltage is necessary for accurate and reproducible calibration and operation. The line voltage should be within manufacturer's specification. During measurement and

operation the line voltage should not vary by more than ± 2 V (Reference 7). Electronic components in most microwave units are matched to the system's function and output. When any part of the high voltage circuit, power source, or control components in the system have been serviced or replaced, it will be necessary to recheck the system's calibration. If the power output has changed significantly (± 10 W), then the entire calibration should be re-evaluated.

11.0 SAMPLE PROCEDURE

11.1 Temperature control of closed vessel microwave instruments provides the main feedback control performance mechanism for the method. Method control requires a temperature sensor in one or more vessels during the entire digestion. The microwave decomposition system should sense the temperature to within ± 2.5 °C and permit adjustment of the microwave output power within 2 seconds.

11.2 All digestion vessels and volumetric ware must be carefully acid washed and rinsed with reagent water. When switching between highly concentrated samples and low concentrated samples, all digestion vessels (fluoropolymer or quartz liners) should be cleaned by leaching with hot (1:1) hydrochloric acid (greater than 80 °C, but less than boiling) for a minimum of two hours followed by hot (1:1) nitric acid (greater than 80 °C, but less than boiling) for a minimum of two hours. The vessels should then be rinsed with reagent water and dried in a clean environment. This cleaning procedure should also be used whenever the prior use of the digestion vessels is unknown or cross contamination from prior sample digestions in vessels is suspected. Polymeric or glass volumetric ware and storage containers should be cleaned by leaching with more dilute acids (approximately 10% V/V) appropriate for the specific material used and then rinsed with reagent water and dried in a clean environment.

11.3 Sample Digestion

11.3.1 Measure a 45 mL aliquot of a well-shaken, homogenized sample using an appropriate volumetric measurement and delivery device, and quantitatively transfer the aliquot to an appropriate vessel equipped with a controlled pressure relief mechanism.

11.3.2 Add 5 ± 0.1 mL concentrated nitric acid or, alternatively, 4 ± 0.1 mL concentrated nitric acid and 1 ± 0.1 mL concentrated hydrochloric acid to the vessel in a fume hood (or fume exhausted enclosure). The addition of concentrated hydrochloric acid to the nitric acid is appropriate for the stabilization of certain analytes, such as Ag, Ba, and Sb and high concentrations of Fe and Al in solution. Improvements and optimal recoveries of antimony and silver upon addition of HCl have been described in the literature (Reference 7). The addition of hydrochloric acid may, however, limit the detection techniques or increase the difficulties of analysis for some detection systems.

CAUTION: The addition of hydrochloric acid must be in the form of concentrated hydrochloric acid and not from a premixed combination of acids. A build-up of chlorine gas, as well as other gases, will result from a premixed acid solution. These gases may be violently released upon heating. This is avoided by adding the acid in the described manner.

CAUTION: Toxic nitrogen oxide(s) and chlorine fumes are usually produced during digestion. Therefore, all steps involving open or the opening of microwave vessels must be performed in a properly operating fume ventilation system.

CAUTION: The analyst should wear protective gloves and face protection.

CAUTION: The use of microwave equipment with temperature feedback control is required to control any unfamiliar reactions that may occur during the leaching of samples of unknown composition. The leaching of these samples may require additional vessel requirements such as increased pressure capabilities.

11.3.3 The analyst should be aware of the potential for a vigorous reaction, especially with samples containing suspended solids composed of volatile or easily oxidized organic species. When digesting a matrix of this type, if a vigorous reaction occurs upon the addition of reagent(s), this sample represents a safety hazard. Do not leach the sample as described in this method due to the high potential for unsafe and uncontrollable reactions.

11.3.4 Seal the vessel according to the manufacturer's directions. Properly place the vessel in the microwave system according to the manufacturer's recommended specifications and, when applicable, connect appropriate temperature and pressure monitoring equipment to vessels according to manufacturer's specifications.

11.3.5 This method is a performance based method, designed to achieve or approach consistent leaching of the sample through achieving specific reaction conditions. The temperature of each sample should rise to 170 ± 5 °C in approximately 10 minutes and remain at 170 ± 5 °C for 10 minutes, or for the remainder of the twenty-minute digestion period (References 3, 4, 6, and 7). The time vs. temperature and pressure profiles for the leaching of three simulated wastewater samples using Method 3015 are shown in Figure 1. The samples are composed of approximately 0.35 g SRM 2704 (Buffalo River Sediment) mixed in 45 mL double-deionized water. The figure demonstrates the temperature and pressure profiles for both the all-nitric digest (5 mL concentrated nitric acid), and the nitric and hydrochloric mixed-acid digest (4 mL concentrated nitric acid and 1 mL concentrated hydrochloric acid). Also shown is the profile for the heating of the wastewater sample without addition of acids. When using temperature feedback control, the number of samples that may be simultaneously digested may vary, from one sample up to the maximum number of vessels that can be heated by the magnetron of the microwave unit according to the heating profile specified in this section. (The number will depend on the power of the unit, the number of vessels, and the heat loss characteristics of the vessels (Reference 7)).

11.3.5.1 Calibration control is applicable in reproducing this method provided the power in watts versus time parameters are determined to reproduce the specifications listed in Section 11.3.5. The calibration settings will be specific to the quantity of reagents, the number of vessels, and the heat loss characteristics of the vessels (Reference 7). If calibration control is being used, any vessels containing acids for analytical blank purposes are counted as sample vessels. When fewer than the recommended number of samples are to be digested, the remaining vessels should be filled with 45 mL water, and the acid mixture added, so that the full complement of vessels is achieved. This provides an energy balance, since the microwave power absorbed is proportional to the total absorbing mass in the cavity (Reference 7). Irradiate each group of vessels using the predetermined calibration settings. (Different vessel types should not be mixed.)

11.3.6 At the end of the microwave program, allow the vessels to cool for a minimum of 5 minutes before removing them from the microwave system. Cooling of the vessels may be accelerated by internal or external cooling devices. When the vessels have cooled to

near room temperature, determine if the microwave vessels have maintained their seal throughout the digestion. Due to the wide variability of vessel designs, a single procedure is not appropriate. For vessels that are sealed as discrete separate entities, the vessel weight may be taken before and after digestion to evaluate seal integrity. If the weight loss of the sample exceeds 1% of the weight of the sample and reagents, then the sample is considered compromised. For vessels with burst disks, a careful visual inspection of the disk, in addition to weighing, may identify compromised vessels. For vessels with resealing pressure relief mechanisms, an auditory or a physical sign that can indicate whether a vessel has vented is appropriate.

11.3.7 Complete the preparation of the sample by carefully uncapping and venting each vessel in a chemical fume hood (or fume exhausted enclosure). Vent the vessels using the procedure recommended by the vessel manufacturer. Quantitatively transfer the sample to an acid-cleaned bottle. If the digested sample contains particulates which may clog nebulizers or interfere with injection of the sample into the instrument, the sample may be centrifuged (Section 11.3.7.1), allowed to settle (Section 11.3.7.2), or filtered (Section 11.3.7.3).

11.3.7.1 Centrifugation: Centrifugation at 2,000 - 3,000 rpm for 10 minutes is usually sufficient to clear the supernatant.

11.3.7.2 Settling: If undissolved material, such as SiO_2, TiO_2, or other refractory oxides, remains, allow the sample to stand until the supernatant is clear. Allowing a sample to stand overnight will usually accomplish this. If it does not, centrifuge or filter the sample.

11.3.7.3 Filtering: If necessary, the filtering apparatus must be thoroughly cleaned and prerinsed with dilute (approximately 10% V/V) nitric acid. Filter the sample through qualitative filter paper into a second acid-cleaned container.

11.3.8 The removal or reduction of the quantity of sample may be desirable for concentration of analytes prior to analysis. The chemistry and volatility of the analytes of interest should be considered and evaluated when using this alternative (Reference 7, 8). Sample evaporation in a controlled environment with controlled purge gas and neutralizing and collection of exhaust interactions is an alternative where appropriate. This manipulation may be performed in the microwave system, if the system is capable of this function, or external to the microwave system in more common apparatus(s). This option must be tested and validated to determine analyte retention and loss and should be accompanied by equipment validation possibly using the standard addition method and standard reference materials. For further information, see References 7 and 8 and Method 3052.

NOTE: The final solution typically requires nitric acid to maintain appropriate sample solution acidity and stability of the elements. Commonly, a 2% (v/v) nitric acid concentration is desirable. Waste minimization techniques should be used to capture reagent fumes. This procedure should be tested and validated in the apparatus and on standards before using on unknown samples.

11.3.9 Transfer or decant the sample into volumetric ware and dilute the digest to a known volume. The digest is now ready for analysis for elements of interest using appropriate elemental analysis techniques and/or SW-846 methods.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Calculations: The concentrations determined are to be reported on the basis of the actual volume of the original sample.

12.2 Prior to use of the method, verify that the temperature sensing equipment is properly reading temperature. A procedure for verification is given in Section 6.1.2. This will establish the accuracy and precision of the temperature sensing equipment, which should be carried throughout the statistical treatment of the quality assurance data.

12.3 In calibrating the microwave unit (Section 10.0), the power absorbed (for each power setting) by 1 kg of reagent water exposed to 120 seconds of microwave energy is determined by the expression:

$$\text{Power (in watts)} = (T_1 - T_2)\ (34.86)$$

where: T_1 = Initial temperature of water (between 21 and 25 °C to nearest 0.1 °C)

T_2 = Final temperature of water (to nearest 0.1 °C)

12.4 Plot the power settings against the absorbed power (calculated in Section 12.3) to obtain a calibration relationship. Alternatively, use a microwave calibration program to analyze the calibration data (References 7 and 8). Interpolate the data to obtain the instrument settings needed to provide the wattage levels specified in Section 12.3.

13.0 METHOD PERFORMANCE

13.1 The fundamental analytical validation of Method 3015 with nitric acid has been performed (Reference 6). The results are shown in Table 1. Variations of 3015 including nitric acid and hydrochloric acid have also been published in the literature (References 5, 7, 9). The method has also been tested on a variety of matrices, including two simulated wastewater matrices, one consisting of ~ 0.35 g sediment (SRM 2704) mixed with 45 mL double-deionized water, and the other consisting of ~ 0.35 g soil (SRM 4355) mixed with 45 mL double-deionized water. The results are shown in Tables 2 and 3, and are published in the literature (Reference 9).

14.0 POLLUTION PREVENTION

14.1 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 operations. 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.

14.2 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 Relations and Science Policy, 1155 16th Street, NW, Washington, DC 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits 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's Department of Government Relations and Science Policy, 1155 16th Street, NW, Washington, DC 20036, (202) 872-4477.

16.0 REFERENCES

1. Kingston, H.M.; Jassie, L.B. In Introduction to Microwave Sample Preparation: Theory and Practice; Kingston, H. M. and Jassie, L. B., Eds.; ACS Professional Reference Book Series; American Chemical Society: Washington, DC, 1988; Chapters 6 and 11.

2. 1985 Annual Book of ASTM Standards, Vol. 11.01; "Standard Specification for Reagent Water"; ASTM, Philadelphia, PA, 1985, D1193-77.

3. Kingston, H. M., Final Report EPA IAG #DW13932541-01-I, September 30, 1988, Appendix A.

4. Shannon, M., Alternate Test Procedure Application, USEPA Region V, Central Regional Laboratory, 536 S. Clark Street, Chicago, IL 60606, 1989.

5. Kingston, H. M., Walter, P. J., "Comparison of Microwave Versus Conventional Dissolution for Environmental Applications", Spectroscopy, Vol. 7 No. 9, 20-27, 1992.

6. Sosinski, P., and Sze, C., "Absolute Accuracy Study, Microwave Digestion Method 3015 (nitric acid only)"; EPA Region III Central Regional Laboratory, 1991.

7. Kingston, H. M., Haswell, S. J., Eds. Microwave Enhanced Chemistry: Fundamentals, Sample Preparation, and Applications; ACS Professional Reference Book Series; American Chemical Society: Washington, DC 1997.

8. Duquesne University. Analytical Sample Preparation and Microwave Chemistry Center. *SamplePrep Web*. Access http://www.sampleprep.duq.edu/

9. Link, D.D., Kingston, H.M., Walter, P.J., "Development and Validation of the New EPA Microwave-Assisted Leach Methods 3051A and 3015A.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain Tables 1 through 3, Figure 1, and a flow diagram of method procedure.

TABLE 1

RESULTS OF VALIDATION STUDY FOR METHOD 3015 (NITRIC ONLY)
(REFERENCE 6)

Element	TM-11		TM-12		T-95		T-107		T-109	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Al	480	26	2800	88.			210	19	120	31
As							13	1	90	11
Ba	140	23	2400	70			200	16		
Be							11.3	0.5	26	1
Ca							12000	783	59000	999
Cd	45	2	240	8			12	1	10	2
Co	240	14	1150	36						
Cr	64	4	350	10			23	1	30	6
Cu	78	4	320	9			42	4	34	4
Fe	290	16	1180	43			60	9	130	7
K					5000	784			2600	383
Mg					35000	1922	2200	110	10200	218
Mn	61	3	300	9			53	3	47	3
Na					20000	10690	2300	1056	13800	516
Ni	280	16	1290	39					61	2
Pb	280	32	1360	35			30.1	0.2	39	1
Se					65.97	2.65	13	1		
V	530	26	2400	61						
Zn	56	3	520	9			31	3	70	4

Element	WP980 #1		WP980 #2		WS378 #4		WS378 #12	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Sb	18.0	0.5	110	34				
Tl	55	2	7.0	0.5				
Ag					ND		19	5

TABLE 2

COMPARISON OF ANALYTE RECOVERIES FROM "SIMULATED WASTEWATER" MIXTURE OF ~ 0.35 G SRM 2704 (BUFFALO RIVER SEDIMENT) AND 45 ML DOUBLE-DEIONIZED WATER USING BOTH DIGEST OPTIONS OF METHOD 3015 (REFERENCE 9)

Element	5 mL HNO_3 digest	4 mL HNO_3 + 1 mL HCl digest	Total Analyte Concentration
Ag	0.31 ± 0.05	0.41 ± 0.09	<4
B	23.8 ± 3.1	30.6 ± 8.3	—*
Be	0.81 ± 0.13	0.91 ± 0.19	—*
Co	12.0 ± 0.30	11.5 ± 0.98	14.0 ± 0.6
Hg	—	1.49 ± 0.03	1.44 ± 0.07
Mo	2.97 ± 0.72	3.15 ± 0.28	—*
Ni	39.6 ± 2.5	41.3 ± 1.7	44.1 ± 3.0
Sr	41.9 ± 1.3	49.0 ± 1.6	(130)
V	6.18 ± 2.5	14.6 ± 2.4	95 ± 4
Zn	418 ± 12	412 ± 31	438 ± 12

Results reported in µg/g analyte (mean ± 95% confidence limit).
Total concentrations are taken from NIST SRM Certificate of Analysis.
Values in parenthesis are reference concentrations.
* The total concentration of this analyte in SRM 2704 is not certified.

TABLE 3

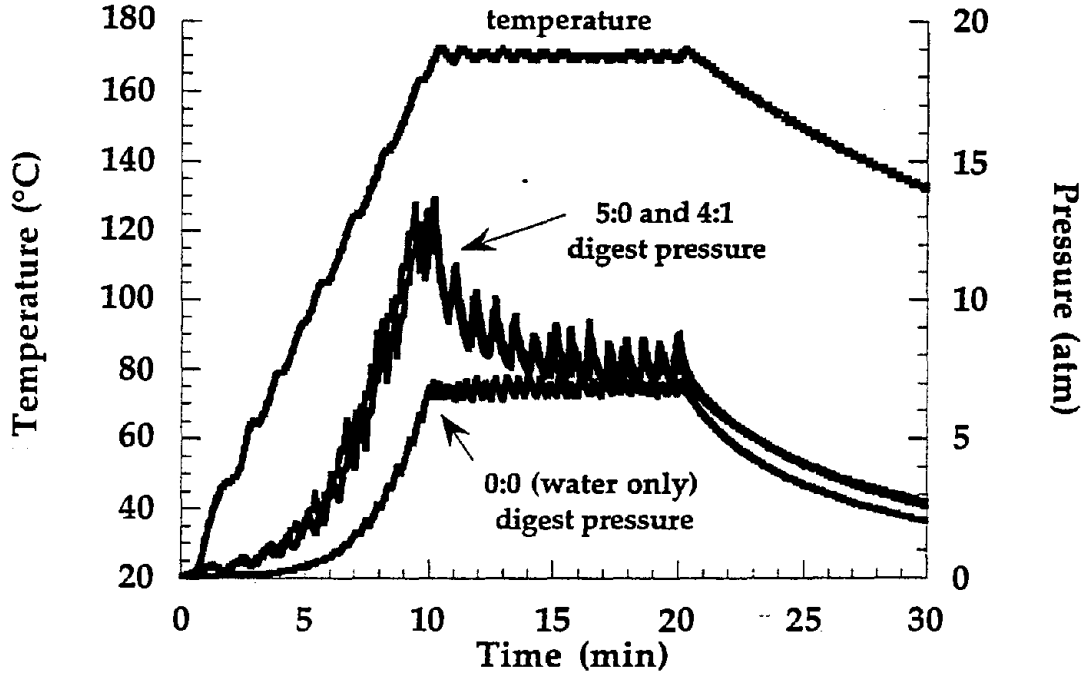
COMPARISON OF ANALYTE RECOVERIES FROM "SIMULATED WASTEWATER" MIXTURE OF ~0.35 G SRM 4355 (PERUVIAN SOIL) AND 45 ML DOUBLE-DEIONIZED WATER USING BOTH DIGEST OPTIONS OF METHOD 3015 (REFERENCE 9)

Element	5 mL HNO_3 digest	4 mL HNO_3 + 1 mL HCl digest	Total Analyte Concentration
Ag	1.31 ± 0.12	1.62 ± 0.11	(1.9)*
B	32.9 ± 2.1	31.8 ± 2.7	(63)*
Co	10.5 ± 0.34	10.4 ± 0.41	14.8 ± 0.76
Mo	0.99 ± 0.06	1.1 ± 0.11	(1.7)*
Ni	12.2 ± 1.2	13.1 ± 1.9	(13)*
Pb	135 ± 4	136 ± 4	129 ± 26
Sb	3.7 ± 0.30	5.2 ± 0.53	14.3 ± 2.2
Sr	140 ± 6	143 ± 7	(330)

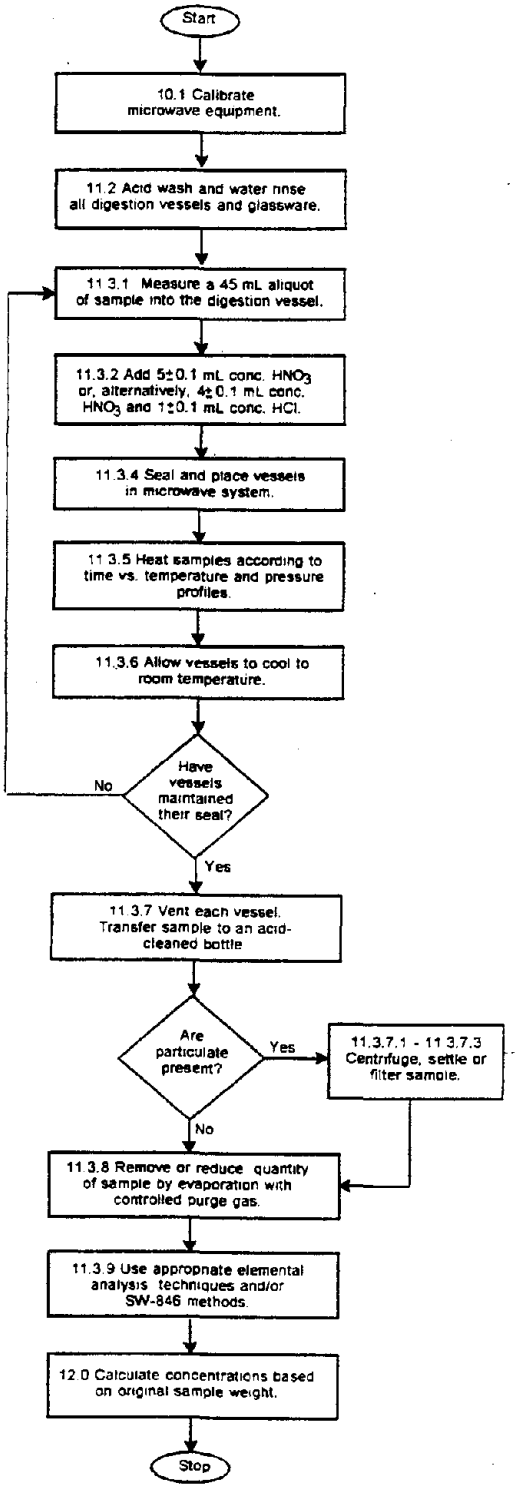
Results reported in µg/g analyte (mean ± 95% confidence limit).
Total concentrations are taken from NIST SRM Certificate of Analysis.
* Values in parenthesis are reference concentrations.

FIGURE 1

THE TYPICAL TEMPERATURE AND PRESSURE PROFILE FOR THE HEATING OF A SIMULATED WASTEWATER SAMPLE (~ 0.35 G SRM 2704 + 45 ML DOUBLE-DEIONIZED WATER) USING BOTH DIGEST OPTIONS (5 ML HNO_3 AND 4 ML HNO_3 + 1 ML HCL) OF METHOD 3015.



MICROWAVE ASSISTED ACID DIGESTION OF AQUEOUS SAMPLES AND EXTRACTS



METHOD 3051A

MICROWAVE ASSISTED ACID DIGESTION OF SEDIMENTS, SLUDGES, SOILS, AND OILS

1.0 SCOPE AND APPLICATION

1.1 This microwave extraction method is designed to mimic extraction using conventional heating with nitric acid (HNO_3), or alternatively, nitric acid and hydrochloric acid (HCl), according to EPA Methods 200.2 and 3050. Since these methods are not intended to accomplish total decomposition of the sample, the extracted analyte concentrations may not reflect the total content in the sample. This method is applicable to the microwave-assisted acid extraction/dissolution‡ of sediments, sludges, soils, and oils for the following elements:

Element		CASRN[a]
Aluminum	(Al)	7429-90-5*
Antimony	(Sb)	7440-36-0*
Arsenic	(As)	7440-38-2
Barium	(Ba)	7440-39-3*
Beryllium	(Be)	7440-41-7*
Boron	(B)	7440-42-8
Cadmium	(Cd)	7440-43-9
Calcium	(Ca)	7440-70-2
Chromium	(Cr)	7440-47-3*
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Iron	(Fe)	7439-89-6*
Lead	(Pb)	7439-92-1
Magnesium	(Mg)	7439-95-4*
Manganese	(Mn)	7439-96-5
Mercury	(Hg)	7439-97-6
Molybdenum	(Mo)	7439-98-7
Nickel	(Ni)	7440-02-0
Potassium	(K)	7440-09-7
Selenium	(Se)	7782-49-2
Silver	(Ag)	7440-22-4*
Sodium	(Na)	7440-23-5
Strontium	(Sr)	7440-24-6
Thallium	(Tl)	7440-28-0
Vanadium	(V)	7440-62-2*
Zinc	(Zn)	7440-66-6

[a]Chemical Abstract Service Registry Number

*Indicates elements which typically require the addition of HCl to achieve equivalent results with EPA Method 3050, as noted in reference 3.

‡Note: For matrices such as certain types of oils, this method may or may not provide total sample dissolution. For other matrices, such as soils and sediments, it should be considered an extraction method. Other elements and matrices may be

analyzed by this method if performance is demonstrated for the analyte of interest, in the matrices of interest, at the concentration levels of interest (see Sec. 9.0).

1.2 This method is provided as an alternative to EPA Methods 200.2 and 3050. This method provides options for improving the performance for certain analytes, such as antimony, iron, aluminum, and silver by the addition of hydrochloric acid, when necessary. It is intended to provide a rapid multi-element acid extraction or dissolution prior to analysis so that decisions can be made about materials and site cleanup levels, the need for TCLP testing of a waste (see EPA Method 1311, Section 1.2, for further details), and whether a BDAT process is providing acceptable performance. Digests produced by the method are suitable for analysis by flame atomic absorption spectrophotometry (FLAA), graphite furnace atomic absorption spectrophotometry (GFAA), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS). However, the addition of HCl may limit the methods of detection, or increase the difficulties of detection with some techniques.

Due to the rapid advances in microwave technology, consult your manufacturer's recommended instructions for guidance on their microwave digestion system.

2.0 SUMMARY OF METHOD

2.1 A representative sample of up to 0.5 g is extracted and/or dissolved in 10 mL concentrated nitric acid, or alternatively, 9 mL concentrated nitric acid and 3 mL concentrated hydrochloric acid for 10 minutes using microwave heating with a suitable laboratory microwave unit. The sample and acid(s) are placed in a fluorocarbon polymer (PFA or TFM) or quartz microwave vessel or vessel liner. The vessel is sealed and heated in the microwave unit. After cooling, the vessel contents are filtered, centrifuged, or allowed to settle and then diluted to volume and analyzed by the appropriate determinative method.

3.0 DEFINITIONS

Please refer to Chapter One and Chapter Three for a listing of applicable definitions.

4.0 INTERFERENCES

4.1 Very reactive samples or volatile materials may create high pressures due to the evolution of gaseous digestion products. This may cause venting of the vessels with potential loss of sample and/or analytes. The complete decomposition of either carbonates, or carbon based samples, may produce enough pressure to vent the vessel if the sample size is greater than 0.25 g (depending on the pressure capability of the vessel). Variations of the method to accommodate very reactive materials are specifically addressed in Section 11.3.3.

4.2 Many types of samples will be dissolved by this method. A few refractory sample matrix compounds, such as quartz, silicates, titanium dioxide, alumina, and other oxides may not be dissolved and in some cases may sequester target analyte elements. These bound elements are considered non-mobile in the environment and are excluded from most aqueous transport mechanisms of pollution.

5.0 SAFETY

5.1 The microwave unit cavity must be corrosion resistant and well ventilated. All electronics must be protected against corrosion for safe operation.

CAUTION: There are many safety and operational recommendations specific to the model and manufacturer of the microwave equipment used in individual laboratories. A listing of these specific suggestions is beyond the scope of this method. The analyst is advised to consult the equipment manual, the equipment manufacturer, and other appropriate literature for proper and safe operation of the microwave equipment and vessels. For further details, see reference 3 and the document of Sec. 13.2.1 for a review of safety in microwave sample preparation.

5.2 The method requires essentially microwave transparent and reagent resistant materials such as fluorocarbon polymers (examples are PFA or TFM) or quartz to contain acids and samples. For higher pressure capabilities the vessel may be contained within layers of different microwave transparent materials for strength, durability, and safety. The internal volume of the vessel should be at least 45 mL, and the vessel must be capable of withstanding pressures of at least 30 atm (435 psi), and capable of controlled pressure relief. These specifications are to provide an appropriate, safe, and durable reaction vessel of which there are many adequate designs by many suppliers.

CAUTION: The reagent combination (9 mL nitric acid to 3 mL hydrochloric acid) results in greater pressures than those resulting from the use of only nitric acid. As demonstrated in Figures 1 and 2, pressures of approximately 12 atm have been reached during the heating of the acid mixture alone (no sample in the vessel). Pressures reached during the actual decomposition of a sediment sample (SRM 2704, a matrix with low organic content) have more than doubled when using the 9 mL nitric and 3 mL hydrochloric acid mixture. These higher pressures necessitate the use of vessels having higher pressure capabilities (30 atm or 435 psi). Matrices having large organic content, such as oils, can produce approximately 25 atm of pressure inside the vessel (as described in EPA Method 3052).

CAUTION: The outer layers of vessels are frequently not as acid or reagent resistant as the liner material. In order to retain the specified performance and safety requirements, these outer layers must not be chemically degraded or physically damaged. Routine examination of the vessel materials is necessary to ensure their safe use.

CAUTION: Another safety concern relates to the use of sealed containers without pressure relief devices. Temperature is the important variable controlling the reaction. Pressure is needed to attain elevated temperatures, but must be safely contained. Some digestion vessels constructed from certain fluorocarbons may crack, burst, or explode in the unit under certain pressures. Only fluorocarbon (such as PFA or TFM and others) or quartz containers with pressure relief mechanisms or containers with fluorocarbon or quartz liners and pressure relief mechanisms are considered acceptable.

CAUTION: Laboratories should not use domestic (kitchen) type microwave ovens for this method because of significant safety issues. When acids such as nitric and hydrochloric are used to effect sample digestion in microwave units in open vessel(s), or sealed vessel(s), there is the potential for any released acid vapors to corrode the safety devices that prevent the microwave magnetron from shutting off when the door is opened. This can result in operator exposure to microwave energy. Use of a system with isolated and corrosion resistant safety devices prevents this from occurring.

Users are therefore advised not to use domestic (kitchen) type microwave ovens or sealed containers which are not equipped with controlled pressure relief mechanisms for microwave acid digestions by this method. Use of laboratory-grade microwave equipment is

required to prevent safety hazards. For further details, consult reference 3 and the document listed in Sec. 13.2.1.

6.0 EQUIPMENT AND SUPPLIES

6.1 Microwave apparatus requirements.

6.1.1 The temperature performance requirements necessitate the microwave decomposition system to sense the temperature to within ± 2.5 °C and automatically adjust the microwave field output power within 2 seconds of sensing. Temperature sensors should be accurate to ± 2 °C (including the final reaction temperature of 175 ± 5 °C). Temperature feedback control provides the primary performance mechanism for the method. Due to the variability in sample matrix types and microwave digestion equipment (i.e., different vessel types and microwave oven designs), temperature feedback control is preferred for reproducible microwave heating. For further details consult reference 3.

Alternatively, for a specific vessel type, specific set of reagent(s), and sample type, a calibration control mechanism can be developed. Through calibration of the microwave power for a specific number and type of vessels, vessel load, and heat loss characteristics of a specific vessel series, the reaction temperature profile described in Sec. 11.3.5 can be reproduced. The calibration settings are specific for the number and type of vessels and microwave system being used, in addition to the specific reagent combination being used. Therefore, no specific calibration settings are provided in this method. These settings may be developed by using temperature monitoring equipment for each specific set of microwave equipment and vessel type. They may be used as previously described in methods such as EPA Methods 3051, 3015, and 3052. In this circumstance, the microwave system provides programmable power, which can be programmed to within ± 12 W of the required power. Typical systems provide a nominal 600 W to 1200 W of power. Calibration control provides backward compatibility with older laboratory microwave systems which may not be equipped for temperature monitoring or feedback control and with lower cost microwave systems for some repetitive analyses. Older vessels with lower pressure capabilities may not be compatible (see refs. 1, 2, and 3 and the documents listed in 13.3.3 and 13.3.5).

6.1.2 The accuracy of the temperature measurement system should be periodically validated at an elevated temperature (see Section 12.2). This can be done using a container of silicon oil (a high temperature oil) and an external, calibrated temperature measurement system. The oil should be adequately stirred to ensure a homogeneous temperature, and both the microwave temperature sensor and the external temperature sensor placed into the oil. After heating the oil to a constant temperature of 180 ± 5 °C, the temperature should be measured using both sensors. If the measured temperatures vary by more than 1 to 2 °C, the microwave temperature measurement system should be calibrated. Consult the microwave manufacturer's instructions about the specific temperature sensor calibration procedure.

6.1.3 A rotating turntable is employed to ensure the homogeneous distribution of microwave radiation within the unit. The speed of the turntable should be a minimum of 3 rpm. Other types of equipment that are used to assist in achieving uniformity of the microwave field may also be appropriate.

6.2 Volumetric graduated cylinder, 50 or 100 mL capacity or equivalent.

6.3 Filter paper, qualitative or equivalent.

6.4 Filter funnel, glass, polypropylene, or other appropriate material.

6.5 Analytical balance, of appropriate capacity and resolution meeting data quality objectives.

7.0 REAGENTS

7.1 All acids should be sub-boiling distilled where possible to minimize the blank levels due to metallic contamination. Other grades may be used, provided it is first ascertained that the reagent is of sufficient purity to permit its use without decreasing the accuracy of the determination. If the purity of a reagent is questionable, the reagent should be analyzed to determine the level of impurities. The reagent blank must be less than the MDL in order to be used.

7.1.1 Concentrated nitric acid (HNO_3). The acid should be analyzed to determine levels of impurity. If the method blank is less than the MDL, the acid can be used.

7.1.2 Concentrated hydrochloric acid (HCl). The acid should be analyzed to determine levels of impurity. If the method blank is less than the MDL, the acid can be used.

7.2 Reagent Water. Reagent water shall be interference free. All references to water in the method refer to reagent water unless otherwise specified. For further details, consult the document listed in Sec. 13.3.3.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of SW-846. Refer to that chapter, as updated, for guidance.

8.2 All sample containers must be prewashed with acids and water, and metal-free detergents, if necessary, depending on the history of use of the container (Ref. 3). Plastic and glass containers are both suitable. For further information, see Chapter Three of SW-846.

8.3 Samples must be refrigerated upon receipt and analyzed as soon as possible.

9.0 QUALITY CONTROL

9.1 All quality control data must be maintained and available for reference or inspection for a period of three years. This method is restricted to use by, or under supervision of, experienced analysts.

9.2 Duplicate samples should be processed on a routine basis. A duplicate sample is a sample brought through the whole sample preparation and analysis process. A duplicate sample should be processed with each analytical batch or every 20 samples, whichever is the greater number. A duplicate sample should be prepared for each matrix type (i.e., soil, sludge, etc.).

9.3 Spiked samples or standard reference materials should be included with each group of samples processed, or every 20 samples, whichever is the greater number. A spiked sample should also be included whenever a new sample matrix is being analyzed.

9.4 Periodically, the accuracy of the temperature measurement system used to control the microwave equipment should be validated per Section 6.1.2.

9.5 (Not necessary if using temperature feedback control.) Each day that samples are extracted, the microwave-power calibration should be verified by heating 1 kg of ASTM Type II water (at 22 ± 3 °C) in a covered, microwave-transparent vessel for 2 min at the setting for 490 W and measuring the water temperature after heating per Section 10.1.5. If the power calculated (per Section 12) differs from 490 W by more than ± 10 W, the microwave settings should be recalibrated per Section 10.0.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Calibration of Microwave Equipment

NOTE: If the microwave unit uses temperature feedback control to control the performance specifications of the method, then performing the calibration procedure is not necessary.

10.1.1 Calibration is the normalization and reproduction of a microwave field strength to permit reagent and energy coupling in a predictable and reproducible manner. It balances reagent heating and heat loss from the vessels and is equipment dependent due to the heat retention and loss characteristics of the specific vessel. Available power is evaluated to permit the microwave field output in watts to be transferred from one microwave system to another.

Use of calibration to control this reaction requires balancing output power, coupled energy, and heat loss to reproduce the temperature heating profile given in Section 11.3.5. The conditions for each acid mixture and each batch containing the same specified number of vessels must be determined individually. Only identical acid mixtures and vessel models and specified numbers of vessels may be used in a given batch.

10.1.2 For cavity type microwave equipment, calibration is accomplished by measuring the temperature rise in 1 kg of water exposed to microwave radiation for a fixed period of time. The analyst can relate power in watts to the partial power setting of the system. The calibration format required for laboratory microwave systems depends on the type of electronic system used by the manufacturer to provide partial microwave power. Few systems have an accurate and precise linear relationship between percent power settings and absorbed power. Where linear circuits have been utilized, the calibration curve can be determined by a three-point calibration method (see Section 10.1.4). Otherwise, the analyst must use the multiple point calibration method (see Section 10.1.3). Assistance in calibration and software guidance of calibration are available in reference 3 and the document listed in Sec. 13.3.5.

10.1.3 The multiple point calibration involves the measurement of absorbed power over a large range of power settings. Typically, for a 600 W unit, the following power settings are measured: 100, 99, 98, 97, 95, 90, 80, 70, 60, 50, and 40% using the procedure described in Section 10.1.5. This data is clustered about the customary working power ranges. Non-linearity has been encountered at the upper end of the calibration. If the system's electronics are known to have nonlinear deviations in any region of proportional power control, it will be necessary to make a set of measurements that bracket the power to be used. The final calibration point should be at the partial power setting that will be used in the test. This setting should be checked periodically to evaluate the integrity of the calibration. If a significant change is detected (± 10 W), then the entire calibration should be re-evaluated.

10.1.4 The three-point calibration involves the measurement of absorbed power at three different power settings. Measure the power at 100% and 50% using the procedure described in Section 10.1.5. From this 2-point line, determine the partial power setting that corresponds to the power, in watts, specified in the procedure to reproduce the heating profile specified in Section 11.3.6. Measure the absorbed power at that partial power setting. If the measured absorbed power does not correspond to the specified power within ± 10 W, use the multiple point calibration in Section 10.1.3. This point should also be used to periodically verify the integrity of the calibration.

10.1.5 Equilibrate a large volume of water to room temperature (22 ± 3 °C). One kg of reagent water is weighed (1,000.0 ± 0.1 g) into a fluorocarbon beaker or a beaker made of some other material that does not significantly absorb microwave energy (glass absorbs microwave energy and is not recommended). The initial temperature of the water should be 22 ± 3 °C measured to ± 0.05 °C. The covered beaker is circulated continuously (in the normal sample path) through the microwave field for 2 minutes at the desired partial power setting with the system's exhaust fan on maximum (as it will be during normal operation). The beaker is removed and the water vigorously stirred. Use a magnetic stirring bar inserted immediately after microwave irradiation (irradiating with the stir bar in the vessel could cause electrical arcing) and record the maximum temperature within the first 30 seconds to ± 0.05 °C. Use a new sample for each additional measurement. If the water is reused (after making adjustments for any loss of weight due to heating), both the water and the beaker must have returned to 22 ± 3 °C. Three measurements at each power setting should be made.

The absorbed power is determined by the following relationship:

Equation 1

$$P=\frac{(K)(C_p)(m)(\Delta T)}{t}$$

Where:

P = the apparent power absorbed by the sample in watts (W) (joule/sec)

K = the conversion factor for thermochemical calories sec^{-1} to watts (K= 4.184)

C_p = the heat capacity, thermal capacity, or specific heat [cal/(g °C)] of water

m = the mass of the water sample in grams (g)

ΔT = the final temperature minus the initial temperature (°C)

t = the time in seconds (s)

Using the experimental conditions of 2 minutes (120 sec) and 1 kg (1000 g) of distilled water [heat capacity at 25 °C is 0.9997 cal/(g °C)] the calibration equation simplifies to:

Equation 2

$$P=(\Delta T)(34.86)$$

NOTE: Stable line voltage is necessary for accurate and reproducible calibration and operation. The line voltage should be within manufacturer's specification, and during measurement and operation should not vary by more than ± 2 V (Reference 3). Electronic components in most microwave units are matched to the system's function and output. When any part of the high voltage circuit, power source, or control components in the system have been serviced or replaced, it will be necessary to recheck the system's calibration. If the power output has changed significantly (± 10 W), then the entire calibration should be re-evaluated.

11.0 PROCEDURE

11.1 Temperature control of closed vessel microwave instruments provides the main feedback control performance mechanism for the method. Method control requires a temperature sensor in one or more vessels during the entire decomposition. The microwave decomposition system should sense the temperature to within ± 2.5 °C and permit adjustment of the microwave output power within 2 seconds.

11.2 All digestion vessels and volumetric ware must be carefully acid washed and rinsed with reagent water. When switching between high concentration samples and low concentration samples, all digestion vessels (fluoropolymer or quartz liners) should be cleaned by leaching with hot (1:1) hydrochloric acid (greater than 80 °C, but less than boiling) for a minimum of two hours followed by hot (1:1) nitric acid (greater than 80 °C, but less than boiling) for a minimum of two hours. The vessels should then be rinsed with reagent water and dried in a clean environment. This cleaning procedure should also be used whenever the prior use of the digestion vessels is unknown or cross contamination from prior sample digestions in vessels is suspected. Polymeric or glass volumetric ware and storage containers should be cleaned by leaching with more dilute acids (approximately 10% V/V) appropriate for the specific material used and then rinsed with reagent water and dried in a clean environment.

11.3 Sample Digestion

11.3.1 Weigh a well-mixed sample to the nearest 0.001 g into an appropriate vessel equipped with a controlled pressure relief mechanism. For soils, sediments, and sludges, use no more than 0.500 g. For oil or oil contaminated soils, initially use no more than 0.250 g. When large samples of oil are necessary, use of EPA Method 3052, which has sample scale-up options, is recommended. If the sample can not be well mixed and homogenized on an as received basis, then air or oven drying at 60°C or less, crushing, sieving, grinding, and mixing should be performed as necessary to homogenize the sample until the subsampling variance is less than the data quality objectives of the analysis. While proper sample preparation generally produces great reduction in analytical variability, be aware that in certain unusual circumstances there could be loss of volatile metals (e.g., Hg, organometallics) or irreversible chemical changes (e.g., precipitation of insoluble species, change in valence state). See Chapter Three for more details.

11.3.2 Add 10 ± 0.1 mL concentrated nitric acid or, alternatively, 9 ± 0.1 mL concentrated nitric acid and 3 ± 0.1 mL concentrated hydrochloric acid to the vessel in a fume hood (or fume exhausted enclosure). The addition of concentrated hydrochloric acid to the nitric acid is appropriate for the stabilization of certain analytes, such as Ag, Ba, and Sb and high concentrations of Fe and Al in solution. Improvements and optimal recoveries of antimony, iron, and silver from a variety of matrices upon addition of HCl are demonstrated in Section 17.0, in Figures 3 through 7. The addition of hydrochloric acid may, however, limit the detection techniques or increase the difficulties of analysis for some detection systems.

CAUTION: The addition of hydrochloric acid must be in the form of concentrated hydrochloric acid and not from a premixed combination of acids as a buildup of chlorine gas, as well as other gases, will result from a premixed acid solution. These gases may be violently released upon heating. This is avoided by adding the acid in the described manner.

CAUTION: Toxic nitrogen oxide(s) and chlorine fumes are usually produced during digestion. Therefore, all steps involving open or the opening of microwave vessels must be performed in a properly operating fume ventilation system.

CAUTION: The analyst should wear protective gloves and face protection.

CAUTION: The use of microwave equipment with temperature feedback control is required to control any unfamiliar reactions that may occur during the leaching of samples of unknown composition. The leaching of these samples may require additional vessel requirements such as increased pressure capabilities.

11.3.3 The analyst should be aware of the potential for a vigorous reaction, especially with samples containing volatile or easily oxidized organic species. When digesting a matrix of this type, initially use no more than 0.100 g of sample. If a vigorous reaction occurs upon the addition of reagent(s), allow the sample to predigest in the uncapped digestion vessel until the reaction ceases. Heat may be added in this step for safety considerations (for example, the rapid release of carbon dioxide from carbonates, easily oxidized organic matter, etc.). Once the initial reaction has ceased, the sample may continue through the digestion procedure. However, if no appreciable reaction occurs, a sample mass of up to 0.250 g for oils, or 0.500 g for solids, may be used.

11.3.4 Seal the vessel according to the manufacturer's directions. Properly place the vessel in the microwave system according to the manufacturer's recommended specifications and, when applicable, connect appropriate temperature and pressure sensors to vessels according to manufacturer's specifications.

11.3.5 This method is a performance based method, designed to achieve or approach consistent leaching of the sample through achieving specific reaction conditions. The temperature of each sample should rise to 175 ± 5 °C in approximately 5.5 ± 0.25 minutes and remain at 175 ± 5 °C for 4.5 minutes, or for the remainder of the ten minute digestion period (see Refs. 2, 3, and 4 and the document listed in 13.3.4). The time versus temperature and pressure profile is given for a standard sediment sample in Figure 2. When using temperature feedback control, the number of samples that may be simultaneously digested may vary, from one sample up to the maximum number of vessels that can be heated by the magnetron of the microwave unit according to the heating profile specified previously in this section. (The number will depend on the power of the unit, the number of vessels, and the heat loss from the vessels (Ref. 3)).

The pressure should peak between 5 and 10 minutes for most samples (see Refs. 1 and 2 and the document listed in 13.3.4). If the pressure exceeds the pressure limits of the vessel, the pressure should be safely and controllably reduced by the pressure relief mechanism of the vessel.

11.3.5.1 Calibration control is applicable in reproducing this method provided the power in watts versus time parameters are determined to reproduce the specifications listed in 11.3.5. The calibration settings will be specific to the quantity

of reagents, the number of vessels, and the heat loss characteristics of the vessels (see Ref. 3 and the document listed in Sec. 13.3.3). If calibration control is being used, any vessels containing acids for analytical blank purposes are counted as sample vessels. When fewer than the recommended number of samples are to be digested, the remaining vessels should be filled with the same acid mixture to achieve the full complement of vessels. This provides an energy balance, since the microwave power absorbed is proportional to the total absorbing mass in the cavity. Irradiate each group of vessels using the predetermined calibration settings. (Different vessel types should not be mixed).

11.3.6 At the end of the microwave program, allow the vessels to cool for a minimum of 5 minutes before removing them from the microwave system. Cooling of the vessels may be accelerated by internal or external cooling devices. When the vessels have cooled to near room temperature, determine if the microwave vessels have maintained their seal throughout the digestion. Due to the wide variability of vessel designs, a single procedure is not appropriate. For vessels that are sealed as discrete separate entities, the vessel weight may be taken before and after digestion to evaluate seal integrity. If the weight loss of sample exceeds 1% of the weight of the sample and reagents, then the sample is considered compromised. For vessels with burst disks, a careful visual inspection of the disk, in addition to weighing, may identify compromised vessels. For vessels with resealing pressure relief mechanisms, an auditory or a physical sign that can indicate whether a vessel has vented is appropriate.

11.3.7 Complete the preparation of the sample by carefully uncapping and venting each vessel in a chemical fume hood (or fume exhausted enclosure). Vent the vessels using the procedure recommended by the vessel manufacturer. Quantitatively transfer the sample to an acid-cleaned bottle. If the digested sample contains particulates which may clog nebulizers or interfere with injection of the sample into the instrument, the sample may be centrifuged (11.3.7.1), allowed to settle (11.3.7.2), or filtered (11.3.7.3).

11.3.7.1 Centrifugation: Centrifugation at 2,000 - 3,000 rpm for 10 minutes is usually sufficient to clear the supernatant.

11.3.7.2 Settling: If undissolved material, such as SiO_2, TiO_2, or other refractory oxides, remains, allow the sample to stand until the supernatant is clear. Allowing a sample to stand overnight will usually accomplish this. If it does not, centrifuge or filter the sample.

11.3.7.3 Filtering: If necessary, the filtering apparatus must be thoroughly cleaned and pre-rinsed with dilute (approximately 10% V/V) nitric acid. Filter the sample through qualitative filter paper into a second acid-cleaned container.

11.3.8 The removal or reduction of the quantity of the nitric and hydrochloric acids prior to analysis may be desirable. The chemistry and volatility of the analytes of interest should be considered and evaluated when using this alternative (Reference 3). Evaporation to near dryness in a controlled environment with controlled purge gas and neutralizing and collection of exhaust interactions is an alternative where appropriate. This manipulation may be performed in the microwave system, if the system is capable of this function, or external to the microwave system in more common apparatus(s). This option must be tested and validated to determine analyte retention and loss and should be accompanied by equipment validation possibly using the standard addition method and standard reference materials. This

alternative may be used to alter either the acid concentration and/or acid composition prior to analysis. (For further information, see reference 3 and Method 3052).

> NOTE: The final solution typically requires nitric acid to maintain appropriate sample solution acidity and stability of the elements. Commonly, a 2% (v/v) nitric acid concentration is desirable. Waste minimization techniques should be used to capture reagent fumes. This procedure should be tested and validated in the apparatus and on standards before using on unknown samples.

11.3.9 Transfer or decant the sample into volumetric ware and dilute the digest to a known volume. The digest is now ready for analysis for elements of interest using appropriate elemental analysis techniques and/or SW-846 methods.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Calculations: The concentrations determined are to be reported on the basis of the actual weight of the original sample.

12.2 Prior to use of the method, verify that the temperature sensing equipment is properly reading temperature. A procedure for verification is given in Section 6.1.2. This will establish the accuracy and precision of the temperature sensing equipment, which should be carried throughout the statistical treatment of the quality assurance data.

12.3 In calibrating the microwave unit (Section 10.0), the power absorbed (for each power setting) by 1 kg of reagent water exposed to 120 seconds of microwave energy is determined by the expression

$$\text{Power (in watts)} = (T_1 - T_2)\ (34.86)$$

where: T_1 = Initial temperature of water (between 21 and 25 °C to nearest 0.1 °C)

T_2 = Final temperature of water (to nearest 0.1 °C)

12.4 Plot the power settings against the absorbed power (calculated in Section 12.3) to obtain a calibration relationship. Alternatively, use a microwave calibration program to analyze the calibration data (see Ref. 3 and the document listed in Sec. 13.3.5). Interpolate the data to obtain the instrument settings needed to provide the wattage levels specified in Section 12.3.

12.5 Calculate the sample dry-weight fraction as follows:

$$\text{Dry-Wt fraction} = \frac{(W_2) - (W_3)}{(W_1) - (W_3)}$$

where: W_1 = Wt for sample + vessel, before drying, g

W_2 = Wt for sample + vessel, after drying, g

W_3 = Wt for empty, dry vessel, g

12.6 Convert the extract concentration obtained from the instrument in mg/L to mg/kg dry-weight of sample by:

$$\text{Sample concentration} = \frac{(C)\,(V)\,(D)}{(W)\,(S)}$$

where: C = Concentration in extract (mg/L)

D = Dilution factor

S = Solid dry-weight fraction for sample, g/g

V = Volume of extract, mL x 0.001

W = Weight of undried sample extracted, g x 0.001

13.0 METHOD PERFORMANCE

13.1 The fundamental chemical basis of Method 3051 with and without HCl has been compared with Method 3050 in several sources (see 13.3.4 and 13.3.5). Several papers have evaluated the leachability of NIST SRMs with this method (Ref. 1 and Sec. 13.3.5). Evaluations and optimizations of this method are being published (Ref. 5 and 6) as well as additional leaches performed on more matrices, which will be addressed in future literature papers. Method 3051 has been determined to be appropriate for enhancing recoveries of certain analytes. This data is contained in Section 17 of this method. Matrices tested include SRM 2710 (Montana Soil - Highly Elevated Concentrations), SRM 2704 (Buffalo River Sediment), and SRM 1084a (Wear Metals in Oil). Analytes demonstrating better recoveries upon addition of HCl include antimony, iron, and silver.

13.2 The following documents may provide additional guidance and insight on this method and technique:

13.2.1 Kingston, H. M. and L. B. Jassie, "Safety Guidelines for Microwave Systems in the Analytical Laboratory". In Introduction to Microwave Acid Decomposition: Theory and Practice; Kingston, H.M. and Jassie, L.B., eds.; ACS Professional Reference Book Series; American Chemical Society: Washington, DC, 1988.

13.2.2 1985 Annual Book of ASTM Standards, Vol. 11.01; "Standard Specification for Reagent Water" ; ASTM, Philadelphia, PA, 1985, D1193-77.

13.3.3 Introduction to Microwave Sample Preparation: Theory and Practice, Kingston, H.M. and Jassie, L.B., Eds.; ACS Professional Reference Book Series; American Chemical Society: Washington, DC, 1988.

13.3.4 Kingston, H.M., Walter, P.J., "Comparison of Microwave Versus Conventional Dissolution for Environmental Applications", Spectroscopy, Vol. 7 No. 9, 20-27, 1992.

13.3.5 Walter, P. J. Special Publication IR4718: *Microwave Calibration Program*, 2.0 ed.; National Institutes of Standards and Technology: Gaithersburg, MD, 1991.

13.3.6 Kingston, H.M., Walter, P.J., Chalk, S.J., Lorentzen, E.M., Link, D.D., "Environmental Microwave Sample Preparation: Fundamentals, Methods, and Applications". In Microwave Enhanced Chemistry: Fundamentals, Sample Preparation, and Applications; ACS Professional Reference Book Series; American Chemical Society: Washington, DC 1997.

14.0 POLLUTION PREVENTION

14.1 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 operations. 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.

14.2 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 Relations and Science Policy, 1155 16th Street, NW, Washington, DC 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits 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's Department of Government Relations and Science Policy, 1155 16th Street, NW, Washington, DC 20036, (202) 872-4477.

16.0 REFERENCES

1. Kingston, H.M. EPA IAG #DWI-393254-01-0 January 1-March 31, 1988, quarterly report.

2. Binstock, D.A., Yeager, W.M., Grohse, P.M. and Gaskill, A. Validation of a Method for Determining Elements in Solid Waste by Microwave Digestion, Research Triangle Institute Technical Report Draft, RTI Project Number 321U-3579-24, November, 1989, prepared for the Office of Solid Waste, U.S. Environmental Protection Agency, Washington, DC 20460.

3. Kingston, H.M., Haswell, S, Microwave Enhanced Chemistry: Fundamentals, Sample Preparation, and Applications; ACS Professional Reference Book Series; American Chemical Society: Washington, DC 1997.

4. Binstock, D.A., Grohse, P.M., Gaskill, A., Sellers, C., Kingston, H.M., Jassie, L.B., "Development and Validation of a Method for Determining Elements in Solid Waste using Microwave Digestion", J. Assoc. Off. Anal. Chem., Vol. 74, 360 - 366 , 1991.

5. Kingston, H.M., Walter, P.J., Lorentzen, E.M.L., Lusnak, G.P. "The Performance of Leaching Studies on Soil SRM's 2710 and 2711"; Final Report to the National Institute of Standards and Technology, Duquesne University: Pittsburgh, PA, April 5, 1994.

6. Link, D.D., Kingston, H.M., Walter, P.J., "Development and Validation of the New EPA Microwave-assisted Leach Method 3051A".

7. Link, DD., Kingston, H.M., Walter, P.J.; *Development and Validation of the EPA Microwave-assisted Methods 3015A and 3051A:* Validation Studies for Updated Microwave Leach Methods, Proceedings for the Waste Testing and Quality Assurance Symposium; July 1997.

8. Kingston, H.M., Walter, P.J., "Comparison of Microwave verses Conventional Dissolution for Environmental Applications", Spectroscopy, Vol. 7 No. 9, 20-27, 1992.

9. Walter, P.J. Special Publication IR7718: Microwave Calibration Program, 2.0 ed.; National Institute of Standards and Technology: Gaithersburg, MD, 1991.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain Tables 1 through 3, Figures 1 through 7, and a flow diagram of method procedure.

TABLE 1

COMPARISON OF ANALYTE RECOVERIES FROM SRM 2704 (BUFFALO RIVER SEDIMENT) USING BOTH DIGEST OPTIONS OF METHOD 3051 (Refs. 5, 6)

Element	10 mL HNO_3 digest	9 mL HNO_3 + 3 mL HCl digest	Total Analyte Concentration
Cd	3.40 ± 0.34	3.62 ± 0.17	3.45 ± 0.22
Cr	84.7 ± 5.6	77.1 ± 12.6	135 ± 5
Ni	45.5 ± 5.9	42.2 ± 3.2	44.1 ± 3.0
Pb	163 ± 9	161 ± 17	161 ± 17

Results reported in µg/g analyte (mean ± 95% confidence limit).
Total concentrations are taken from NIST SRM Certificate of Analysis.

TABLE 2

COMPARISON OF ANALYTE RECOVERIES FROM SRM 4355 (PERUVIAN SOIL) USING BOTH DIGEST OPTIONS OF METHOD 3051 (Ref. 6).

Element	10 mL HNO_3 digest	9 mL HNO_3 + 3 mL HCl digest	Total Analyte Concentration
Cd	0.86 ± 0.16	0.85 ± 0.17	(1.50)
Cr	14.6 ± 0.47	19.0 ± 0.69	28.9 ± 2.8
Ni	9.9 ± 0.33	11.2 ± 0.44	(13)
Pb	124 ± 5.3	130 ± 3.6	129 ± 26

Results reported in µg/g analyte (mean ± 95% confidence limit).
Total concentrations are taken from NIST SRM Certificate of Analysis.
Values in parenthesis are reference concentrations.

TABLE 3

COMPARISON OF ANALYTE RECOVERIES FROM SRM 1084A (WEAR METALS IN OIL) USING BOTH DIGEST OPTIONS OF METHOD 3051 (Ref. 6)

Element	10 mL HNO_3 digest	9 mL HNO_3 + 3 mL HCl digest	Total Analyte Concentration
Cu	91.6 ± 4.0	93.0 ± 2.6	100.0 ± 1.9
Cr	91.2 ± 3.3	94.3 ± 3.1	98.3 ± 0.8
Mg	93.2 ± 3.6	93.5 ± 2.8	99.5 ± 1.7
Ni	91.6 ± 3.9	92.9 ± 3.4	99.7 ± 1.6
Pb	104 ± 4.1	99.5 ± 5.1	101.1 ± 1.3

Results reported in µg/g analyte (mean ± 95% confidence limit).
Total concentrations are taken from NIST SRM Certificate of Analysis.

FIGURE 1

TEMPERATURE AND PRESSURE PROFILES FOR THE HEATING OF DIFFERENT RATIOS OF NITRIC ACID TO HYDROCHLORIC ACID USING METHOD 3051

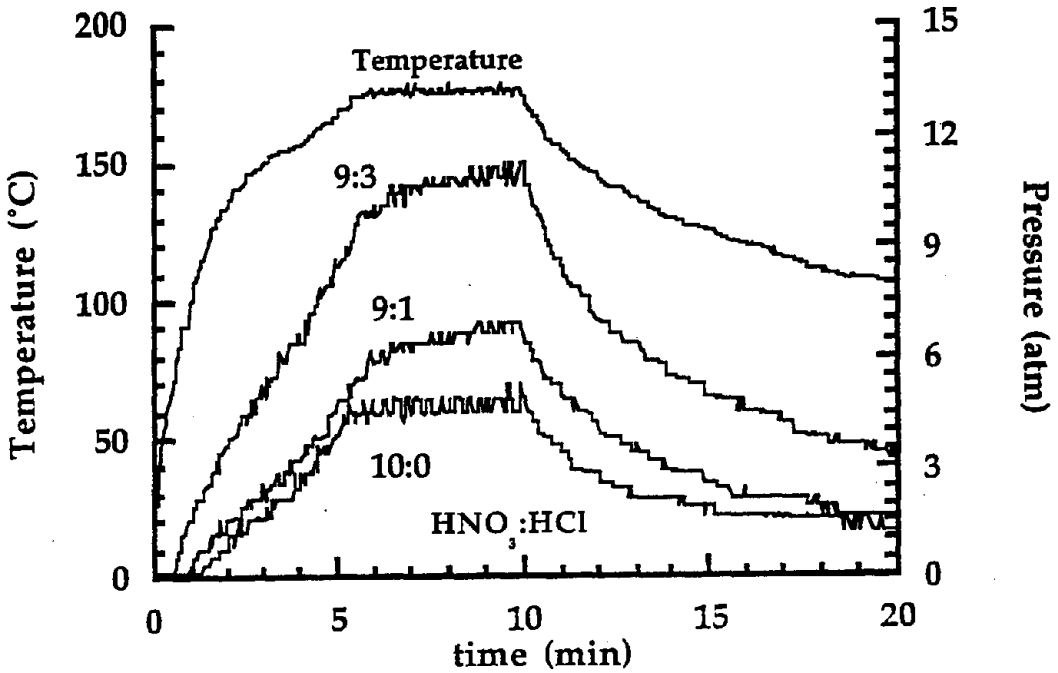


FIGURE 2

TEMPERATURE AND PRESSURE PROFILE FOR THE EXTRACTION AND DISSOLUTION OF NIST SRM 2704 (BUFFALO RIVER SEDIMENT) USING DIFFERENT RATIOS OF NITRIC ACID TO HYDROCHLORIC ACID

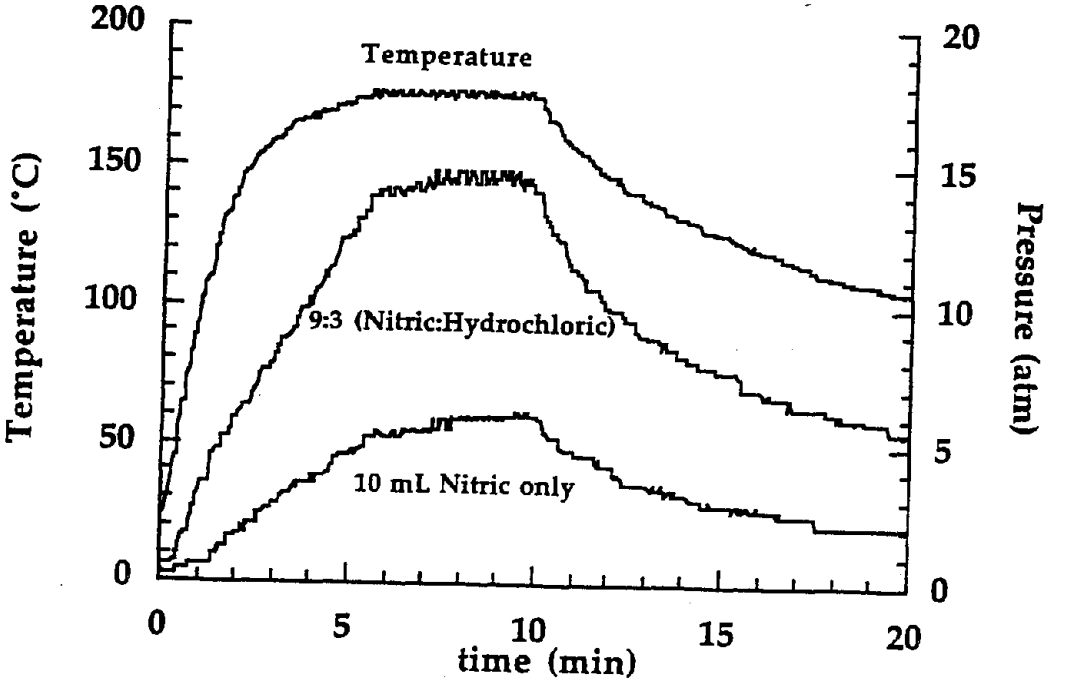


FIGURE 3

PERCENT RECOVERY OF ANTIMONY FROM NIST SRM 2710 (MONTANA SOIL) VERSUS VARIOUS COMBINATIONS OF NITRIC AND HYDROCHLORIC ACIDS (N=6) (Refs. 6, 7)

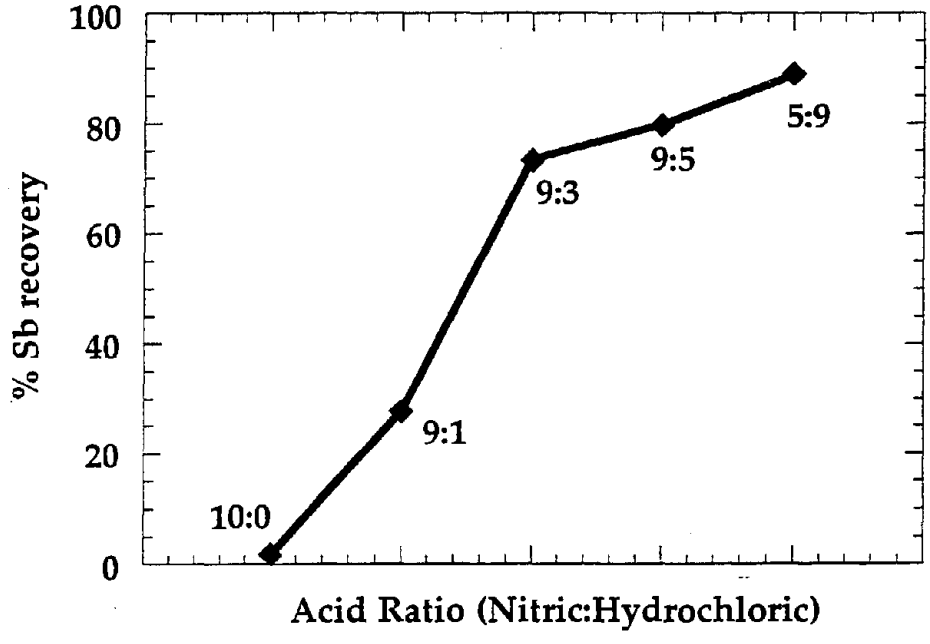


FIGURE 4

PERCENT RECOVERY OF ANTIMONY FROM NIST SRM 2704 (BUFFALO RIVER SEDIMENT) VERSUS VARIOUS COMBINATIONS OF NITRIC AND HYDROCHLORIC ACIDS (N=6) (Refs. 6, 7).

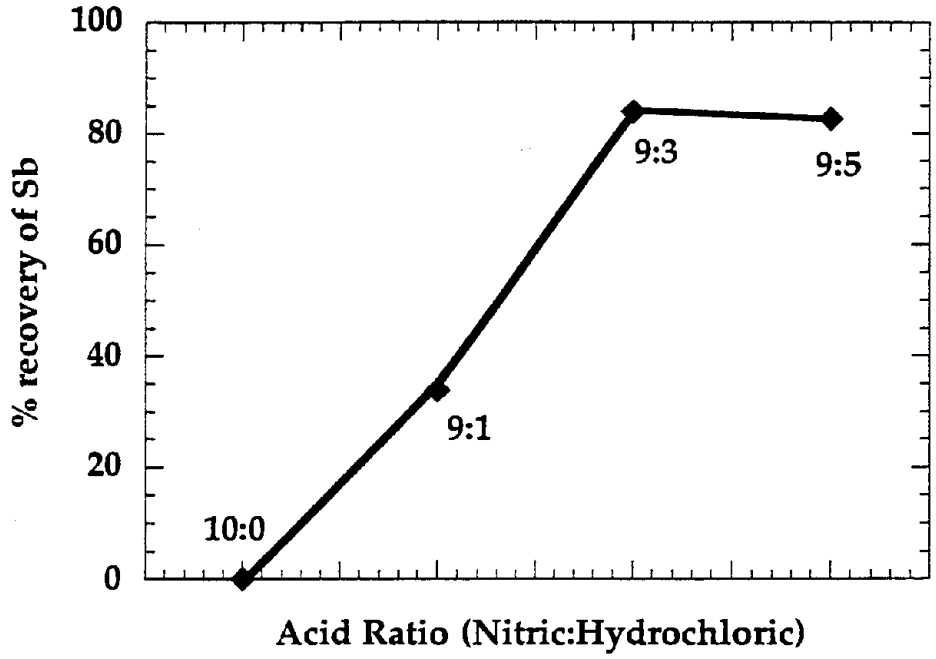


FIGURE 5

PERCENT RECOVERY OF IRON FROM NIST SRM 2704 (BUFFALO RIVER SEDIMENT) VERSUS VARIOUS COMBINATIONS OF NITRIC AND HYDROCHLORIC ACIDS (N=6) (Refs. 6, 7).

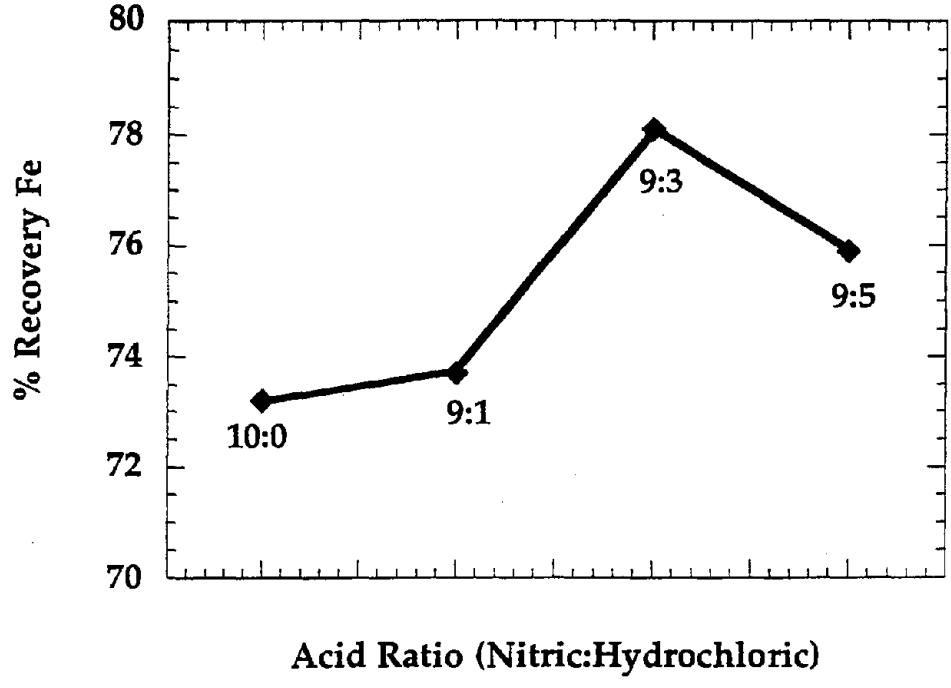


FIGURE 6

PERCENT RECOVERY OF SILVER FROM NIST SRM 2710 (MONTANA SOIL) VERSUS VARIOUS COMBINATIONS OF NITRIC AND HYDROCHLORIC ACIDS (N=6) (Refs. 6, 7)

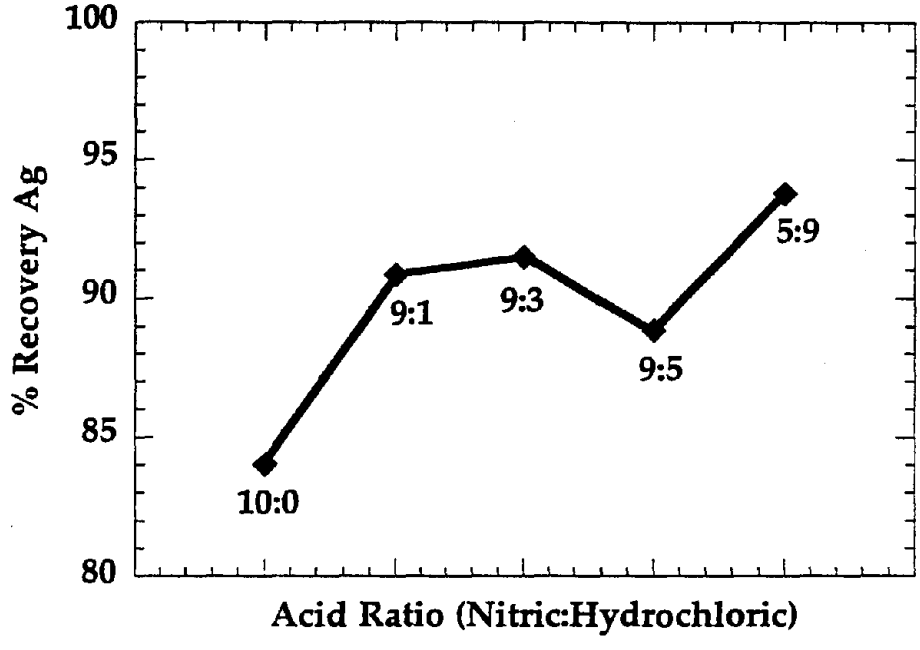
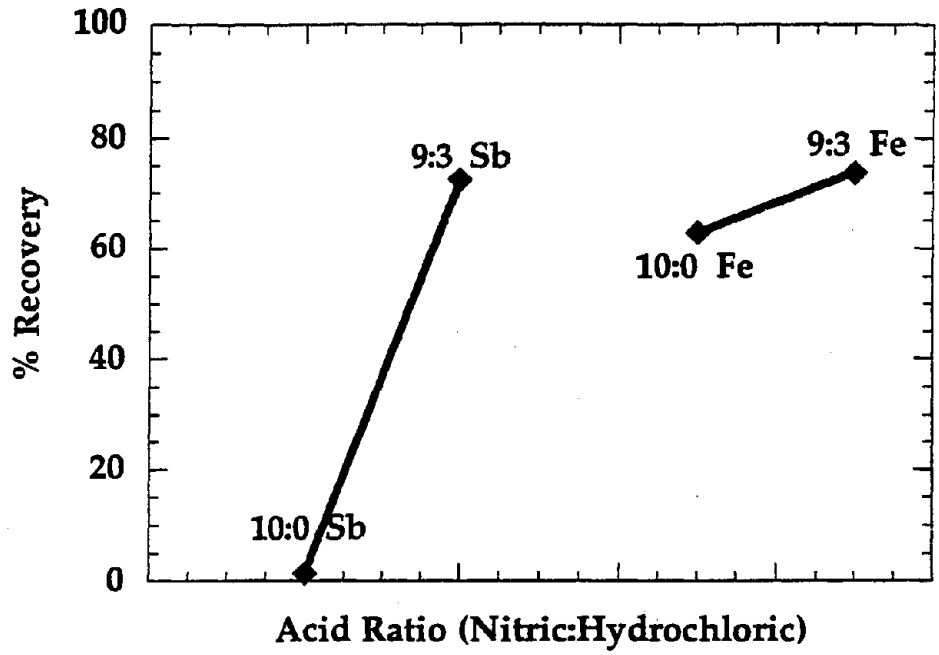
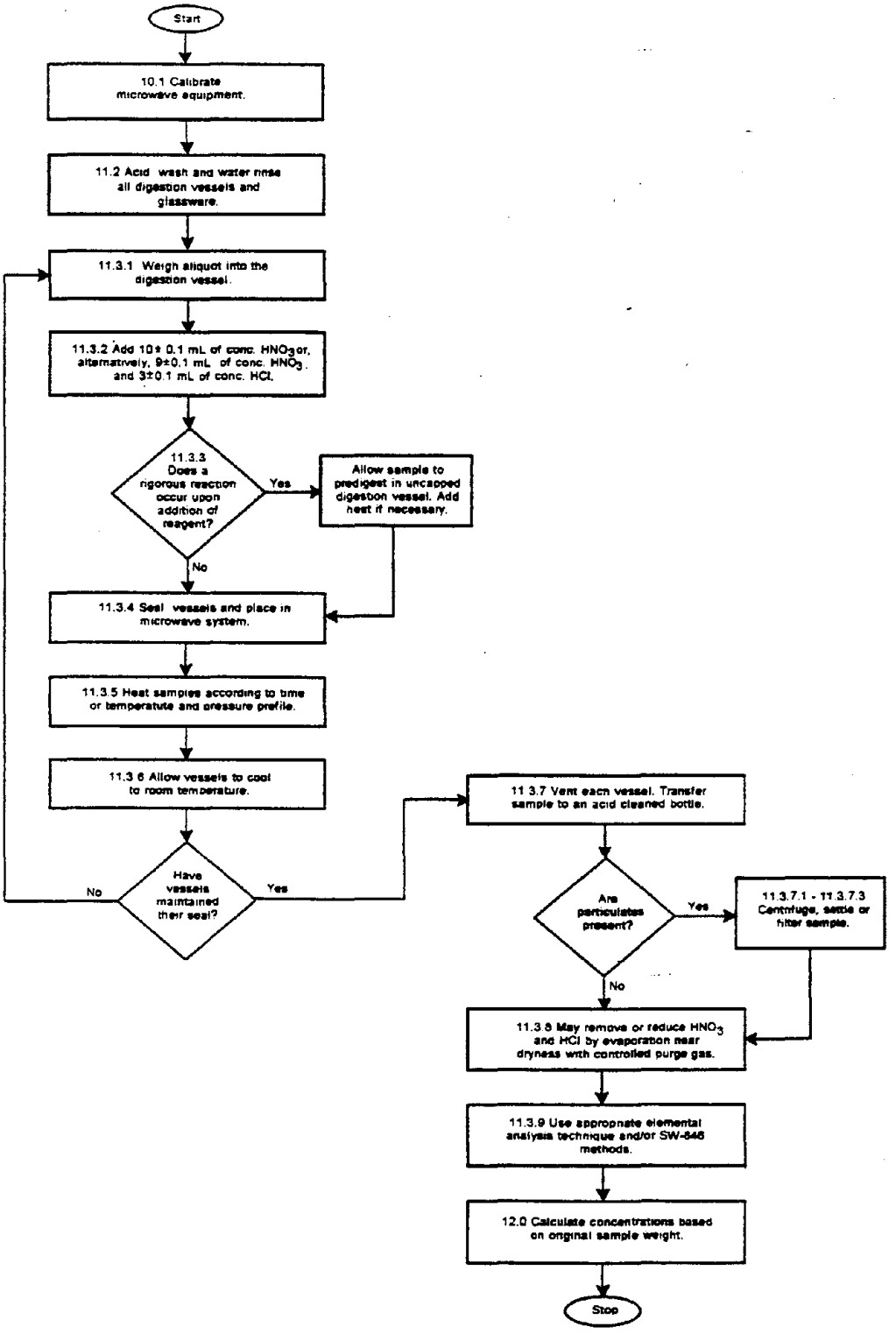


FIGURE 7

PERCENT RECOVERY OF ANTIMONY AND IRON, RESPECTIVELY, FROM SRM 4355 (PERUVIAN SOIL) USING BOTH DIGEST OPTIONS (10 ML HNO_3 AND 9 ML HNO_3 + 3 ML HCL) OF METHOD 3051 (N=6) (Refs. 6, 7)



MICROWAVE ASSISTED ACID DIGESTION OF SEDIMENTS, SLUDGES, SOILS, AND OILS



3.3 METHODS FOR DETERMINATION OF INORGANIC ANALYTES

This section of the manual contains analytical techniques for trace inorganic analyte determinations. Examples of the techniques included in this section are: inductively coupled argon plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), direct-aspiration or flame atomic absorption spectrophotometry (FLAA), graphite-furnace atomic absorption spectrophotometry (GFAA), hydride-generation atomic absorption spectrometry (HGAA), cold-vapor atomic absorption spectrometry (CVAA), and several procedures for hexavalent chromium analysis. Each of these is briefly discussed below in terms of advantages, disadvantages, and cautions for analysis of wastes.

ICP allows simultaneous or rapid sequential determination of many elements in a short time. A primary disadvantage of ICP is the occurrance of background radiation from other elements and the plasma gases. Although all ICP instruments utilize high-resolution optics and back-ground correction to minimize these interferences, analysis for traces of inorganic analytes in the presence of a large excess of a single analyte is difficult. Examples would be traces of inorganic analytes in an alloy or traces of metals in a limed (high calcium) waste. ICP and Flame AA have comparable detection limits (within a factor of 4) except that ICP exhibits greater sensitivity for refractories (Al, Ba, etc.). Furnace AA, in general, will exhibit lower detection limits than either ICP or FLAA. Detection limits are drastically improved when ICP-MS is used. In general ICP-MS exhibits greater sensitivity than either GFAA or FLAA for most elements. The greatest disadvantage of ICP-MS is isobaric elemental interferences. These are caused by different elements forming atomic ions with the same nominal mass-to-charge ratio. Mathematical correction for interfering ions can minimize these interferences.

Flame AAS (FLAA) direct aspiration determinations, as opposed to ICP, are normally completed as single element analyses and are relatively free of interelement spectral interferences. Either a nitrous-oxide/acetylene or air/acetylene flame is used as an energy source for dissociating the aspirated sample into the free atomic state, making analyte atoms available for absorption of light. In the analysis of some elements, the temperature or type of flame used is critical. If the proper flame and analytical conditions are not used, chemical and ionization interferences can occur.

Graphite furnace AAS (GFAA) replaces the flame with an electrically heated graphite furnace. The furnace allows for gradual heating of the sample aliquot in several stages. Thus, the processes of dissolution, drying, decomposition of organic and inorganic molecules and salts, and formation of atoms which must occur in a flame or ICP in a few milliseconds may be allowed to occur over a much longer time period and at controlled temperatures in the furnace. This allows an experienced analyst to remove unwanted matrix components by using temperature programming and/or matrix modifiers. The major advantage of this technique is that it affords extremely low detection limits. It is the easiest to perform on relatively clean samples. Because this technique is so sensitive, interferences can be a real problem; finding the optimum combination of digestion, heating times and temperatures, and matrix modifiers can be a challenge for complex matrices.

Hydride AA utilizes a chemical reduction to reduce and separate arsenic or selenium selectively from a sample digestate. The technique therefore has the advantage of being able to isolate these two elements from complex samples which may cause interferences for other analytical procedures. Significant interferences have been reported when any of the following is present: (1) easily reduced metals (Cu, Ag, Hg); (2) high concentrations of transition metals (>200 mg/L); (3) oxidizing agents (oxides of nitrogen) remaining following sample digestion.

Cold-Vapor AA uses a chemical reduction to reduce mercury selectively. The procedure is extremely sensitive but is subject to interferences from some volatile organics, chlorine, and sulfur compounds.

Prior to employing the above methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgements necessary to meet the data quality objectives or needs for the intended use of the data.

The following methods are included in this section:

Method 4500:	Mercury in Soil by Immunoassay
Method 6010B:	Inductively Coupled Plasma-Atomic Emission Spectrometry
Method 6020A:	Inductively Coupled Plasma - Mass Spectrometry
Method 6200:	Field Portable X-Ray Fluorescence Spectrometry for the Determination of Elemental Concentrations in Soil and Sediment
Method 6500:	Dissolved Inorganic Anions in Aqueous Matrices by Capillary Ion Electrophoresis
Method 6800:	Elemental and Speciated Isotope Dilution Mass Spectrometry
Method 7000B:	Flame Atomic Absorption Spectrophotometry
Method 7010:	Graphite Furnace Atomic Absorption Spectrophotometry
Method 7061A:	Arsenic (Atomic Absorption, Gaseous Hydride)
Method 7062:	Antimony and Arsenic (Atomic Absorption, Borohydride Reduction)
Method 7063:	Arsenic in Aqueous Samples and Extracts by Anodic Stripping Voltammetry (ASV)
Method 7195:	Chromium, Hexavalent (Coprecipitation)
Method 7196A:	Chromium, Hexavalent (Colorimetric)
Method 7197:	Chromium, Hexavalent (Chelation/Extraction)
Method 7198:	Chromium, Hexavalent (Differential Pulse Polarography)
Method 7199:	Determination of Hexavalent Chromium in Drinking Water, Groundwater and Industrial Wastewater Effluents by Ion Chromatography
Method 7470A:	Mercury in Liquid Waste (Manual Cold-Vapor Technique)
Method 7471B:	Mercury in Solid or Semisolid Waste (Manual Cold-Vapor Technique)
Method 7472:	Mercury in Aqueous Samples and Extracts by Anodic Stripping Voltammetry (ASV)
Method 7473:	Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry
Method 7474:	Mercury in Sediment and Tissue Samples by Atomic Fluorescence Spectrometry
Method 7580:	White Phosphorus (P_4) by Solvent Extraction and Gas Chromatography
Method 7741A:	Selenium (Atomic Absorption, Gaseous Hydride)
Method 7742:	Selenium (Atomic Absorption, Borohydride Reduction)
Method 9000:	Determination of Water in Waste Materials by Karl Fischer Titration
Method 9001:	Determination of Water in Waste Materials by Quantitative Calcium Hydride Reaction

METHOD 4500

MERCURY IN SOIL BY IMMUNOASSAY

1.0 SCOPE AND APPLICATION

1.1 This method provides a screening procedure for the determination of mercury in soils at concentrations as low as 0.5 mg/Kg.

1.2 This procedure describes the analysis of soil samples for the detection of mercury by an Enzyme-Linked Immunosorbent Assay (ELISA). This method is conducted as a screening technique, the sample's concentration is estimated through comparison of the sample to a standard. Other solid matrices (see Table 5) may be analyzed by this technique as long as the QC parameters detailed in this method are achievable.

1.3 In conjunction with this technique, Method 7471 should be used to determine the exact concentration of mercury when required or for confirmatory purposes. This is especially true near regulatory or action levels. A minimum of 10% confirmatory analyses is suggested.

1.4 This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method.

1.5 Tubes or reagents from different kits or separate batches are NOT interchangeable.

2.0 SUMMARY OF THE METHOD

2.1 Test kits are commercially available for this method. The manufacturer's directions should be followed.

2.2 In general, the method is performed using an extract of a solid sample. Solid samples are prepared by extraction with a mixture of hydrochloric and nitric acids for ten minutes and then buffered prior to analysis. The sample is added to a tube (treated with BSA-glutathione) and incubated at ambient temperatures for five minutes. The mercuric ions bound to the sulfihydryl groups of the BSA-glutathione are now reacted with a reconstituted antibody specific for mercury and incubated for five more minutes. A peroxidase conjugate is added to the sample, reacting with any mercury specific antibody. The substrate is then added forming a color that is in proportion to the amount of mercury originally present in the sample. The color produced is then spectrophotometrically compared with the control standards.

3.0 DEFINITIONS

3.1 Antibody - A binding protein which is produced in response to an antigen, and which has the ability to bond with the antigen that stimulated its production.

3.2 Cross-Reactivity - The relative concentration of an untargeted substance that would produce a response equivalent to a specified concentration of the targeted compound. In a semi-quantitative immunoassay, it provides an indication of the concentration of cross-reactant that would produce a positive response. Cross-reactivity for individual compounds is often calculated as the ratio of target substance concentration to the cross-reacting substance concentration at 50% inhibition of the immunoassay's maximum signal x 100%.

3.3 Dose-Response Curve - Representation of the signal generated by an immunoassay (y axis) plotted against the concentration of the target compound (x axis) in a series of standards of known concentration. When plotting a competitive immunoassay in a rectilinear format, the dose-response will have a hyperbolic character. When the $\log_{10}$ of concentration is used, the plot assumes a sigmoidal shape, when the log of signal is plotted against the logit transformation (linear representation of calibration data) of concentration, a straight line is produced.

3.4 ELISA - *Enzyme Linked Immunosorbent Assay* is an enzyme immunoassay method that uses an immobilized reagent (e.g., antibody adsorbed to a plastic tube) to facilitate the separation of targeted analytes (antibody-bound components) from non-targeted substances (free reaction components), using a washing step and an enzyme conjugate to generate the signal used for the interpretation of results.

3.5 Enzyme Conjugate - A molecule produced by the coupling of an enzyme molecule to an immunoassay component that is responsible for acting upon a substrate to produce a detectable signal.

3.6 Enzyme Immunoassay - An immunoassay method that uses an enzyme conjugate reagent to generate the signal used for interpretation of results. The enzyme mediated response may take the form of a chromogenic, fluorogenic, chemiluminescent or potentiometric reaction. (see Immunoassay and ELISA)

3.7 False Negatives - A negative interpretation of the sample containing the target analytes at or above the action level. Ideally, an immunoassay test product should produce no false negatives. The maximum permissible false negative rate is 5%, as measured by analyzing split samples using both the test product and a reference method.

3.8 False Positives - A positive interpretation for a sample is defined as a positive response for a sample that contains analytes below the action level.

3.9 Immunoassay - An analytical technique that uses an antibody molecule as a binding agent in the detection and quantitation of substances in a sample. (see Enzyme Immunoassay and ELISA)

3.10 Immunogen - A substance having a minimum size and complexity, and that is sufficiently foreign to a genetically competent host to stimulate an immune response.

4.0 INTERFERENCES

4.1 Refer to Table 3 for a comparison of the effects of other metals on the procedure.

4.2 Consult the information provided by the manufacturer of the kit used for additional information regarding cross reactivity with other compounds.

4.3 Temperature range in which test can be reliably conducted (refer to test-kit instructions).

5.0 SAFETY

Refer to Chapter Three for guidance.

6.0 EQUIPMENT AND SUPPLIES

6.1 BiMelyze® Soil Extraction Kit and BiMelyze® Mercury Assay Tube Kit for Solid Matrices (BioNebraska, Inc. 3820 NW 46th St., Lincoln, NE 68524) or equivalent. Each commercially available test kit will supply or specify the apparatus and materials necessary for successful completion of the test.

6.2 Analytical balance - capable of measuring 5 g ± 0.1 g.

6.3 Differential photometer or equivalent - capable of reading the absorbance at 405 nm.

6.4 Timer.

6.5 Permanent marking pen.

6.6 Cleaning and waste supplies - lab tissues, disposable gloves, waste container.

6.7 Micropipets - capable of accurate delivery volumes at 105 and 500 µL.

6.8 Squirt bottle - 500 mL or equivalent.

6.9 Graduated cylinder - 500 mL or equivalent.

7.0 REAGENTS AND STANDARDS

7.1 Each commercially available test kit will supply or specify the reagents necessary for successful completion of the test.

7.2 Nitric acid, concentrated - reagent grade or equivalent.

7.3 Hydrochloric acid, concentrated - reagent grade or equivalent.

7.4 Acid mixture - Add 36 mL HCl to 18 mL of reagent water and then add 18 mL of HNO_3 to the HCl/reagent water solution.

7.5 Reagent water - All references to water in this method refer to reagent water unless otherwise specified. Reagent grade water is defined in Chapter One.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Environmental samples may be contaminated, and should therefore be considered hazardous and handled accordingly.

8.2 All test kits must be stored under the conditions described by the manufacturer.

8.3 Sample Collection - Sufficient sample should be collected to ensure a representative sample. Samples should be collected in pre-cleaned glass or plastic containers.

8.4 All samples that are not immediately analyzed must be stored under the conditions described in Chapter Three.

9.0 QUALITY CONTROL

9.1 Follow the manufacturer's instructions for the test kit being used for quality control procedures specific to the test kit used. Additionally, guidance provided in Method 4000 and Chapter One should be followed.

9.2 Use of replicate analyses, particularly when results indicate concentrations near the action level, is recommended to refine information gathered with the tube kit.

9.3 Do not use test kits past their expiration date.

9.4 Do not use tubes or reagents designated for use with other manufacturer's test kits and **do not use tubes or reagents from separate batches of test kits.**

9.5 Use the test kits within their specified storage temperature and operating temperature limits.

9.6 Although Method 4500 is intended as a field screening method, the appropriate level of quality assurance should accompany the application of this method to document data quality. These include but are not limited to positive and negative controls.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Follow the instrument manufacturer's instruction when conducting the calibration.

10.2 All analyses must be accompanied by at least a reference standard (NIST 2709, NIST 2704, or equivalent).

10.3 All analyses must be accompanied by at least one control. Additional controls may be used to refine the data.

11.0 PROCEDURE

11.1 Follow all of the manufacturer's specific instruction when conducting analyses by the immunoassay technique. A general overview of the technique follows.

11.2 Prepare all assay solutions, standards, and controls prior to beginning the analysis. Appropriately label all vials.

11.3 Soil Extraction

11.3.1 Weigh out 5 ± 0.1 g of soil sample and place into the extraction vessel.

11.3.2 Add a 4 mL volume of the acid mixture to the extraction vessel for all samples, standards, and controls.

11.3.3 Cap the vessels and swirl the samples for 15 seconds of each minute during the 10-minute extraction period and then add 7 mL of the buffer included with the test-kit.

11.3.4 Place bottle filter tops firmly onto extraction bottles. Squeeze bottle and discard the first few drops. Add three drops (105 µL) into the corresponding dilution bottles.

11.4 Sample Analysis

11.4.1 Add diluted samples to the black line on each mercury assay tube (500 µL) Incubate tubes for five minutes at ambient temperature. Occasionally swirl and after five minutes empty and then rinse tubes three times with approximately 2 mL of reagent water.

11.4.2 Add antibody to the black line on each mercury assay tube (500 µL), incubate for 5 minutes at ambient temperature, wash and rinse tubes with buffer three times.

11.4.3 Add conjugate to the black line on each mercury tube (500 µL), incubate for 5 minutes at ambient temperature, wash and rinse tubes with buffer three times.

11.4.4 Add substrate to the black line on each mercury tube (500 µL), and incubate for 5 minutes at ambient temperature. Add three drops (105 µL) of stop solution to each tube in the same order as the substrate was added.

11.4.5 Read absorbance of each sample and standard at 405 nm.

11.4.5.1 Samples with absorbances less than that of a control should be reported as "<[control concentration]." For example, if the control is 4 ppm and the sample's absorbance is less than that of the 4 ppm control then the result should be reported as "<4 ppm."

11.4.5.2 Samples with absorbances greater than that of a control should be reported as ">[control concentration]." For example, if the control is 4 ppm and the sample's absorbance is greater than that of the 4 ppm control then the result should be reported as ">4 ppm."

11.4.5.3 Results may be bracketed between two controls to further refine the data. For example, if a 4 ppm control and a 20 ppm control are used, potentially three results are possible: <4 ppm, 4-20 ppm, and >20 ppm.

12.0 DATA ANALYSIS AND CALCULATIONS

Ensure that all sample containers, dilution bottles, extraction vials, and tubes have been labeled properly prior to analysis. Proper laboratory protocols, including documentation and notetaking, should be conducted according to good laboratory practices.

13.0 METHOD PERFORMANCE

13.1 Table 1 displays data obtained from analyzing samples using both the tube kit and CVAA at a Superfund Site. Sixty-nine samples were analyzed, nine samples analyzed by the tube kit had results greater than that of the CVAA analyses. This gives a false positive rate of 13%. Two of the samples analyzed by the tube kit gave results lower than the CVAA analyses. This gives a false negative result of 2.8%.

13.2 Table 2 displays data concerning false positives and false negatives at the detection limit of 0.5 ppm. Twenty samples were prepared using NIST Standard Reference Material 8407 (soil standard) diluted gravimetrically with mercury free soils to levels of 1.0, 0.50, 0.25, and 0.00 ppm. Four sets of the soil samples were extracted in five separate experiments. Within each of the five experiments, a 0.5 ppm sample that gave an absorbance greater than that of the 0.5 ppm standard would be considered a false positive. An absorbance greater than that of a 0.5 ppm standard would

be determined to be a false negative. None of the experiments yielded false positives or false negative results.

13.3 Table 3 displays the effects of other metals on the quantitation of mercury by ELISA and the concentration at which cross-reactivity may be observed. Elements with values designated "as greater than" displayed no interference at the levels shown in the table.

13.4 Table 4 provides comparison of the tube kit with eight different certified reference materials.

13.5 Table 5 provides data for a set of analyses conducted at an abandoned battery reclamation site.

13.6 Figure 1 displays the tube-kit's 10 month stability claims at 4°C with a control (fresh kit every analysis period), a kit stored at room temperature (22-25°C), and a kit stored at 4°C.

13.7 The MDL at the 99% confidence level was derived from the data in Table 2. The standard deviation of the lowest standard analyzed (0.067 = SD for the 0.25 ppm standard) was multiplied by the t-statistic for 20 samples (2.54). The calculated MDL is 0.17 ppm. For the purposes of this methodology the detection limit will be listed as 0.5 ppm.

13.8 The following documents may provide additional guidance and insight on this method and technique:

13.8.1 Wylie, D.E., Lu, D., Carlson, L.D., Carlson, R., Babacan, K.F., Schuster, S.M., and Wagner, F., "Monoclonal Antibodies Specific for Mercuric Ions", *Proc. Natl. Acad. Sci.*, Vol. 89, pp 4104-4108, May 1992.

13.8.2 Wylie, D.E., Lu, D., Carlson, L.D., Carlson, Schuster, S.M., and Wagner, F., "Detection of Mercuric Ions in Water by ELISA with a Mercury-Specific Antibody", Analytical Biochemistry 194, 381-387 (1991).

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/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.

14.2 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 as listed in.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits 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, Department of Government Relations and Science Policy, 1155 16th Street, NW, Washington, D.C., 20036, (202) 872-4477.

16.0 REFERENCES

1. BiMelyze® Mercury Assay Kit and BiMelyze® Mercury Assay Soil Extraction Kit, BioNebraska, Inc.

2. Schweitzer, Craig, et. al.; "Enzyme-Linked Immunoassay (ELISA) for the Detection of Mercury in Environmental Matrices."

3. Letter to Frank Calovini, SAIC; data submission from Craig Schweitzer, BioNebraska, Inc.; August 16, 1995.

4. Letter and data submission attachments, O.M. Fordham, USEPA; from Craig Schweitzer, BioNebraska, Inc.; March 20, 1995.

5. California Environmental Protection Agency, Department of Toxic Substances Control, Environmental Technology Certification Program, "BiMelyze® Field Screening Assay for Mercury ("Tube Assay") and Soil Extraction Kit, with a Partial Evaluation of BiMelyze® Laboratory Screening Test for Mercury ("Plate Assay")", BioNebraska, Inc. Lincoln, NB; Contract No. 93-T0470, June 1995.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain Tables 1 through 5, Figure 1, and a flow diagram for this method's procedure.

TABLE 1

CORRELATION OF IMMUNOASSAY AND CVAA ANALYSES
FOR MERCURY IN SOIL AT A SUPERFUND SITE

Hg conc. by CVAA (ppm)	Hg conc. by Immunoassay (ppm)	Agreement [a]
1.0	<5	Y
0.2	<5	Y
0.02	<5	Y
0.03	<5	Y
<0.02	<5	Y
<0.02	<5	Y
0.02	<5	Y
36.2	>15	Y
7.4	5-15	Y
0.03	<5	Y
0.3	<5	Y
0.03	<5	Y
0.03	<5	Y
0.1	<5	Y
<0.03	<5	Y
0.9	<5	Y
0.03	<5	Y
0.04	<5	Y
<0.02	<5	Y
39.4	>15	Y
46.5	>15	Y
18.2	>15	Y
139	>15	Y
106	>15	Y
4.7	>15	N - FP
0.4	<5	Y

(Continued)

TABLE 1 (Continued)

Hg conc. by CVAA (ppm)	Hg conc. by Immunoassay (ppm)	Agreement[a]
1.0	<5	Y
0.2	<5	Y
0.3	<5	Y
4.1	5-15	N - FP
<0.02	<5	Y
0.05	<5	Y
56.6	>15	Y
0.5	<5	Y
0.2	<5	Y
0.1	<5	Y
0.3	<5	Y
0.02	<5	Y
0.04	<5	Y
0.08	<5	Y
0.03	<5	Y
0.02	<5	Y
<0.01	<5	Y
0.2	<5	Y
0.06	<5	Y
<0.01	<5	Y
<0.01	<5	Y
28.1	5-15	N - FN
51.8	>15	Y
21.8	5-15	N - FN
7.7	5-15	Y
0.4	<5	Y
18.0	>15	Y

(Continued)

TABLE 1 (Continued)

Hg conc. by CVAA (ppm)	Hg conc. by Immunoassay (ppm)	Agreement [a]
0.8	<5	Y
2.2	<5	Y
4.4	5-15	N - FP
1.1	5-15	N - FP
0.05	<5	Y
1.3	5-15	N - FP
0.06	<5	Y
<0.02	<5	Y
<0.01	<5	Y
3.1	5-15	N - FP
3.4	5-15	N - FP
0.3	<5	Y
3.4	5-15	N - FP
2.0	5-15	N - FP
0.13	<5	Y
0.06	<5	Y

[a] Y = Yes, N = No, FN = False Negative, FP = False Positive

Source: Reference 4

TABLE 2

MERCURY ANALYSIS OF SOIL NEAR THE IMMUNOASSAY DETECTION LIMIT OF 0.50 PPM

		ABSORBANCE OBTAINED BY IMMUNOASSAY				CONCENTRATION BY CVAA			
		SAMPLE CONC. (ppm)				SAMPLE CONC. (ppm)			
		1.0	0.50	0.25	0.00	1.0	0.50	0.25	0.00
#1)	A)	0.97	0.75	0.53	0.08	0.99	0.48	0.24	0.00
	B)	0.93	0.74	0.50	0.06	1.00	0.50	0.25	0.00
	C)	0.84	0.73	0.51	0.08	0.98	0.53	0.25	0.00
	D)	0.88	0.70	0.51	0.08	0.98	0.49	0.24	0.00
#2)	A)	1.05	0.81	0.58	0.10	1.02	0.52	0.24	0.00
	B)	0.99	0.83	0.43	0.10	0.90	0.50	0.24	0.00
	C)	1.00	0.65	0.57	0.10	0.97	0.49	0.25	0.00
	D)	1.08	0.88	0.48	0.12	0.94	0.53	0.25	0.00
#3)	A)	1.17	0.80	0.58	0.08	1.06	0.55	0.26	0.00
	B)	1.11	0.82	0.52	0.11	1.03	0.54	0.28	0.00
	C)	0.95	0.62	0.43	0.10	1.00	0.52	0.28	0.00
	D)	0.99	0.80	0.51	0.11	1.03	0.54	0.27	0.00
#4)	A)	0.91	0.76	0.36	0.09	1.07	0.57	0.28	0.00
	B)	0.87	0.66	0.49	0.07	1.15	0.58	0.31	0.00
	C)	0.78	0.67	0.42	0.06	1.26	0.57	0.30	0.00
	D)	0.90	0.69	0.39	0.06	1.16	0.57	0.28	0.00
#5)	A)	1.15	0.61	0.46	0.07	0.88	0.47	0.24	0.00
	B)	1.11	0.67	0.35	0.07	0.94	0.48	0.23	0.00
	C)	1.07	0.66	0.48	0.07	0.90	0.47	0.26	0.00
	D)	1.09	0.54	0.50	0.09	0.88	0.47	0.24	0.00

Source: Reference 5

TABLE 3

CROSS-REACTIVITY OF ELEMENTS WITH THE MERCURY IMMUNOASSAY

ELEMENT	SOIL EQUIVALENT CONCENTRATION REQUIRED TO YIELD A POSITIVE RESULT (ppm)
Mercury	0.36
Arsenic	>55,000
Barium	>100,000
Cadmium	>82,000
Chromium	38,000
Copper	47,000
Gold	144,000
Iron	>41,000
Lead	>150,000
Nickel	>43,000
Silver	79,000
Sodium	>17,000
Strontium	>64,000
Thallium	>150,000
Zinc	>48,000

Source: Reference 3

TABLE 4

ANALYSIS OF CERTIFIED REFERENCE SOILS USING IMMUNOASSAY

REFERENCE SAMPLE	[Hg] (ppm)	ABSORBANCE @ 405-nm exp.1	exp.2	exp.3	INTERPRETATION
ERA Inorganic Blank Soil	<0.10	0.12	0.05	0.08	---[3]
NIST 2709	1.40	1.01	0.64	0.47	<4
NIST 2704	1.47	0.78	0.41	0.47	<4
ERA CLP Lot #216	2.36	1.54	0.84	0.93[2]	<4
ERA Custom Mercury Std.[1]					
dil. 1	4	1.76	1.01	0.83	---[3]
dil. 2	15	1.99	1.45	1.59	---[3]
dil. 3	50	2.04	1.73	2.02	>15
NIST 8408	122	2.55	2.55	2.55	>15

Source: Reference 2

[1] dilutions from 107 ppm.
[2] only value that gives incorrect conclusion.
[3] standard reference point, no interpretation.

TABLE 5

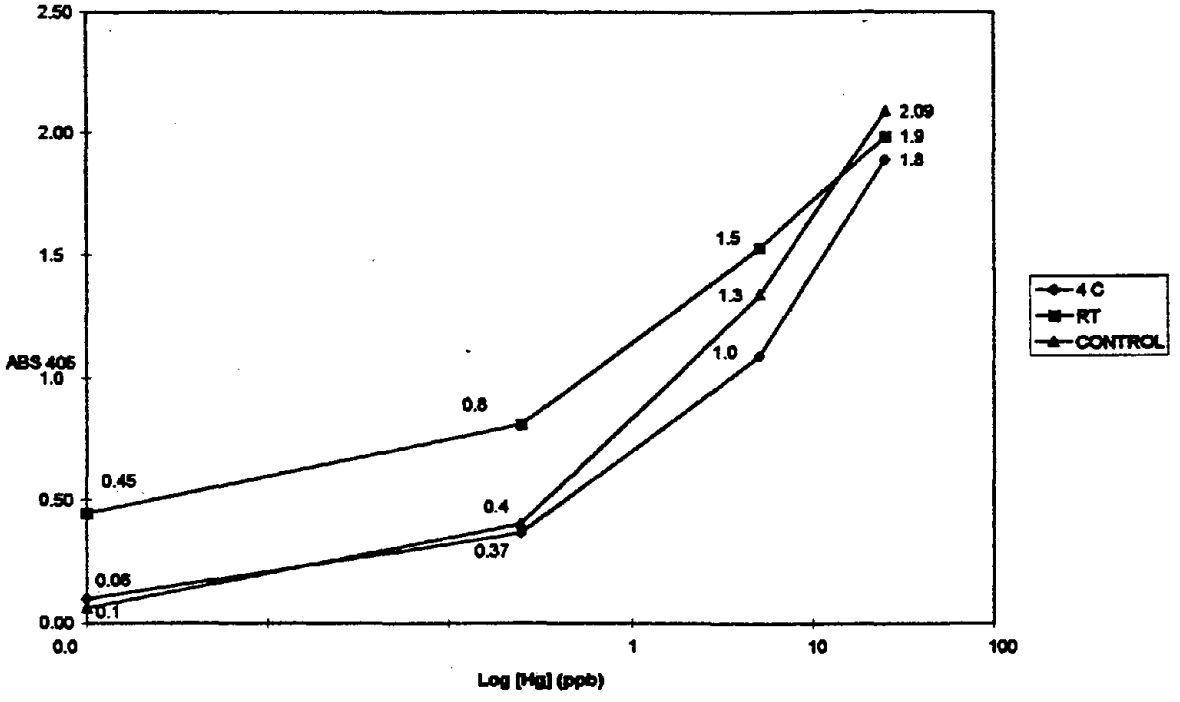
ANALYSIS OF MULTIPLE MATRICES AT AN ABANDONED BATTERY RECLAMATION SITE USING IMMUNOASSAY

SAMPLE DESCRIPTION	IMMMUNOASSAY RESULTS	CVAA RESULTS
Process Room	< 5 ppm	0.83 ppm
Dust from process room	< 5 ppm	> 4.5 ppm
Groundwater - unfiltered	< 0.5 ppb	< 0.4 ppb
Soil, alkaline	< 5 ppm	0.93 ppm
Sludge from tank	> 15 ppm	4,400 ppm
Sump sludge	5 > 15 ppm	14 ppm
Cinder block	< 5 ppm	3 ppm
Cinderblock duplicate	< 5 ppm	
Soil	5 > 15 ppm	14 ppm
Paint	> 15 ppm	34 ppm
Background cinderblock	< 5 ppm	1.4 ppm
Background paint	> 15 ppm	14 ppm
Debris from CO_2 blast	> 15 ppm	19 ppm

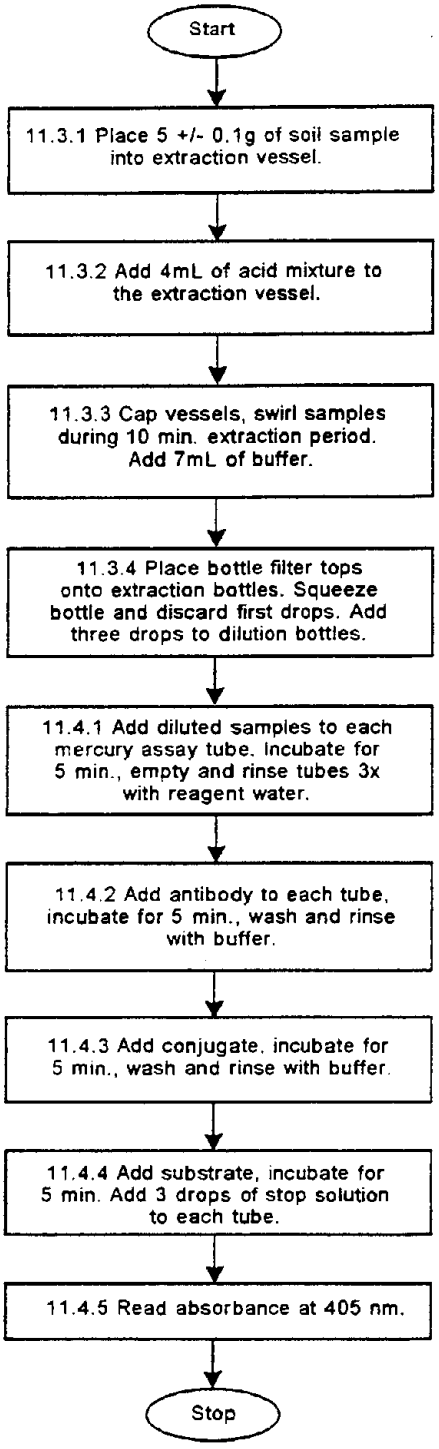
Source: Reference 2

FIGURE 1

TEN-MONTH IMMUNOASSAY KIT STABILITY



MERCURY IN SOIL BY IMMUNOASSAY



METHOD 6020A

INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY

1.0 SCOPE AND APPLICATION

1.1 Inductively coupled plasma-mass spectrometry (ICP-MS) is applicable to the determination of sub-µg/L concentrations of a large number of elements in water samples and in waste extracts or digests (References 1 and 2). When dissolved constituents are required, samples must be filtered and acid-preserved prior to analysis. No digestion is required prior to analysis for dissolved elements in water samples. Acid digestion prior to filtration and analysis is required for groundwater, aqueous samples, industrial wastes, soils, sludges, sediments, and other solid wastes for which total (acid-leachable) elements are required.

1.2 ICP-MS has been applied to the determination of over 60 elements in various matrices. Analytes for which EPA has demonstrated the acceptability of Method 6020 in a multi-laboratory study on solid and aqueous wastes are listed below.

Element		CASRN[a]
Aluminum	(Al)	7429-90-5
Antimony	(Sb)	7440-36-0
Arsenic	(As)	7440-38-2
Barium	(Ba)	7440-39-3
Beryllium	(Be)	7440-41-7
Cadmium	(Cd)	7440-43-9
Calcium	(Ca)	7440-70-2
Chromium	(Cr)	7440-47-3
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Iron	(Fe)	7439-89-6
Lead	(Pb)	7439-92-1
Magnesium	(Mg)	7439-95-4
Manganese	(Mn)	7439-96-5
Mercury	(Hg)	7439-97-6
Nickel	(Ni)	7440-02-0
Potassium	(K)	7440-09-7
Selenium	(Se)	7782-49-2
Silver	(Ag)	7440-22-4
Sodium	(Na)	7440-23-5
Thallium	(Tl)	7440-28-0
Vanadium	(V)	7440-62-2
Zinc	(Zn)	7440-66-6

[a]Chemical Abstract Service Registry Number

Acceptability of the method for an element was based upon the multi-laboratory performance compared with that of either furnace atomic absorption spectrophotometry or inductively coupled

plasma-atomic emission spectrometry. It should be noted that one multi-laboratory study was conducted in 1988 and advances in ICP-MS instrumentation and software have been made since that time and additional studies have been added with validation and improvements in performance of the method. Performance, in general, exceeds the multi-laboratory performance data for the listed elements. It is expected that current performance will exceed the multi-laboratory performance data for the listed elements (and others) that are provided in Section 13.0. Instrument detection limits, sensitivities, and linear ranges will vary with the matrices, instrumentation, and operating conditions. In relatively simple matrices, detection limits will generally be below 0.1 µg/L. Less sensitive elements (like Se and As) and desensitized major elements may be 1.0 µg/L or higher.

1.3 If Method 6020 is used to determine any analyte not listed in Section 1.2, it is the responsibility of the analyst to demonstrate the accuracy and precision of the method in the waste to be analyzed. The analyst is always required to monitor potential sources of interferences and take appropriate action to ensure data of known quality (see Section 9.4). Other elements and matrices may be analyzed by this method if performance is demonstrated for the analyte of interest, in the matrices of interest, at the concentration levels of interest in the same manner as the listed elements and matrices (see Sec. 9.0).

1.4 Use of this method should be relegated to spectroscopists who are knowledgeable in the recognition and in the correction of spectral, chemical, and physical interferences in ICP-MS.

1.5 An appropriate internal standard is required for each analyte determined by ICP-MS. Recommended internal standards are ^{6}Li, ^{45}Sc, ^{89}Y, ^{103}Rh, ^{115}In, ^{159}Tb, ^{165}Ho, and ^{209}Bi. The lithium internal standard should have an enriched abundance of ^{6}Li, so that interference from lithium native to the sample is minimized. Other elements may need to be used as internal standards when samples contain significant native amounts of the recommended internal standards.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, samples which require total ("acid-leachable") values must be digested using appropriate sample preparation methods (such as Methods 3005 - 3052).

2.2 Method 6020 describes the multi-elemental determination of analytes by ICP-MS in environmental samples. The method measures ions produced by a radio-frequency inductively coupled plasma. Analyte species originating in a liquid are nebulized and the resulting aerosol is transported by argon gas into the plasma torch. The ions produced by high temperatures are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier. Interferences must be assessed and valid corrections applied or the data flagged to indicate problems. Interference correction must include compensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix.

3.0 DEFINITIONS

Refer to Chapter One and Chapter Three for a listing of applicable definitions.

4.0 INTERFERENCES

4.1 Isobaric elemental interferences in ICP-MS are caused by isotopes of different elements forming atomic ions with the same nominal mass-to-charge ratio (m/z). A data system must be used to correct for these interferences. This involves determining the signal for another isotope

of the interfering element and subtracting the appropriate signal from the analyte isotope signal. Since commercial ICP-MS instruments nominally provide unit resolution at 10% of the peak height, very high ion currents at adjacent masses can also contribute to ion signals at the mass of interest. Although this type of interference is uncommon, it is not easily corrected, and samples exhibiting a significant problem of this type could require resolution improvement, matrix separation, or analysis using another verified and documented isotope, or use of another method.

4.2 Isobaric molecular and doubly-charged ion interferences in ICP-MS are caused by ions consisting of more than one atom or charge, respectively. Most isobaric interferences that could affect ICP-MS determinations have been identified in the literature (References 3 and 4). Examples include $^{75}ArCl^+$ ion on the ^{75}As signal and MoO^+ ions on the cadmium isotopes. While the approach used to correct for molecular isobaric interferences is demonstrated below using the natural isotope abundances from the literature (Reference 5), the most precise coefficients for an instrument can be determined from the ratio of the net isotope signals observed for a standard solution at a concentration providing suitable (<1 percent) counting statistics. Because the ^{35}Cl natural abundance of 75.77 percent is 3.13 times the ^{37}Cl abundance of 24.23 percent, the chloride correction for arsenic can be calculated (approximately) as follows (where the $^{38}Ar^{37}Cl^+$ contribution at m/z 75 is a negligible 0.06 percent of the $^{40}Ar^{35}Cl^+$ signal):

Corrected arsenic signal (using natural isotopes abundances for coefficient approximations) =
(m/z 75 signal) - (3.13) (m/z 77 signal) + (2.73) (m/z 82 signal),

where the final term adjusts for any selenium contribution at 77 m/z,

NOTE: Arsenic values can be biased high by this type of equation when the net signal at m/z 82 is caused by ions other than $^{82}Se^+$, (e.g., $^{81}BrH^+$ from bromine wastes [Reference 6]).

Similarly,

Corrected cadmium signal (using natural isotopes abundances for coefficient approximations) =
(m/z 114 signal) - (0.027)(m/z 118 signal) - (1.63)(m/z 108 signal),

where last 2 terms adjust for any $^{114}Sn^+$ or $^{114}MoO^+$ contributions at m/z 114.

NOTE: Cadmium values will be biased low by this type of equation when $^{92}ZrO^+$ ions contribute at m/z 108, but use of m/z 111 for Cd is even subject to direct ($^{94}ZrOH^+$) and indirect ($^{90}ZrO^+$) additive interferences when Zr is present.

NOTE: As for the arsenic equation above, the coefficients could be improved. The most appropriate coefficients for a particular instrument can be determined from the ratio of the net isotope signals observed for a standard solution at a concentration providing suitable (<1 percent) counting precision.

The accuracy of these types of equations is based upon the constancy of the OBSERVED isotopic ratios for the interfering species. Corrections that presume a constant fraction of a molecular ion relative to the "parent" ion have not been found (Ref. 7) to be reliable, e.g., oxide levels can vary with operating conditions. If a correction for an oxide ion is based upon the ratio of parent-to-oxide ion intensities, the correction must be adjusted for the degree of oxide formation by the use of an appropriate oxide internal standard previously demonstrated to form a similar level of oxide as the interferent. For example, this type of correction has been reported (Ref. 7) for oxide-ion corrections using ThO^+/Th^+ for the determination of rare earth elements. The use of aerosol desolvation and/or

mixed gas plasmas have been shown to greatly reduce molecular interferences (Ref. 8). These techniques can be used provided that method detection limits, accuracy, and precision requirements for analysis of the samples can be met.

4.3 Additionally, solid phase chelation may be used to eliminate isobaric interferences from both element and molecular sources. An on-line method has been demonstrated for environmental waters such as sea water, drinking water and acid decomposed samples. Acid decomposed samples refer to samples decomposed by methods similar to methods 3052, 3051, 3050 or 3015. Samples with percent levels of iron and aluminum should be avoided. The method also provides a method for preconcentration to enhance detection limits simultaneously with elimination of isobaric interferences. The method relies on chelating resins such as imminodiacetate or other appropriate resins and selectively concentrates the elements of interest while eliminating interfering elements from the sample matrix. By eliminating the elements that are direct isobaric interferences or those that form isobaric interfering molecular masses, the mass region is simplified and these interferences can not occur. The method has been proven effective for the certification of standard reference materials and validated using SRMs (References 13-15). The method has the potential to be used on-line or off-line as an effective sample preparation method specifically designed to address interference problems.

4.4 Physical interferences are associated with the sample nebulization and transport processes as well as with ion-transmission efficiencies. Nebulization and transport processes can be affected if a matrix component causes a change in surface tension or viscosity. Changes in matrix composition can cause significant signal suppression or enhancement (Ref. 9). Dissolved solids can deposit on the nebulizer tip of a pneumatic nebulizer and on the interface skimmers (reducing the orifice size and the instrument performance). Total solid levels below 0.2% (2,000 mg/L) have been currently recommended (Ref. 10) to minimize solid deposition. An internal standard can be used to correct for physical interferences, if it is carefully matched to the analyte so that the two elements are similarly affected by matrix changes (Ref. 11). When intolerable physical interferences are present in a sample, a significant suppression of the internal standard signals (to less than 30 % of the signals in the calibrations standard) will be observed. Dilution of the sample fivefold (1+4) will usually eliminate the problem (see Sec. 9.3).

4.5 Memory interferences or carry-over can occur when there are large concentration differences between samples or standards which are analyzed sequentially. Sample deposition on the sampler and skimmer cones, spray chamber design, and the type of nebulizer affect the extent of the memory interferences which are observed. The rinse period between samples must be long enough to eliminate significant memory interference.

5.0 SAFETY

Refer to Chapter Three for a discussion on safety related references and issues.

6.0 EQUIPMENT AND SUPPLIES

6.1 Inductively coupled plasma-mass spectrometer:

6.1.1 A system capable of providing resolution, better than or equal to 1.0 amu at 10% peak height is required. The system must have a mass range from at least 6 to 240 amu and a data system that allows corrections for isobaric interferences and the application of the internal standard technique. Use of a mass-flow controller for the nebulizer argon and a peristaltic pump for the sample solution are recommended.

6.1.2 Argon gas supply: high-purity grade (99.99%).

7.0 REAGENTS AND STANDARDS

7.1 Acids used in the preparation of standards and for sample processing must be of high purity. Redistilled acids are recommended because of the high sensitivity of ICP-MS. Nitric acid at less than 2 per cent (v/v) is required for ICP-MS to minimize damage to the interface and to minimize isobaric molecular-ion interferences with the analytes. Many more molecular-ion interferences are observed when hydrochloric and sulfuric acids are used (References 3 and 4). Concentrations of antimony and silver between 50-500 µg/L require 1% (v/v) HCl for stability; for concentrations above 500 µg/L Ag, additional HCl will be needed. Consequently, accuracy of analytes requiring significant chloride molecular ion corrections (such as As and V) will degrade.

7.2 Reagent water: All references to water in the method refer to reagent water unless otherwise specified. Refer to Chapter One for a definition of reagent water.

7.3 Standard stock solutions for each analyte may be purchased or prepared from ultra-high purity grade chemicals or metals (99.99 or greater purity). See Method 6010 for instructions on preparing standard solutions from solids.

7.3.1 Bismuth internal standard stock solution (1 mL = 100 µg Bi): Dissolve 0.1115 g Bi_2O_3 in a minimum amount of dilute HNO_3. Add 10 mL conc. HNO_3 and dilute to 1,000 mL with reagent water.

7.3.2 Holmium internal standard stock solution (1 mL = 100 µg Ho): Dissolve 0.1757 g $Ho_2(CO_3)_2 \cdot 5H_2O$ in 10 mL reagent water and 10 mL HNO_3. After dissolution is complete, warm the solution to degas. Add 10 mL conc. HNO_3 and dilute to 1,000 mL with reagent water.

7.3.3 Indium internal standard stock solution (1 mL = 100 µg In): Dissolve 0.1000 g indium metal in 10 mL conc. HNO_3. Dilute to 1,000 mL with reagent water.

7.3.4 Lithium internal standard stock solution (1 mL = 100 µg ^6Li): Dissolve 0.6312 g 95-atom-% ^6Li, Li_2CO_3 in 10 mL of reagent water and 10 mL HNO_3. After dissolution is complete, warm the solution to degas. Add 10 mL conc. HNO_3 and dilute to 1,000 mL with reagent water.

7.3.5 Rhodium internal standard stock solution (1 mL = 100 µg Rh): Dissolve 0.3593 g ammonium hexachlororhodate (III) $(NH_4)_3RhCl_6$ in 10 mL reagent water. Add 100 mL conc. HCl and dilute to 1,000 mL with reagent water.

7.3.6 Scandium internal standard stock solution (1 mL = 100 µg Sc): Dissolve 0.15343 g Sc_2O_3 in 10 mL (1+1) hot HNO_3. Add 5 mL conc. HNO_3 and dilute to 1,000 mL with reagent water.

7.3.7 Terbium internal standard stock solution (1 mL = 100 µg Tb): Dissolve 0.1828 g $Tb_2(CO_3)_3 \cdot 5H_2O$ in 10 mL (1+1) HNO_3. After dissolution is complete, warm the solution to degas. Add 5 mL conc. HNO_3 and dilute to 1,000 mL with reagent water.

7.3.8 Yttrium internal standard stock solution (1 mL = 100 µg Y): Dissolve 0.2316 g $Y_2(CO_3)_3.3H_2O$ in 10 mL (1+1) HNO_3. Add 5 mL conc. HNO_3 and dilute to 1,000 mL with reagent water.

7.3.9 Titanium interference stock solution (1 mL = 100 µg Ti): Dissolve 0.4133 g $(NH_4)_2TiF_6$ in reagent water. Add 2 drops conc. HF and dilute to 1,000 mL with reagent water.

7.3.10 Molybdenum interference stock solution (1 mL = 100 µg Mo): Dissolve 0.2043 g $(NH_4)_2MoO_4$ in reagent water. Dilute to 1,000 mL with reagent water.

7.3.11 Gold preservative stock solution for mercury (1 mL = 100 µg): Recommend purchasing as high purity prepared solution of $AuCl_3$ in dilute hydrochloric acid matrix.

7.4 Mixed calibration standard solutions are prepared by diluting the stock-standard solutions to levels in the linear range for the instrument in a solvent consisting of 1 percent (v/v) HNO_3 in reagent water. The calibration standard solutions must contain a suitable concentration of an appropriate internal standard for each analyte. Internal standards may be added on-line at the time of analysis using a second channel of the peristaltic pump and an appropriate mixing manifold. Generally, an internal standard should be no more than 50 amu removed from the analyte. Recommended internal standards include ^{6}Li, ^{45}Sc, ^{89}Y, ^{103}Rh, ^{115}In, ^{159}Tb, ^{169}Ho, and ^{209}Bi. Prior to preparing the mixed standards, each stock solution must be analyzed separately to determine possible spectral interferences or the presence of impurities. Care must be taken when preparing the mixed standards that the elements are compatible and stable. Transfer the mixed standard solutions to freshly acid-cleaned FEP fluorocarbon bottles for storage. Fresh mixed standards must be prepared as needed with the realization that concentrations can change on aging. Calibration standards must be initially verified using a quality control standard (see Section 7.7).

7.5 Blanks: Three types of blanks are required for the analysis. The calibration blank is used in establishing the calibration curve. The preparation blank is used to monitor for possible contamination resulting from the sample preparation procedure. The rinse blank is used to flush the system between all samples and standards.

7.5.1 The calibration blank consists of the same concentration(s) of the same acid(s) used to prepare the final dilution of the calibrating solutions of the analytes [often 1 percent HNO_3 (v/v) in reagent water] along with the selected concentrations of internal standards such that there is an appropriate internal standard element for each of the analytes. Use of HCl for antimony and silver is cited in Section 7.1.

7.5.2 The preparation (or reagent) blank must be carried through the complete preparation procedure and contain the same volumes of reagents as the sample solutions.

7.5.3 The rinse blank consists of 1 to 2 percent HNO_3 (v/v) in reagent water. Prepare a sufficient quantity to flush the system between standards and samples. If mercury is to be analyzed, the rinse blank should also contain 2 µg/mL (ppm) $AuCl_3$ solution.

7.6 The interference check solution (ICS) is prepared to contain known concentrations of interfering elements that will demonstrate the magnitude of interferences and provide an adequate test of any corrections. Chloride in the ICS provides a means to evaluate software corrections for chloride-related interferences such as $^{35}Cl^{16}O^{+}$ on $^{51}V^{+}$ and $^{40}Ar^{35}Cl^{+}$ on $^{75}As^{+}$. Iron is used to demonstrate adequate resolution of the spectrometer for the determination of manganese.

Molybdenum serves to indicate oxide effects on cadmium isotopes. The other components are present to evaluate the ability of the measurement system to correct for various molecular-ion isobaric interferences. The ICS is used to verify that the interference levels are corrected by the data system within quality control limits.

NOTE: The final ICS solution concentrations in Table 1 are intended to evaluate corrections for known interferences on only the analytes in Sec. 1.2. If Method 6020 is used to determine an element not listed in Sec. 1.2, it is the responsibility of the analyst to modify the ICS solutions, or prepare an alternative ICS solution, to allow adequate verification of correction of interferences on the unlisted element (see Section 9.4).

7.6.1 These solutions must be prepared from ultra-pure reagents. They can be obtained commercially or prepared by the following procedure.

7.6.1.1 Mixed ICS solution I may be prepared by adding 13.903 g $Al(NO_3)_3 \cdot 9H_2O$, 2.498 g $CaCO_3$ (dried at 180 °C for 1 hour before weighing), 1.000 g Fe, 1.658 g MgO, 2.305 g Na_2CO_3, and 1.767 g K_2CO_3 to 25 mL of reagent water. Slowly add 40 mL of (1+1) HNO_3. After dissolution is complete, warm the solution to degas. Cool and dilute to 1,000 mL with reagent water.

7.6.1.2 Mixed ICS solution II may be prepared by slowly adding 7.444 g 85 % H_3PO_4, 6.373 g 96% H_2SO_4, 40.024 g 37% HCl, and 10.664 g citric acid $C_6O_7H_8$ to 100 mL of reagent water. Dilute to 1,000 mL with reagent water.

7.6.1.3 Mixed ICS solution III may be prepared by adding 1.00 mL each of 100-µg/mL arsenic, cadmium, selenium, chromium, cobalt, copper, manganese, nickel, silver, vanadium, and zinc stock solutions to about 50 mL reagent water. Add 2.0 mL concentrated HNO_3, and dilute to 100.0 mL with reagent water.

7.6.1.4 Working ICS Solutions

7.6.1.4.1 ICS-A may be prepared by adding 10.0 mL of mixed ICS solution I (Sec. 7.6.1.1), 2.0 mL each of 100-µg/mL titanium stock solution (Sec. 7.3.9) and molybdenum stock solution (Sec. 7.3.10), and 5.0 mL of mixed ICS solution II (Sec. 7.6.1.2). Dilute to 100 mL with reagent water. ICS solution A must be prepared fresh weekly.

7.6.1.4.2 ICS-AB may be prepared by adding 10.0 mL of mixed ICS solution I (Sec. 7.6.1.1), 2.0 mL each of 100-µg/mL titanium stock solution (Sec. 7.3.9) and molybdenum stock solution (Sec. 7.3.10), 5.0 mL of mixed ICS solution II (Sec. 7.6.1.2), and 2.0 mL of Mixed ICS solution III (Sec. 7.6.1.3). Dilute to 100 mL with reagent water. Although the ICS solution AB must be prepared fresh weekly, the analyst should be aware that the solution may precipitate silver more quickly.

7.7 The quality control standard is the initial calibration verification solution (ICV), which must be prepared in the same acid matrix as the calibration standards. This solution must be an independent standard near the midpoint of the linear range at a concentration other than that used for instrument calibration. An independent standard is defined as a standard composed of the analytes from a source different from those used in the standards for instrument calibration.

7.8 Mass spectrometer tuning solution. A solution containing elements representing all of the mass regions of interest (for example, 10 µg/L of Li, Co, In, and Tl) must be prepared to verify that the resolution and mass calibration of the instrument are within the required specifications (see Section 10.1). This solution is also used to verify that the instrument has reached thermal stability (see Section 11.4).

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Sample collection procedures should address the considerations described in Chapter Nine.

8.2 See the introductory material in Chapter Three, Inorganic Analytes, for information on sample handling, storage, holding times and preservation. Only polyethylene or fluorocarbon (TFE or PFA) containers are recommended for use in this method.

9.0 QUALITY CONTROL

9.1 All quality control data should be maintained and be available for easy reference or inspection.

9.2 Instrument detection limits (IDLs) in µg/L can be estimated by calculating the average of the standard deviations of three runs on three non-consecutive days from the analysis of a reagent blank solution with seven consecutive measurements per day. Each measurement must be performed as though it were a separate analytical sample (i.e., each measurement must be followed by a rinse and/or any other procedure normally performed between the analysis of separate samples). IDLs must be determined at least every three months and kept with the instrument log book. Refer to Chapter One for additional guidance.

9.3 The intensities of all internal standards must be monitored for every analysis. If the intensity of any internal standard in a sample falls below 30 percent of the intensity of that internal standard in the initial calibration standard, a significant matrix effect must be suspected. Under these conditions, the detection limit has degraded and the correction ability of the internal standardization technique becomes questionable. The following procedure is followed: First, make sure the instrument has not just drifted by observing the internal standard intensities in the nearest clean matrix (calibration blank, Section 7.5.1). If the low internal standard intensities are also seen in the nearest calibration blank, terminate the analysis, correct the problem, recalibrate, verify the new calibration, and reanalyze the affected samples. If drift has not occurred, matrix effects need to be removed by dilution of the affected sample. The sample must be diluted fivefold (1+4) and reanalyzed with the addition of appropriate amounts of internal standards. If the first dilution does not eliminate the problem, this procedure must be repeated until the internal-standard intensities rise above the 30 percent limit. Reported results must be corrected for all dilutions.

9.4 To obtain analyte data of known quality, it is necessary to measure more than the analytes of interest in order to apply corrections or to determine whether interference corrections are necessary. For example, tungsten oxide moleculars can be very difficult to distinguish from mercury isotopes. If the concentrations of interference sources (such as C, Cl, Mo, Zr, W) are such that, at the correction factor, the analyte is less than the limit of quantification and the concentration of interferents are insignificant, then the data may go uncorrected. Note that monitoring the interference sources does not necessarily require monitoring the interferant itself, but that a molecular species may be monitored to indicate the presence of the interferent. When correction equations are used, all QC criteria must also be met. Extensive QC for interference corrections are

required at all times. The monitored masses must include those elements whose hydrogen, oxygen, hydroxyl, chlorine, nitrogen, carbon and sulfur molecular ions could impact the analytes of interest. Unsuspected interferences may be detected by adding pure major matrix components to a sample to observe any impact on the analyte signals. When an interference source is present, the sample elements impacted must be flagged to indicate (a) the percentage interference correction applied to the data or (b) an uncorrected interference by virtue of the elemental equation used for quantitation. The isotope proportions for an element or molecular-ion cluster provide information useful for quality assurance.

NOTE: Only isobaric elemental, molecular, and doubly charged interference corrections which use the observed isotopic-response ratios or parent-to-oxide ratios (provided an oxide internal standard is used as described in Section 4.2) for each instrument system are acceptable corrections for use in Method 6020.

9.5 Dilution test (serial dilution): If the analyte concentration is within the linear dynamic range of the instrument and sufficiently high (minimally, a factor of at least 100 times greater than the concentration in the reagent blank, refer to Section 7.5.2), an analysis of a fivefold (1+4) dilution must agree within ± 10% of the original determination. If not, an interference effect must be suspected. One dilution test must be included for each twenty samples (or less) of each matrix in a batch.

9.6 Post-digestion spike addition: An analyte spike added to a portion of a prepared sample, or its dilution, should be recovered to within 75 to 125 percent of the known value or within the laboratory derived acceptance criteria. The spike addition should be based on the indigenous concentration of each element of interest in the sample. If the spike is not recovered within the specified limits, the sample must be diluted and reanalyzed to compensate for the matrix effect. Results must agree to within 10% of the original determination. The use of a standard-addition analysis procedure may also be used to compensate for this effect (refer to Method 7000).

9.7 A laboratory control sample (LCS) should be analyzed for each analyte using the same sample preparations, analytical methods and QA/QC procedures employed for the test samples. One LCS should be prepared and analyzed for each sample batch at a frequency of one LCS for each 20 samples or less.

9.8 Check the instrument calibration by analyzing appropriate quality control solutions as follows:

9.8.1 Check instrument calibration using a calibration blank (Section 7.5.1) and the initial calibration verification solution (Sections 7.7 and 11.6).

9.8.2 Verify calibration at a frequency of every 10 analytical samples with the instrument check standard (Section 7.6) and the calibration blank (Section 7.5.1). These solutions must also be analyzed for each analyte at the beginning of the analysis and after the last sample.

9.8.3 The results of the initial calibration verification solution and the instrument check standard must agree within ± 10% of the expected value. If not, terminate the analysis, correct the problem, and recalibrate the instrument. Any sample analyzed under an out-of-control calibration must be reanalyzed .

9.8.4 The results of the calibration blank must be less than 3 times the current IDL for each element. If this is not the case, the reason for the out-of-control condition must be found and corrected, and affected samples must be reanalyzed. If the laboratory consistently has concentrations greater than 3 times the IDL, the IDL may be indicative of an estimated IDL and should be re-evaluated.

9.9 Verify the magnitude of elemental and molecular-ion isobaric interferences and the adequacy of any corrections at the beginning of an analytical run or once every 12 hours, whichever is more frequent. Do this by analyzing the interference check solutions A and AB. The analyst should be aware that precipitation from solution AB may occur with some elements, specifically silver. Refer to Section 4.0 for a discussion on interferences and potential solutions to those interferences if additional guidance is needed.

9.10 Analyze one duplicate sample for every matrix in a batch at a frequency of one matrix duplicate for every 20 samples.

9.10.1 The relative percent difference (RPD) between duplicate determinations must be calculated as follows:

$$RPD = \frac{|D_1 - D_2|}{(D_1 + D_2)/2} \times 100$$

where:

RPD = relative percent difference.
D_1 = first sample value.
D_2 = second sample value (duplicate)

A control limit of 20% RPD should not be exceeded for analyte values greater than 100 times the instrumental detection limit. If this limit is exceeded, the reason for the out-of-control situation must be found and corrected, and any samples analyzed during the out-of-control condition must be reanalyzed.

9.11 Ultra-trace analysis requires the use of clean chemistry. Several suggestions for reduction on the analytical blank are provided in Chapter Three.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Conduct mass calibration and resolution checks in the mass regions of interest. The mass calibration and resolution parameters are required criteria which must be met prior to any samples being analyzed. If the mass calibration differs more than 0.1 amu from the true value, then the mass calibration must be adjusted to the correct value. The resolution must also be verified to be less than 0.9 amu full width at 10 percent peak height.

10.2 Calibrate the instrument for the analytes of interest (recommended isotopes for the analytes in Sec. 1.2 are provided in Table 2), using the calibration blank and at least a single initial calibration standard according to the instrument manufacturer's procedure. Flush the system with the rinse blank (Sec. 7.5.3) between each standard solution. Use the average of at least three integrations for both calibration and sample analyses.

NOTE: Analysts have noted improved performance in calibration stability if the instrument is exposed to the interference check solution after cleaning sampler and skimmer cones. Improved performance is also realized if the instrument is allowed to rinse for 5 or 10 minutes before the calibration blank is run.

10.3 All masses which could affect data quality should be monitored to determine potential effects from matrix components on the analyte peaks. The recommended isotopes to be monitored are listed in Table 2.

10.4 Immediately after the calibration has been established, the calibration must be verified and documented for every analyte by the analysis of the calibration verification solution (Section 7.7). When measurements exceed ± 10% of the accepted value, the analyses must be terminated, the problem corrected, the instrument recalibrated, and the new calibration verified. Any samples analyzed under an out-of-control calibration must be reanalyzed. During the course of an analytical run, the instrument may be "resloped" or recalibrated to correct for instrument drift but resloping must not be used as an alternative to reanalyzing samples following an unacceptable QC sample, such as a CCV. A recalibration must then be followed immediately by a new analysis of a CCV and CCB before any further samples may be analyzed.

11.0 PROCEDURE

11.1 Solubilization and digestion procedures are presented in Chapter Three (e.g., Methods 3005 - 3052).

NOTE: If mercury is to be analyzed, the digestion procedure must use mixed nitric and hydrochloric acids through all steps of the digestion. Mercury will be lost if the sample is digested when hydrochloric acid is not present. If it has not already been added to the sample as a preservative, Au should be added to give a final concentration of 2 mg/L (use 2.0 mL of 5.3.11 per 100 mL of sample) to preserve the mercury and to prevent it from plating out in the sample introduction system.

11.2 Initiate appropriate operating configuration of the instruments computer according to the instrument manufacturer's instructions.

11.3 Set up the instrument with the proper operating parameters according to the instrument manufacturer's instructions.

11.4 Operating conditions: The analyst should follow the instructions provided by the instrument manufacturer. Allow at least 30 minutes for the instrument to equilibrate before analyzing any samples. This must be verified by analyzing a tuning solution (Section 7.8) at least four times with relative standard deviations of $\leq$ 5% for the analytes contained in the tuning solution.

NOTE: The instrument should have features that protect itself from high ion currents. If not, precautions must be taken to protect the detector from high ion currents. A channel electron multiplier or active film multiplier suffer from fatigue after being exposed to high ion currents. This fatigue can last from several seconds to hours depending on the extent of exposure. During this time period, response factors are constantly changing, which invalidates the calibration curve, causes instability, and invalidates sample analyses.

11.5 Calibrate the instrument following the procedure outlined in Section 10.0.

11.6 Flush the system with the rinse blank solution (Sec. 7.5.3) until the signal levels return to the DQO or method's levels of quantitation (usually about 30 seconds) before the analysis of each sample (see Section 10.3). Nebulize each sample until a steady-state signal is achieved (usually about 30 seconds) prior to collecting data. Analyze the calibration verification solution (Section 7.6) and the calibration blank (Section 7.5.1) at a frequency of at least once every 10 analytical samples. Flow-injection systems may be used as long as they can meet the performance criteria of this method.

11.7 Dilute and reanalyze samples that are more concentrated than the linear range for an analyte (or species needed for a correction) or measure an alternate but less-abundant isotope. The linearity at the alternate mass must be confirmed by appropriate calibration (see Sec. 10.2 and 10.4). Alternatively apply solid phase chelation chromatography to eliminate the matrix as described in Sec. 4.3.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 The quantitative values shall be reported in appropriate units, such as micrograms per liter (µg/L) for aqueous samples and milligrams per kilogram (mg/kg) for solid samples. If dilutions were performed, the appropriate corrections must be applied to the sample values.

12.1.1 If appropriate, or required, calculate results for solids on a dry-weight basis as follows:

(1) A separate determination of percent solids must be performed.

(2) The concentrations determined in the digest are to be reported on the basis of the dry weight of the sample.

$$\text{Concentration (dry weight)(mg/kg)} = \frac{C \times V}{W \times S}$$

Where,

C = Digest Concentration (mg/L)
V = Final volume in liters after sample preparation
W = Weight in kg of wet sample

$$S = \frac{\%\ \text{Solids}}{100}$$

Calculations must include appropriate interference corrections (see Section 4.2 for examples), internal-standard normalization, and the summation of signals at 206, 207, and 208 m/z for lead (to compensate for any differences in the abundances of these isotopes between samples and standards).

13.0 METHOD PERFORMANCE

13.1 In an EPA multi-laboratory study (Ref. 12), twelve laboratories applied the ICP-MS technique to both aqueous and solid samples. Table 3 summarizes the method performance data for aqueous samples. Performance data for solid samples are provided in Table 4.

13.2 Table 5 summarizes the method performance data for aqueous and sea water samples with interfering elements removed and samples preconcentrated prior to analysis. Table 6 summarizes the performance data for a simulated drinking water standard.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/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.

14.2 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 Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits 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 Sec. 14.2.

16.0 REFERENCES

1. Horlick, G., et al., Spectrochim. Acta 40B, 1555 (1985).

2. Gray, A.L., Spectrochim. Acta 40B, 1525 (1985); 41B, 151 (1986).

3. Tan, S.H., and Horlick, G., Appl. Spectrosc. 40, 445 (1986).

4. Vaughan, M.A., and Horlick, G., Appl. Spectrosc. 40, 434 (1986).

5. Holden, N.E., "Table of the Isotopes," in Lide, D.R., Ed., CRC Handbook of Chemistry and Physics, 74th Ed., CRC Press, Boca Raton, FL, 1993.

6. Hinners, T.A., Heithmar, E., Rissmann, E., and Smith, D., Winter Conference on Plasma Spectrochemistry, Abstract THP18; p. 237, San Diego, CA (1994).

7. Lichte, F.E., et al., Anal. Chem. 59, 1150 (1987).

8. Evans E.H., and Ebdon, L., J. Anal. At. Spectrom. 4, 299 (1989).

9. Beauchemin, D., et al., Spectrochim. Acta 42B, 467 (1987).

10. Houk, R.S., Anal. Chem. 58, 97A (1986).

11. Thompson, J.J., and Houk, R.S., Appl. Spectrosc. 41, 801 (1987).

12. Newberry, W.R., Butler, L.C., Hurd, M.L., Laing, G.A., Stapanian, M.A., Aleckson, K.A., Dobb, D.E., Rowan, J.T., and Garner, F.C., Final Report of the Multi-Laboratory Evaluation of Method 6020 CLP-M Inductively Coupled Plasma-Mass Spectrometry (1989).

13. Taylor, Daniel B., Kingston, H.M., Nogay, Donald J., Koller, Dagmar, and Hutton, Robert. "On-Line Solid-phase Chelation for the Determination of Eight Metals in Environmental Waters by Inductively Coupled Plasma Mass Spectrometry".

14. Kingston, H. M., Siriraks, A., and Riviello, J. M., Patent Number 5,126,272, "A Method and Apparatus for Detecting Transition and Rare Earth Elements in a Matrix", U.S. Patent, Filed U.S. Patent Office, March 1989, 31 pages, Granted June 30, 1992, Patent held by US Government.

15. Kingston, H. M., Siriraks, A., and Riviello, J. M., Patent Number 5,244,634 , "A Method and Apparatus for Detecting Transition and Rare Earth Elements in a Matrix", U.S. Patent , Filed U.S. Patent Office, March 1989, 31 pages, Granted Sept. 14, 1993, Patent held by US Government.

16. Dobb, David E., Rowan, J.T., and Cardenas, D., Lockheed Environmental Systems and Technologies Co., Las Vegas, NV; and Butler, L.C., and Heithman, E.M., U.S.EPA, Las Vegas, NV; "Determination of Mercury by ICP-MS".

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain Tables 1 through 7, and a flow diagram of the method procedure.

TABLE 1

RECOMMENDED INTERFERENCE CHECK SAMPLE COMPONENTS AND CONCENTRATIONS

Solution Component	Solution A Concentration (mg/L)	Solution AB Concentration (mg/L)
Al	100.0	100.0
Ca	300.0	300.0
Fe	250.0	250.0
Mg	100.0	100.0
Na	250.0	250.0
P	100.0	100.0
K	100.0	100.0
S	100.0	100.0
C	200.0	200.0
Cl	2000.0	2000.0
Mo	2.0	2.0
Ti	2.0	2.0
As	0.0	0.100
Cd	0.0	0.100
Cr	0.0	0.200
Co	0.0	0.200
Cu	0.0	0.200
Mn	0.0	0.200
Hg	0.0	0.020
Ni	0.0	0.200
Se	0.0	0.100
Ag	0.0	0.050
V	0.0	0.200
Zn	0.0	0.100

TABLE 2
RECOMMENDED ISOTOPES FOR SELECTED ELEMENTS

Element of interest	Mass
Aluminum	**27**
Antimony	121, **123**
Arsenic	**75**
Barium	138, 137, 136, **135**, 134
Beryllium	**9**
Bismuth (IS)	209
Cadmium	**114**, 112, **111**, 110, 113, 116, 106
Calcium (I)	42, 43, **44**, 46, 48
Chlorine (I)	35, 37, (77, 82)[a]
Chromium	**52**, **53**, **50**, 54
Cobalt	**59**
Copper	**63**, **65**
Holmium (IS)	165
Indium (IS)	**115**, 113
Iron (I)	**56**, **54**, **57**, 58
Lanthanum (I)	139
Lead	**208**, **207**, **206**, 204
Lithium (IS)	6[b], 7
Magnesium (I)	24, **25**, **26**
Manganese	**55**
Mercury	202, **200**, 199, 201
Molybdenum (I)	98, 96, 92, **97**, 94, (108)[a]
Nickel	58, **60**, 62, **61**, 64
Potassium (I)	**39**
Rhodium (IS)	103
Scandium (IS)	45
Selenium	80, **78**, **82**, **76**, **77**, 74
Silver	**107**, **109**
Sodium (I)	**23**
Terbium (IS)	159
Thallium	**205**, 203
Vanadium	**51**, **50**
Tin (I)	120, **118**
Yttrium (IS)	89
Zinc	64, **66**, **68**, **67**, 70

NOTE: Method 6020 is recommended for only those analytes listed in Sec.1.2. Other elements are included in this table because they are potential interferents (labeled I) in the determination of recommended analytes, or because they are commonly used internal standards (labeled IS). Isotopes are listed in descending order of natural abundance. The most generally useful isotopes are underlined and in boldface, although certain matrices may require the use of alternative isotopes.

[a] These masses are also useful for interference correction (Section 4.2).

[b] Internal standard must be enriched in the ^{6}Li isotope. This minimizes interference from indigenous lithium.

TABLE 3

ICP-MS MULTI-LABORATORY PRECISION AND ACCURACY DATA FOR AQUEOUS SOLUTIONS

Element	Comparability[a] Range	%RSD Range	N[b]	S[c]
Aluminum	95 - 100	11 - 14	14 - 14	4
Antimony	d	5.0 - 7.6	16 - 16	3
Arsenic	97 - 114	7.1 - 48	12 - 14	4
Barium	91 - 99	4.3 - 9.0	16 - 16	5
Beryllium	103 - 107	8.6 - 14	13 - 14	3
Cadmium	98 - 102	4.6 - 7.2	18 - 20	3
Calcium	99 - 107	5.7 - 23	17 - 18	5
Chromium	95 - 105	13 - 27	16 - 18	4
Cobalt	101 - 104	8.2 - 8.5	18 - 18	3
Copper	85 - 101	6.1 - 27	17 - 18	5
Iron	91 - 900	11 - 150	10 - 12	5
Lead	71 - 137	11 - 23	17 - 18	6
Magnesium	98 - 102	10 - 15	16 - 16	5
Manganese	95 - 101	8.8 - 15	18 - 18	4
Nickel	98 - 101	6.1 - 6.7	18 - 18	2
Potassium	101 - 114	9.9 - 19	11 - 12	5
Selenium	102 - 107	15 - 25	12 - 12	3
Silver	104 - 105	5.2 - 7.7	13 - 16	2
Sodium	82 - 104	24 - 43	9 - 10	5
Thallium	88 - 97	9.7 - 12	18 - 18	3
Vanadium	107 - 142	23 - 68	8 - 13	3
Zinc	93 - 102	6.8 - 17	16 - 18	5

Data obtained from reference 12.

[a] Comparability refers to the percent agreement of mean ICP-MS values to those of the reference technique (ICP-AES or GFAA).
[b] N is the range of the number of ICP-MS measurements where the analyte values exceed the limit of quantitation (3.3 times the average IDL value). A larger number gives a more reliable comparison.
[c] S is the number of samples with results greater than the limit of quantitation.
[d] No comparability values are provided for antimony because of evidence that the reference data is affected by an interference.

TABLE 4

ICP-MS MULTI-LABORATORY PRECISION AND ACCURACY DATA FOR SOLID MATRICES

Element	Comparability[a] Range	%RSD Range	N[b]	S[c]
Aluminum	83 - 101	11 - 39	13 - 14	7
Antimony	d	12 - 21	15 - 16	2
Arsenic	79 - 102	12 - 23	16 - 16	7
Barium	100 - 102	4.3 - 17	15 - 16	7
Beryllium	50 - 87	19 - 34	12 - 14	5
Cadmium	93 - 100	6.2 - 25	19 - 20	5
Calcium	95 - 109	4.1 - 27	15 - 17	7
Chromium	77 - 98	11 - 32	17 - 18	7
Cobalt	43 - 102	15 - 30	17 - 18	6
Copper	90 - 109	9.0 - 25	18 - 18	7
Iron	87 - 99	6.7 - 21	12 - 12	7
Lead	90 - 104	5.9 - 28	15 - 18	7
Magnesium	89 - 111	7.6 - 37	15 - 16	7
Manganese	80 - 108	11 - 40	16 - 18	7
Nickel	87 - 117	9.2 - 29	16 - 18	7
Potassium	97 - 137	11 - 62	10 - 12	5
Selenium	81	39	12	1
Silver	43 - 112	12 - 33	15 - 15	3
Sodium	100 - 146	14 - 77	8 - 10	5
Thallium	91	33	18	1
Vanadium	83 - 147	20 - 70	6 - 14	7
Zinc	84 - 124	14 - 42	18 - 18	7

Data obtained from reference 12.

[a] Comparability refers to the percent agreement of mean ICP-MS values to those of the reference technique.
[b] N is the range of the number of ICP-MS measurements where the analyte values exceed the limit of quantitation (3.3 times the average IDL value).
[c] S is the number of samples with results greater than the limit of quantitation.
[d] No comparability values are provided for antimony because of evidence that the reference data is affected by an interference.

TABLE 5

METHOD PERFORMANCE DATA FOR AQUEOUS AND SEA WATER SAMPLES[A]
WITH INTERFERING ELEMENTS REMOVED
AND SAMPLES PRECONCENTRATED PRIOR TO ANALYSIS

ELEMENT	ISOTOPE	CONCENTRATION (ng/mL)[B] 9.0 mL	27.0 mL	CERTFIED
Manganese	55	1.8±0.05	1.9±0.2	1.99±0.15
Nickel	58	0.32±0.018	0.32±0.04	0.30±0.04
Cobalt	59	0.033±0.002	0.028±0.003	0.025±0.006
Copper	63	0.68±0.03	0.63±0.03	0.68±0.04
Zinc	64	1.6±0.05	1.8±0.15	1.97±0.12
Copper	65	0.67±0.03	0.6±0.05	0.68±0.04
Zinc	66	1.6±0.06	1.8±0.2	1.97±0.12
Cadmium	112	0.020±0.0015	0.019±0.0018	0.019±0.004
Cadmium	114	0.020±0.0009	0.019±0.002	0.019±0.004
Lead	206	0.013±0.0009	0.019±0.0011	0.019±0.006
Lead	207	0.014±0.0005	0.019±0.004	0.019±0.006
Lead	208	0.014±0.0006	0.019±0.002	0.019±0.006

Data obtained from reference 12.

[A] The dilution of the sea-water during the adjustment of pH produced 10 mL samples containing 9 mL of sea-water and 30 mL samples containing 27 mL of sea-water. Samples containing 9.0 mL of CASS-2, n=5; samples containing 27.0 mL of CASS-2, n=3.

[B] Concentration (ng/mL) ± 95% confidence limits.

TABLE 6

ANALYSIS OF NIST SRM 1643b, TRACE METALS IN WATER[A]

ELEMENT	ISOTOPE	CONCENTRATION (ng/mL)[B] DETERMINED	CERTFIED
Manganese	55	30±1.3	28±2
Nickel	58	50±2	49±3
Cobalt	59	27±1.3	26±1
Nickel	60	51±2	49±3
Copper	63	23±1.0	21.9±0.4
Zinc	64	67±1.4	66±2
Copper	65	22±0.9	21.9±0.4
Zinc	66	67±1.8	66±2
Cadmium	111	20±0.5	20±1
Cadmium	112	19.9±0.3	20±1
Cadmium	114	19.8±0.4	20±1
Lead	206	23±0.5	23.7±0.7
Lead	207	23.9±0.4	23.7±0.7
Lead	208	24.2±0.4	23.7±0.7

Data obtained from reference 12.

[A] 5.0 mL samples, n=5.
[B] Concentration (ng/mL) ± 95% confidence limits.

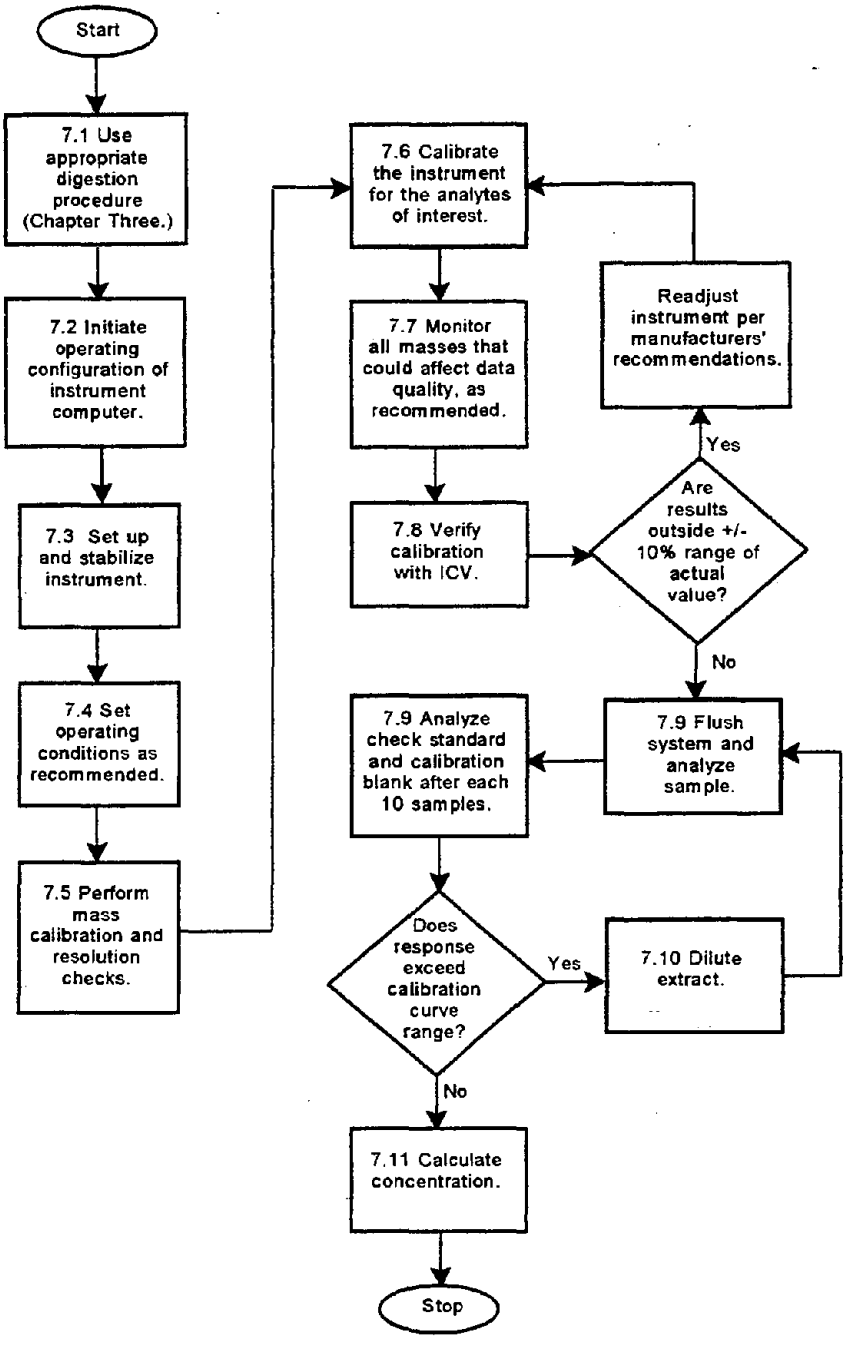
TABLE 7

COMPARISON OF TOTAL MERCURY RESULTS IN HEAVILY CONTAMINATED SOILS

Soil Sample	Mercury in µg/g	
	ICP-MS	CVAA
1	27.8	29.2
2	442	376
3	64.7	58.2
4	339	589
5	281	454
6	23.8	21.4
7	217	183
8	157	129
9	1670	1360
10	73.5	64.8
11	2090	1830
12	96.4	85.8
13	1080	1190
14	294	258
15	3300	2850
16	301	281
17	2130	2020
18	247	226
19	2630	2080

Source: Reference 16.

INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY



METHOD 6200

FIELD PORTABLE X-RAY FLUORESCENCE SPECTROMETRY FOR THE DETERMINATION OF ELEMENTAL CONCENTRATIONS IN SOIL AND SEDIMENT

1.0 SCOPE AND APPLICATION

1.1 This method is applicable to the in situ and intrusive analysis of the 26 analytes listed in Table 1 for soil and sediment samples. Some common elements are not listed in Table 1 because they are considered "light" elements that cannot be detected by field portable x-ray fluorescence (FPXRF). They are: lithium, beryllium, sodium, magnesium, aluminum, silicon, and phosphorus. Most of the analytes listed in Table 1 are of environmental concern, while a few others have interference effects or change the elemental composition of the matrix, affecting quantitation of the analytes of interest. Generally elements of atomic number 16 or greater can be detected and quantitated by FPXRF.

1.2 Detection limits depend on several factors, the analyte of interest, the type of detector used, the type of excitation source, the strength of the excitation source, count times used to irradiate the sample, physical matrix effects, chemical matrix effects, and interelement spectral interferences. General instrument detection limits for analytes of interest in environmental applications are shown in Table 1. These detection limits apply to a clean matrix of quartz sand (silicon dioxide) free of interelement spectral interferences using long (600-second) count times. These detection limits are given for guidance only and will vary depending on the sample matrix, which instrument is used, and operating conditions. A discussion of field performance-based detection limits is presented in Section 13.4 of this method. The clean matrix and field performance-based detection limits should be used for general planning purposes, and a third detection limit discussed, based on the standard deviation around single measurements, should be used in assessing data quality. This detection limit is discussed in Sections 9.7 and 11.3.

1.3 Use of this method is restricted to personnel either trained and knowledgeable in the operation of an XRF instrument or under the supervision of a trained and knowledgeable individual. This method is a screening method to be used with confirmatory analysis using EPA-approved methods. This method's main strength is as a rapid field screening procedure. The method detection limits (MDL) of FPXRF are above the toxicity characteristic regulatory level for most RCRA analytes. If the precision, accuracy, and detection limits of FPXRF meet the data quality objectives (DQOs) of your project, then XRF is a fast, powerful, cost effective technology for site characterization.

2.0 SUMMARY OF METHOD

2.1 The FPXRF technologies described in this method use sealed radioisotope sources to irradiate samples with x-rays. X-ray tubes are used to irradiate samples in the laboratory and are beginning to be incorporated into field portable instruments. When a sample is irradiated with x-rays, the source x-rays may undergo either scattering or absorption by sample atoms. This later process is known as the photoelectric effect. When an atom absorbs the source x-rays, the incident radiation dislodges electrons from the innermost shells of the atom, creating vacancies. The electron vacancies are filled by electrons cascading in from outer electron shells. Electrons in outer shells have higher energy states than inner shell electrons, and the outer shell electrons give off energy as they cascade down into the inner shell vacancies. This rearrangement of electrons results in emission of x-rays characteristic of the given atom. The emission of x-rays, in this manner, is termed x-ray fluorescence.

Three electron shells are generally involved in emission of x-rays during FPXRF analysis of environmental samples: the K, L, and M shells. A typical emission pattern, also called an emission spectrum, for a given metal has multiple intensity peaks generated from the emission of K, L, or M shell electrons. The most commonly measured x-ray emissions are from the K and L shells; only metals with an atomic number greater than 57 have measurable M shell emissions.

Each characteristic x-ray line is defined with the letter K, L, or M, which signifies which shell had the original vacancy and by a subscript alpha (α) or beta (β), which indicates the higher shell from which electrons fell to fill the vacancy and produce the x-ray. For example, a K_α line is produced by a vacancy in the K shell filled by an L shell electron, whereas a K_β line is produced by a vacancy in the K shell filled by an M shell electron. The K_α transition is on average 6 to 7 times more probable than the K_β transition; therefore, the K_α line is approximately 7 times more intense than the K_β line for a given element, making the K_α line the choice for quantitation purposes.

The K lines for a given element are the most energetic lines and are the preferred lines for analysis. For a given atom, the x-rays emitted from L transitions are always less energetic than those emitted from K transitions. Unlike the K lines, the main L emission lines (L_α and L_β) for an element are of nearly equal intensity. The choice of one or the other depends on what interfering element lines might be present. The L emission lines are useful for analyses involving elements of atomic number (Z) 58 (cerium) through 92 (uranium).

An x-ray source can excite characteristic x-rays from an element only if the source energy is greater than the absorption edge energy for the particular line group of the element, that is, the K absorption edge, L absorption edge, or M absorption edge energy. The absorption edge energy is somewhat greater than the corresponding line energy. Actually, the K absorption edge energy is approximately the sum of the K, L, and M line energies of the particular element, and the L absorption edge energy is approximately the sum of the L and M line energies. FPXRF is more sensitive to an element with an absorption edge energy close to but less than the excitation energy of the source. For example, when using a cadmium-109 source, which has an excitation energy of 22.1 kiloelectron volts (keV), FPXRF would exhibit better sensitivity for zirconium which has a K line energy of 15.7 keV than to chromium, which has a K line energy of 5.41 keV.

2.2 Under this method, inorganic analytes of interest are identified and quantitated using a field portable energy-dispersive x-ray fluorescence spectrometer. Radiation from one or more radioisotope sources or an electrically excited x-ray tube is used to generate characteristic x-ray emissions from elements in a sample. Up to three sources may be used to irradiate a sample. Each source emits a specific set of primary x-rays that excite a corresponding range of elements in a sample. When more than one source can excite the element of interest, the source is selected according to its excitation efficiency for the element of interest.

For measurement, the sample is positioned in front of the probe window. This can be done in two manners using FPXRF instruments: in situ or intrusive. If operated in the in situ mode, the probe window is placed in direct contact with the soil surface to be analyzed. When an FPXRF instrument is operated in the intrusive mode, a soil or sediment sample must be collected, prepared, and placed in a sample cup. The sample cup is then placed on top of the window inside a protective cover for analysis.

Sample analysis is then initiated by exposing the sample to primary radiation from the source. Fluorescent and backscattered x-rays from the sample enter through the detector window and are converted into electric pulses in the detector. The detector in FPXRF instruments is usually either a solid-state detector or a gas-filled proportional counter. Within the detector, energies of the characteristic x-rays are converted into a train of electric pulses, the amplitudes of which are linearly

proportional to the energy of the x-rays. An electronic multichannel analyzer (MCA) measures the pulse amplitudes, which is the basis of qualitative x-ray analysis. The number of counts at a given energy per unit of time is representative of the element concentration in a sample and is the basis for quantitative analysis. Most FPXRF instruments are menu-driven from software built into the units or from personal computers (PC).

The measurement time of each source is user-selectable. Shorter source measurement times (30 seconds) are generally used for initial screening and hot spot delineation, and longer measurement times (up to 300 seconds) are typically used to meet higher precision and accuracy requirements.

FPXRF instruments can be calibrated using the following methods: internally using fundamental parameters determined by the manufacturer, empirically based on site-specific calibration standards (SSCS), or based on Compton peak ratios. The Compton peak is produced by backscattering of the source radiation. Some FPXRF instruments can be calibrated using multiple methods.

3.0 DEFINITIONS

3.1 FPXRF: Field portable x-ray fluorescence.

3.2 MCA: Multichannel analyzer for measuring pulse amplitude.

3.3 SSCS: Site specific calibration standard.

3.4 FP: Fundamental parameter.

3.5 ROI: Region of interest.

3.6 SRM: Standard reference material. A standard containing certified amounts of metals in soil or sediment.

3.7 eV: Electron Volt. A unit of energy equivalent to the amount of energy gained by an electron passing through a potential difference of one volt.

3.8 Refer to Chapter One and Chapter Three for additional definitions.

4.0 INTERFERENCES

4.1 The total method error for FPXRF analysis is defined as the square root of the sum of squares of both instrument precision and user- or application-related error. Generally, instrument precision is the least significant source of error in FPXRF analysis. User- or application-related error is generally more significant and varies with each site and method used. Some sources of interference can be minimized or controlled by the instrument operator, but others cannot. Common sources of user- or application-related error are discussed below.

4.2 Physical matrix effects result from variations in the physical character of the sample. These variations may include such parameters as particle size, uniformity, homogeneity, and surface condition. For example, if any analyte exists in the form of very fine particles in a coarser-grained matrix, the analyte's concentration measured by the FPXRF will vary depending on how fine particles are distributed within the coarser-grained matrix. If the fine particles "settle" to the bottom of the sample cup, the analyte concentration measurement will be higher than if the fine particles are not

mixed in well and stay on top of the coarser-grained particles in the sample cup. One way to reduce such error is to grind and sieve all soil samples to a uniform particle size thus reducing sample-to-sample particle size variability. Homogeneity is always a concern when dealing with soil samples. Every effort should be made to thoroughly mix and homogenize soil samples before analysis. Field studies have shown heterogeneity of the sample generally has the largest impact on comparability with confirmatory samples.

4.3 Moisture content may affect the accuracy of analysis of soil and sediment sample analyses. When the moisture content is between 5 and 20 percent, the overall error from moisture may be minimal. However, moisture content may be a major source of error when analyzing samples of surface soil or sediment that are saturated with water. This error can be minimized by drying the samples in a convection or toaster oven. Microwave drying is not recommended because field studies have shown that microwave drying can increase variability between FPXRF data and confirmatory analysis and because metal fragments in the sample can cause arcing to occur in a microwave.

4.4 Inconsistent positioning of samples in front of the probe window is a potential source of error because the x-ray signal decreases as the distance from the radioactive source increases. This error is minimized by maintaining the same distance between the window and each sample. For the best results, the window of the probe should be in direct contact with the sample, which means that the sample should be flat and smooth to provide a good contact surface.

4.5 Chemical matrix effects result from differences in the concentrations of interfering elements. These effects occur as either spectral interferences (peak overlaps) or as x-ray absorption and enhancement phenomena. Both effects are common in soils contaminated with heavy metals. As examples of absorption and enhancement effects; iron (Fe) tends to absorb copper (Cu) x-rays, reducing the intensity of the Cu measured by the detector, while chromium (Cr) will be enhanced at the expense of Fe because the absorption edge of Cr is slightly lower in energy than the fluorescent peak of iron. The effects can be corrected mathematically through the use of fundamental parameter (FP) coefficients. The effects also can be compensated for using SSCS, which contain all the elements present on site that can interfere with one another.

4.6 When present in a sample, certain x-ray lines from different elements can be very close in energy and, therefore, can cause interference by producing a severely overlapped spectrum. The degree to which a detector can resolve the two different peaks depends on the energy resolution of the detector. If the energy difference between the two peaks in electron volts is less than the resolution of the detector in electron volts, then the detector will not be able to fully resolve the peaks.

The most common spectrum overlaps involve the K_β line of element Z-1 with the K_α line of element Z. This is called the K_α/K_β interference. Because the K_α:K_β intensity ratio for a given element usually is about 7:1, the interfering element, Z-1, must be present at large concentrations to cause a problem. Two examples of this type of spectral interference involve the presence of large concentrations of vanadium (V) when attempting to measure Cr or the presence of large concentrations of Fe when attempting to measure cobalt (Co). The V K_α and K_β energies are 4.95 and 5.43 keV, respectively, and the Cr K_α energy is 5.41 keV. The Fe K_α and K_β energies are 6.40 and 7.06 keV, respectively, and the Co K_α energy is 6.92 keV. The difference between the V K_β and Cr K_α energies is 20 eV, and the difference between the Fe K_β and the Co K_α energies is 140 eV. The resolution of the highest-resolution detectors in FPXRF instruments is 170 eV. Therefore, large amounts of V and Fe will interfere with quantitation of Cr or Co, respectively. The presence of Fe is a frequent problem because it is often found in soils at tens of thousands of parts per million (ppm).

4.7 Other interferences can arise from K/L, K/M, and L/M line overlaps, although these overlaps are less common. Examples of such overlap involve arsenic (As) K_α/lead (Pb) L_α and sulfur (S) K_α/Pb M_α. In the As/Pb case, Pb can be measured from the Pb L_β line, and As can be measured from either the As K_α or the As K_β line; in this way the interference can be corrected. If the As K_β line is used, sensitivity will be decreased by a factor of two to five times because it is a less intense line than the As K_α line. If the As K_α line is used in the presence of Pb, mathematical corrections within the instrument software can be used to subtract out the Pb interference. However, because of the limits of mathematical corrections, As concentrations cannot be efficiently calculated for samples with Pb:As ratios of 10:1 or more. This high ratio of Pb to As may result in no As being reported regardless of the actual concentration present.

No instrument can fully compensate for this interference. It is important for an operator to understand this limitation of FPXRF instruments and consult with the manufacturer of the FPXRF instrument to evaluate options to minimize this limitation. The operator's decision will be based on action levels for metals in soil established for the site, matrix effects, capabilities of the instrument, data quality objectives, and the ratio of lead to arsenic known to be present at the site. If a site is encountered that contains lead at concentrations greater than ten times the concentration of arsenic it is advisable that all critical soil samples be sent off site for confirmatory analysis by an EPA-approved method.

4.8 If SSCS are used to calibrate an FPXRF instrument, the samples collected must be representative of the site under investigation. Representative soil sampling ensures that a sample or group of samples accurately reflects the concentrations of the contaminants of concern at a given time and location. Analytical results for representative samples reflect variations in the presence and concentration ranges of contaminants throughout a site. Variables affecting sample representativeness include differences in soil type, contaminant concentration variability, sample collection and preparation variability, and analytical variability, all of which should be minimized as much as possible.

4.9 Soil physical and chemical effects may be corrected using SSCS that have been analyzed by inductively coupled plasma (ICP) or atomic absorption (AA) methods. However, a major source of error can be introduced if these samples are not representative of the site or if the analytical error is large. Another concern is the type of digestion procedure used to prepare the soil samples for the reference analysis. Analytical results for the confirmatory method will vary depending on whether a partial digestion procedure, such as SW-846 Method 3050, or a total digestion procedure, such as Method 3052 is used. It is known that depending on the nature of the soil or sediment, Method 3050 will achieve differing extraction efficiencies for different analytes of interest. The confirmatory method should meet the project data quality objectives.

XRF measures the total concentration of an element; therefore, to achieve the greatest comparability of this method with the reference method (reduced bias), a total digestion procedure should be used for sample preparation. However, in the study used to generate the performance data for this method, the confirmatory method used was Method 3050, and the FPXRF data compared very well with regression correlation coefficients (r^2 often exceeding 0.95, except for barium and chromium. See Table 9 in Section 17.0). The critical factor is that the digestion procedure and analytical reference method used should meet the data quality objectives (DQOs) of the project and match the method used for confirmation analysis.

4.10 Ambient temperature changes can affect the gain of the amplifiers producing instrument drift. Gain or drift is primarily a function of the electronics (amplifier or preamplifier) and not the detector as most instrument detectors are cooled to a constant temperature. Most FPXRF instruments have a built-in automatic gain control. If the automatic gain control is allowed to make

periodic adjustments, the instrument will compensate for the influence of temperature changes on its energy scale. If the FPXRF instrument has an automatic gain control function, the operator will not have to adjust the instrument's gain unless an error message appears. If an error message appears, the operator should follow the manufacturer's procedures for troubleshooting the problem. Often, this involves performing a new energy calibration. The performance of an energy calibration check to assess drift is a quality control measure discussed in Section 9.2.

If the operator is instructed by the manufacturer to manually conduct a gain check because of increasing or decreasing ambient temperature, it is standard to perform a gain check after every 10 to 20 sample measurements or once an hour whichever is more frequent. It is also suggested that a gain check be performed if the temperature fluctuates more than 10 to 20°F. The operator should follow the manufacturer's recommendations for gain check frequency.

5.0 SAFETY

5.1 Proper training for the safe operation of the instrument and radiation training should be completed by the analyst prior to analysis. Radiation safety for each specific instrument can be found in the operators manual. Protective shielding should never be removed by the analyst or any personnel other than the manufacturer. The analyst should be aware of the local state and national regulations that pertain to the use of radiation-producing equipment and radioactive materials with which compliance is required. Licenses for radioactive materials are of two types; (1) general license which is usually provided by the manufacturer for receiving, acquiring, owning, possessing, using, and transferring radioactive material incorporated in a device or equipment, and (2) specific license which is issued to named persons for the operation of radioactive instruments as required by local state agencies. There should be a person appointed within the organization that is solely responsible for properly instructing all personnel, maintaining inspection records, and monitoring x-ray equipment at regular intervals. A copy of the radioactive material licenses and leak tests should be present with the instrument at all times and available to local and national authorities upon request. X-ray tubes do not require radioactive material licenses or leak tests, but do require approvals and licenses which vary from state to state. In addition, fail-safe x-ray warning lights should be illuminated whenever an x-ray tube is energized. Provisions listed above concerning radiation safety regulations, shielding, training, and responsible personnel apply to x-ray tubes just as to radioactive sources. In addition, a log of the times and operating conditions should be kept whenever an x-ray tube is energized. Finally, an additional hazard present with x-ray tubes is the danger of electric shock from the high voltage supply. The danger of electric shock is as substantial as the danger from radiation but is often overlooked because of its familiarity.

5.2 Radiation monitoring equipment should be used with the handling of the instrument. The operator and the surrounding environment should be monitored continually for analyst exposure to radiation. Thermal luminescent detectors (TLD) in the form of badges and rings are used to monitor operator radiation exposure. The TLDs should be worn in the area of most frequent exposure. The maximum permissible whole-body dose from occupational exposure is 5 Roentgen Equivalent Man (REM) per year. Possible exposure pathways for radiation to enter the body are ingestion, inhaling, and absorption. The best precaution to prevent radiation exposure is distance and shielding.

5.3 Refer to Chapter Three for guidance on some proper safety protocols.

6.0 EQUIPMENT AND SUPPLIES

6.1 FPXRF Spectrometer: An FPXRF spectrometer consists of four major components: (1) a source that provides x-rays; (2) a sample presentation device; (3) a detector that converts x-

ray-generated photons emitted from the sample into measurable electronic signals; and (4) a data processing unit that contains an emission or fluorescence energy analyzer, such as an MCA, that processes the signals into an x-ray energy spectrum from which elemental concentrations in the sample may be calculated, and a data display and storage system. These components and additional, optional items, are discussed below.

6.1.1 Excitation Sources: Most FPXRF instruments use sealed radioisotope sources to produce x-rays in order to irradiate samples. The FPXRF instrument may contain between one and three radioisotope sources. Common radioisotope sources used for analysis for metals in soils are iron (Fe)-55, cadmium (Cd)-109, americium (Am)-241, and curium (Cm)-244. These sources may be contained in a probe along with a window and the detector; the probe is connected to a data reduction and handling system by means of a flexible cable. Alternatively, the sources, window, and detector may be included in the same unit as the data reduction and handling system.

The relative strength of the radioisotope sources is measured in units of millicuries (mCi). All other components of the FPXRF system being equal, the stronger the source, the greater the sensitivity and precision of a given instrument. Radioisotope sources undergo constant decay. In fact, it is this decay process that emits the primary x-rays used to excite samples for FPXRF analysis. The decay of radioisotopes is measured in "half-lives." The half-life of a radioisotope is defined as the length of time required to reduce the radioisotopes strength or activity by half. Developers of FPXRF technologies recommend source replacement at regular intervals based on the source's half-life. The characteristic x-rays emitted from each of the different sources have energies capable of exciting a certain range of analytes in a sample. Table 2 summarizes the characteristics of four common radioisotope sources.

X-ray tubes have higher radiation output, no intrinsic lifetime limit, produce constant output over their lifetime, and do not have the disposal problems of radioactive sources but are just now appearing in FPXRF instruments An electrically-excited x-ray tube operates by bombarding an anode with electrons accelerated by a high voltage. The electrons gain an energy in electron volts equal to the accelerating voltage and can excite atomic transitions in the anode, which then produces characteristic x-rays. These characteristic x-rays are emitted through a window which contains the vacuum required for the electron acceleration. An important difference between x-ray tubes and radioactive sources is that the electrons which bombard the anode also produce a continuum of x-rays across a broad range of energies in addition to the characteristic x-rays. This continuum is weak compared to the characteristic x-rays but can provide substantial excitation since it covers a broad energy range. It has the undesired property of producing background in the spectrum near the analyte x-ray lines when it is scattered by the sample. For this reason a filter is often used between the x-ray tube and the sample to suppress the continuum radiation while passing the characteristic x-rays from the anode. This filter is sometimes incorporated into the window of the x-ray tube. The choice of accelerating voltage is governed by the anode material, since the electrons must have sufficient energy to excite the anode, which requires a voltage greater than the absorption edge of the anode material. The anode is most efficiently excited by voltages 2 to 2.5 times the edge energy (most x-rays per unit power to the tube), although voltages as low as 1.5 times the absorption edge energy will work. The characteristic x-rays emitted by the anode are capable of exciting a range of elements in the sample just as with a radioactive source. Table 3 gives the recommended operating voltages and the sample elements excited for some common anodes.

6.1.2 Sample Presentation Device: FPXRF instruments can be operated in two modes: in situ and intrusive. If operated in the in situ mode, the probe window is placed in direct contact with the soil surface to be analyzed. When an FPXRF instrument is operated in the intrusive mode, a soil or sediment sample must be collected, prepared, and placed in a sample cup. For most FPXRF instruments operated in the intrusive mode, the probe is rotated so that the window faces upward. A protective sample cover is placed over the window, and the sample cup is placed on top of the window inside the protective sample cover for analysis.

6.1.3 Detectors: The detectors in the FPXRF instruments can be either solid-state detectors or gas-filled, proportional counter detectors. Common solid-state detectors include mercuric iodide (HgI_2), silicon pin diode and lithium-drifted silicon Si(Li). The HgI_2 detector is operated at a moderately subambient temperature controlled by a low power thermoelectric cooler. The silicon pin diode detector also is cooled via the thermoelectric Peltier effect. The Si(Li) detector must be cooled to at least -90 °C either with liquid nitrogen or by thermoelectric cooling via the Peltier effect. Instruments with a Si(Li) detector have an internal liquid nitrogen dewar with a capacity of 0.5 to 1.0 liter. Proportional counter detectors are rugged and lightweight, which are important features of a field portable detector. However, the resolution of a proportional counter detector is not as good as that of a solid-state detector. The energy resolution of a detector for characteristic x-rays is usually expressed in terms of full width at half-maximum (FWHM) height of the manganese K_α peak at 5.89 keV. The typical resolutions of the above mentioned detectors are as follows: HgI_2-270 eV; silicon pin diode-250 eV; Si(Li)–170 eV; and gas-filled, proportional counter-750 eV.

During operation of a solid-state detector, an x-ray photon strikes a biased, solid-state crystal and loses energy in the crystal by producing electron-hole pairs. The electric charge produced is collected and provides a current pulse that is directly proportional to the energy of the x-ray photon absorbed by the crystal of the detector. A gas-filled, proportional counter detector is an ionization chamber filled with a mixture of noble and other gases. An x-ray photon entering the chamber ionizes the gas atoms. The electric charge produced is collected and provides an electric signal that is directly proportional to the energy of the x-ray photon absorbed by the gas in the detector.

6.1.4 Data Processing Units: The key component in the data processing unit of an FPXRF instrument is the MCA. The MCA receives pulses from the detector and sorts them by their amptitudes (energy level). The MCA counts pulses per second to determine the height of the peak in a spectrum, which is indicative of the target analyte's concentration. The spectrum of element peaks are built on the MCA. The MCAs in FPXRF instruments have from 256 to 2,048 channels. The concentrations of target analytes are usually shown in parts per million on a liquid crystal display (LCD) in the instrument. FPXRF instruments can store both spectra and from 100 to 500 sets of numerical analytical results. Most FPXRF instruments are menu-driven from software built into the units or from PCs. Once the data–storage memory of an FPXRF unit is full, data can be downloaded by means of an RS-232 port and cable to a PC.

6.2 Spare battery chargers.

6.3 Polyethylene sample cups: 31 millimeters (mm) to 40 mm in diameter with collar, or equivalent (appropriate for FPXRF instrument).

6.4 X-ray window film: Mylar™, Kapton™, Spectrolene™, polypropylene, or equivalent; 2.5 to 6.0 micrometers (µm) thick.

6.5 Mortar and pestle: glass, agate, or aluminum oxide; for grinding soil and sediment samples.

6.6 Containers: glass or plastic to store samples.

6.7 Sieves: 60-mesh (0.25 mm), stainless-steel, Nylon, or equivalent for preparing soil and sediment samples.

6.8 Trowels: for smoothing soil surfaces and collecting soil samples.

6.9 Plastic bags: used for collection and homogenization of soil samples.

6.10 Drying oven: standard convection or toaster oven, for soil and sediment samples that require drying.

7.0 REAGENTS AND STANDARDS

7.1 Pure Element Standards: Each pure, single-element standard is intended to produce strong characteristic x-ray peaks of the element of interest only. Other elements present must not contribute to the fluorescence spectrum. A set of pure element standards for commonly sought analytes is supplied by the instrument manufacturer, if required for the instrument; not all instruments require the pure element standards. The standards are used to set the region of interest (ROI) for each element. They also can be used as energy calibration and resolution check samples.

7.2 Site-specific Calibration Standards: Instruments that employ fundamental parameters (FP) or similar mathematical models in minimizing matrix effects may not require SSCS. If the FP calibration model is to be optimized or if empirical calibration is necessary, then SSCSs must be collected, prepared, and analyzed.

7.2.1 The SSCS must be representative of the matrix to be analyzed by FPXRF. These samples must be well homogenized. A minimum of ten samples spanning the concentration ranges of the analytes of interest and of the interfering elements must be obtained from the site. A sample size of 4 to 8 ounces is recommended, and standard glass sampling jars should be used.

7.2.2 Each sample should be oven-dried for 2 to 4 hours at a temperature of less than 150°C. If mercury is to be analyzed, a separate sample portion must remain undried, as heating may volatilize the mercury. When the sample is dry, all large, organic debris and nonrepresentative material, such as twigs, leaves, roots, insects, asphalt, and rock should be removed. The sample should be ground with a mortar and pestle and passed through a 60-mesh sieve. Only the coarse rock fraction should remain on the screen.

7.2.3 The sample should be homogenized by using a riffle splitter or by placing 150 to 200 grams of the dried, sieved sample on a piece of kraft or butcher paper about 1.5 by 1.5 feet in size. Each corner of the paper should be lifted alternately, rolling the soil over on itself and toward the opposite corner. The soil should be rolled on itself 20 times. Approximately 5 grams of the sample should then be removed and placed in a sample cup for FPXRF analysis. The rest of the prepared sample should be sent off site for ICP or AA analysis. The method use for confirmatory analysis should meet the data quality objectives of the project.

7.3 Blank Samples: The blank samples should be from a "clean" quartz or silicon dioxide matrix that is free of any analytes at concentrations above the method detection limits. These

samples are used to monitor for cross-contamination and laboratory-induced contaminants or interferences.

7.4 Standard Reference Materials: Standard reference materials (SRM) are standards containing certified amounts of metals in soil or sediment. These standards are used for accuracy and performance checks of FPXRF analyses. SRMs can be obtained from the National Institute of Standards and Technology (NIST), the U.S. Geological Survey (USGS), the Canadian National Research Council, and the national bureau of standards in foreign nations. Pertinent NIST SRMs for FPXRF analysis include 2704, Buffalo River Sediment; 2709, San Joaquin Soil; and 2710 and 2711, Montana Soil. These SRMs contain soil or sediment from actual sites that has been analyzed using independent inorganic analytical methods by many different laboratories.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample handling and preservation procedures used in FPXRF analyses should follow the guidelines in Chapter Three, Inorganic Analytes.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for additional guidance on quality assurance protocols. All field data sheets and quality control data should be maintained for reference or inspection.

9.2 Energy Calibration Check: To determine whether an FPXRF instrument is operating within resolution and stability tolerances, an energy calibration check should be run. The energy calibration check determines whether the characteristic x-ray lines are shifting, which would indicate drift within the instrument. As discussed in Section 4.10, this check also serves as a gain check in the event that ambient temperatures are fluctuating greatly (> 10 to 20°F).

The energy calibration check should be run at a frequency consistent with manufacturers recommendations. Generally, this would be at the beginning of each working day, after the batteries are changed or the instrument is shut off, at the end of each working day, and at any other time when the instrument operator believes that drift is occurring during analysis. A pure element such as iron, manganese, copper, or lead is often used for the energy calibration check. A manufacturer-recommended count time per source should be used for the check.

9.2.1 The instrument manufacturer's manual specifies the channel or kiloelectron volt level at which a pure element peak should appear and the expected intensity of the peak. The intensity and channel number of the pure element as measured using the radioactive source should be checked and compared to the manufacturer's recommendation. If the energy calibration check does not meet the manufacturer's criteria, then the pure element sample should be repositioned and reanalyzed. If the criteria are still not met, then an energy calibration should be performed as described in the manufacturer's manual. With some FPXRF instruments, once a spectrum is acquired from the energy calibration check, the peak can be optimized and realigned to the manufacturer's specifications using their software.

9.3 Blank Samples: Two types of blank samples should be analyzed for FPXRF analysis: instrument blanks and method blanks. An instrument blank is used to verify that no contamination exists in the spectrometer or on the probe window.

9.3.1 The instrument blank can be silicon dioxide, a Teflon block, a quartz block, "clean" sand, or lithium carbonate. This instrument blank should be analyzed on each working day before and after analyses are conducted and once per every twenty samples. An

instrument blank should also be analyzed whenever contamination is suspected by the analyst. The frequency of analysis will vary with the data quality objectives of the project. A manufacturer-recommended count time per source should be used for the blank analysis. No element concentrations above the method detection limits should be found in the instrument blank. If concentrations exceed these limits, then the probe window and the check sample should be checked for contamination. If contamination is not a problem, then the instrument must be "zeroed" by following the manufacturer's instructions.

9.3.2 A method blank is used to monitor for laboratory-induced contaminants or interferences. The method blank can be "clean" silica sand or lithium carbonate that undergoes the same preparation procedure as the samples. A method blank must be analyzed at least daily. The frequency of analysis will depend on the data quality objectives of the project. To be acceptable, a method blank must not contain any analyte at a concentration above its method detection limit. If an analyte's concentration exceeds its method detection limit, the cause of the problem must be identified, and all samples analyzed with the method blank must be reanalyzed.

9.4 Calibration Verification Checks: A calibration verification check sample is used to check the accuracy of the instrument and to assess the stability and consistency of the analysis for the analytes of interest. A check sample should be analyzed at the beginning of each working day, during active sample analyses, and at the end of each working day. The frequency of calibration checks during active analysis will depend on the data quality objectives of the project. The check sample should be a well characterized soil sample from the site that is representative of site samples in terms of particle size and degree of homogeneity and that contains contaminants at concentrations near the action levels. If a site-specific sample is not available, then an NIST or other SRM that contains the analytes of interest can be used to verify the accuracy of the instrument. The measured value for each target analyte should be within ±20 percent (%D) of the true value for the calibration verification check to be acceptable. If a measured value falls outside this range, then the check sample should be reanalyzed. If the value continues to fall outside the acceptance range, the instrument should be recalibrated, and the batch of samples analyzed before the unacceptable calibration verification check must be reanalyzed.

9.5 Precision Measurements: The precision of the method is monitored by analyzing a sample with low, moderate, or high concentrations of target analytes. The frequency of precision measurements will depend on the data quality objectives for the data. A minimum of one precision sample should be run per day. Each precision sample should be analyzed 7 times in replicate. It is recommended that precision measurements be obtained for samples with varying concentration ranges to assess the effect of concentration on method precision. Determining method precision for analytes at concentrations near the site action levels can be extremely important if the FPXRF results are to be used in an enforcement action; therefore, selection of at least one sample with target analyte concentrations at or near the site action levels or levels of concern is recommended. A precision sample is analyzed by the instrument for the same field analysis time as used for other project samples. The relative standard deviation (RSD) of the sample mean is used to assess method precision. For FPXRF data to be considered adequately precise, the RSD should not be greater than 20 percent with the exception of chromium. RSD values for chromium should not be greater than 30 percent.

The equation for calculating RSD is as follows:

$$RSD = (SD/Mean\ Concentration) \times 100$$

where:

RSD	=	Relative standard deviation for the precision measurement for the analyte
SD	=	Standard deviation of the concentration for the analyte
Mean Concentration	=	Mean concentration for the analyte

The precision or reproducibility of a measurement will improve with increasing count time, however, increasing the count time by a factor of 4 will provide only 2 times better precision, so there is a point of diminishing return. Increasing the count time also improves the detection limit, but decreases sample throughput.

9.6 Detection Limits: Results for replicate analyses of a low-concentration sample, SSCS, or SRM can be used to generate an average site-specific method detection and quantitation limits. In this case, the method detection limit is defined as 3 times the standard deviation of the results for the low-concentration samples and the method quantitation limit is defined as 10 times the standard deviation of the same results. Another means of determining method detection and quantitation limits involves use of counting statistics. In FPXRF analysis, the standard deviation from counting statistics is defined as $SD = (N)^{1/2}$, where SD is the standard deviation for a target analyte peak and N is the net counts for the peak of the analyte of interest (i.e., gross counts minus background under the peak). Three times this standard deviation would be the method detection limit and 10 times this standard deviation would be the method quantitation limit. If both of the above mentioned approaches are used to calculate method detection limits, the larger of the standard deviations should be used to provide the more conservative detection limits.

This SD based detection limit criteria must be used by the operator to evaluate each measurement for its useability. A measurement above the average calculated or manufacturer's detection limit, but smaller than three times its associated SD, should not be used as a quantitative measurement. Conversely, if the measurement is below the average calculated or manufacturer's detection limit, but greater than three times its associated SD. It should be coded as an estimated value.

9.7 Confirmatory Samples: The comparability of the FPXRF analysis is determined by submitting FPXRF-analyzed samples for analysis at a laboratory. The method of confirmatory analysis must meet the project and XRF measurement data quality objectives. The confirmatory samples must be splits of the well homogenized sample material. In some cases the prepared sample cups can be submitted. A minimum of 1 sample for each 20 FPXRF-analyzed samples should be submitted for confirmatory analysis. This frequency will depend on data quality objectives. The confirmatory analyses can also be used to verify the quality of the FPXRF data. The confirmatory samples should be selected from the lower, middle, and upper range of concentrations measured by the FPXRF. They should also include samples with analyte concentrations at or near the site action levels. The results of the confirmatory analysis and FPXRF analyses should be evaluated with a least squares linear regression analysis. If the measured concentrations span more than one order of magnitude, the data should be log-transformed to standardize variance which is proportional to the magnitude of measurement. The correlation coefficient (r^2) for the results should be 0.7 or greater for the FPXRF data to be considered screening level data. If the r^2 is 0.9 or greater and inferential statistics indicate the FPXRF data and the confirmatory data are statistically equivalent at a 99 percent confidence level, the data could potentially meet definitive level data criteria.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Instrument Calibration: Instrument calibration procedures vary among FPXRF instruments. Users of this method should follow the calibration procedures outlined in the operator's manual for each specific FPXRF instrument. Generally, however, three types of calibration procedures exist for FPXRF instruments: FP calibration, empirical calibration, and the Compton peak ratio or normalization method. These three types of calibration are discussed below.

10.2 Fundamental Parameters Calibration: FP calibration procedures are extremely variable. An FP calibration provides the analyst with a "standardless" calibration. The advantages of FP calibrations over empirical calibrations include the following:

- No previously collected site-specific samples are required, although site-specific samples with confirmed and validated analytical results for all elements present could be used.

- Cost is reduced because fewer confirmatory laboratory results or calibration standards are required.

However, the analyst should be aware of the limitations imposed on FP calibration by particle size and matrix effects. These limitations can be minimized by adhering to the preparation procedure described in Section 7.2. The two FP calibration processes discussed below are based on an effective energy FP routine and a back scatter with FP (BFP) routine. Each FPXRF FP calibration process is based on a different iterative algorithmic method. The calibration procedure for each routine is explained in detail in the manufacturer's user manual for each FPXRF instrument; in addition, training courses are offered for each instrument.

10.2.1 Effective Energy FP Calibration: The effective energy FP calibration is performed by the manufacturer before an instrument is sent to the analyst. Although SSCS can be used, the calibration relies on pure element standards or SRMs such as those obtained from NIST for the FP calibration. The effective energy routine relies on the spectrometer response to pure elements and FP iterative algorithms to compensate for various matrix effects.

Alpha coefficients are calculated using a variation of the Sherman equation, which calculates theoretical intensities from the measurement of pure element samples. These coefficients indicate the quantitative effect of each matrix element on an analyte's measured x-ray intensity. Next, the Lachance Traill algorithm is solved as a set of simultaneous equations based on the theoretical intensities. The alpha coefficients are then downloaded into the specific instrument.

The working effective energy FP calibration curve must be verified before sample analysis begins on each working day, after every 20 samples are analyzed, and at the end of sampling. This verification is performed by analyzing either an NIST SRM or an SSCS that is representative of the site-specific samples. This SRM or SSCS serves as a calibration check. A manufacturer-recommended count time per source should be used for the calibration check. The analyst must then adjust the y-intercept and slope of the calibration curve to best fit the known concentrations of target analytes in the SRM or SSCS.

A percent difference (%D) is then calculated for each target analyte. The %D should be within ±20 percent of the certified value for each analyte. If the %D falls outside this acceptance range, then the calibration curve should be adjusted by varying the slope of the

line or the y-intercept value for the analyte. The SRM or SSCS is reanalyzed until the %D falls within ±20 percent. The group of 20 samples analyzed before an out-of-control calibration check should be reanalyzed.

The equation to calibrate %D is as follows:

$$\%D = ((C_s - C_k) / C_k) \times 100$$

where:

%D = Percent difference
C_k = Certified concentration of standard sample
C_s = Measured concentration of standard sample

10.2.2 BFP Calibration: BFP calibration relies on the ability of the liquid nitrogen-cooled, Si(Li) solid-state detector to separate the coherent (Compton) and incoherent (Rayleigh) backscatter peaks of primary radiation. These peak intensities are known to be a function of sample composition, and the ratio of the Compton to Rayleigh peak is a function of the mass absorption of the sample. The calibration procedure is explained in detail in the instrument manufacturer's manual. Following is a general description of the BFP calibration procedure.

The concentrations of all detected and quantified elements are entered into the computer software system. Certified element results for an NIST SRM or confirmed and validated results for an SSCS can be used. In addition, the concentrations of oxygen and silicon must be entered; these two concentrations are not found in standard metals analyses. The manufacturer provides silicon and oxygen concentrations for typical soil types. Pure element standards are then analyzed using a manufacturer-recommended count time per source. The results are used to calculate correction factors in order to adjust for spectrum overlap of elements.

The working BFP calibration curve must be verified before sample analysis begins on each working day, after every 20 samples are analyzed, and at the end of the analysis. This verification is performed by analyzing either an NIST SRM or an SSCS that is representative of the site-specific samples. This SRM or SSCS serves as a calibration check. The standard sample is analyzed using a manufacturer-recommended count time per source to check the calibration curve. The analyst must then adjust the y-intercept and slope of the calibration curve to best fit the known concentrations of target analytes in the SRM or SSCS.

A %D is then calculated for each target analyte. The %D should fall within ±20 percent of the certified value for each analyte. If the %D falls outside this acceptance range, then the calibration curve should be adjusted by varying the slope of the line the y-intercept value for the analyte. The standard sample is reanalyzed until the %D falls within ±20 percent. The group of 20 samples analyzed before an out-of-control calibration check should be reanalyzed.

10.3 Empirical Calibration: An empirical calibration can be performed with SSCS, site-typical standards, or standards prepared from metal oxides. A discussion of SSCS is included in Section 7.2; if no previously characterized samples exist for a specific site, site-typical standards can be used. Site-typical standards may be selected from commercially available characterized soils or from SSCS prepared for another site. The site-typical standards should closely approximate the site's soil matrix with respect to particle size distribution, mineralogy, and contaminant analytes. If neither SSCS nor site-typical standards are available, it is possible to make gravimetric standards

by adding metal oxides to a "clean" sand or silicon dioxide matrix that simulates soil. Metal oxides can be purchased from various chemical vendors. If standards are made on site, a balance capable of weighing items to at least two decimal places is required. Concentrated ICP or AA standard solutions can also be used to make standards. These solutions are available in concentrations of 10,000 parts per million, thus only small volumes have to be added to the soil.

An empirical calibration using SSCS involves analysis of SSCS by the FPXRF instrument and by a conventional analytical method such as ICP or AA. A total acid digestion procedure should be used by the laboratory for sample preparation. Generally, a minimum of 10 and a maximum of 30 well characterized SSCS, site-typical standards, or prepared metal oxide standards are required to perform an adequate empirical calibration. The number of required standards depends on the number of analytes of interest and interfering elements. Theoretically, an empirical calibration with SSCS should provide the most accurate data for a site because the calibration compensates for site-specific matrix effects.

The first step in an empirical calibration is to analyze the pure element standards for the elements of interest. This enables the instrument to set channel limits for each element for spectral deconvolution. Next the SSCS, site-typical standards, or prepared metal oxide standards are analyzed using a count time of 200 seconds per source or a count time recommended by the manufacturer. This will produce a spectrum and net intensity of each analyte in each standard. The analyte concentrations for each standard are then entered into the instrument software; these concentrations are those obtained from the laboratory, the certified results, or the gravimetrically determined concentrations of the prepared standards. This gives the instrument analyte values to regress against corresponding intensities during the modeling stage. The regression equation correlates the concentrations of an analyte with its net intensity.

The calibration equation is developed using a least squares fit regression analysis. After the regression terms to be used in the equation are defined, a mathematical equation can be developed to calculate the analyte concentration in an unknown sample. In some FPXRF instruments, the software of the instrument calculates the regression equation. The software uses calculated intercept and slope values to form a multiterm equation. In conjunction with the software in the instrument, the operator can adjust the multiterm equation to minimize interelement interferences and optimize the intensity calibration curve.

It is possible to define up to six linear or nonlinear terms in the regression equation. Terms can be added and deleted to optimize the equation. The goal is to produce an equation with the smallest regression error and the highest correlation coefficient. These values are automatically computed by the software as the regression terms are added, deleted, or modified. It is also possible to delete data points from the regression line if these points are significant outliers or if they are heavily weighing the data. Once the regression equation has been selected for an analyte, the equation can be entered into the software for quantitation of analytes in subsequent samples. For an empirical calibration to be acceptable, the regression equation for a specific analyte should have a correlation coefficient of 0.98 or greater or meet the DQOs of the project.

In an empirical calibration, one must apply the DQOs of the project and ascertain critical or action levels for the analytes of interest. It is within these concentration ranges or around these action levels that the FPXRF instrument should be calibrated most accurately. It may not be possible to develop a good regression equation over several orders of analyte concentration.

10.4 Compton Normalization Method: The Compton normalization method is based on analysis of a single, certified standard and normalization for the Compton peak. The Compton peak is produced from incoherent backscattering of x-ray radiation from the excitation source and is present in the spectrum of every sample. The Compton peak intensity changes with differing matrices. Generally, matrices dominated by lighter elements produce a larger Compton peak, and those dominated by heavier elements produce a smaller Compton peak. Normalizing to the Compton peak can reduce problems with varying matrix effects among samples. Compton normalization is similar to the use of internal standards in organics analysis. The Compton normalization method may not be effective when analyte concentrations exceed a few percent.

The certified standard used for this type of calibration could be an NIST SRM such as 2710 or 2711. The SRM must be a matrix similar to the samples and must contain the analytes of interests at concentrations near those expected in the samples. First, a response factor has to be determined for each analyte. This factor is calculated by dividing the net peak intensity by the analyte concentration. The net peak intensity is gross intensity corrected for baseline interference. Concentrations of analytes in samples are then determined by multiplying the baseline corrected analyte signal intensity by the normalization factor and by the response factor. The normalization factor is the quotient of the baseline corrected Compton K_α peak intensity of the SRM divided by that of the samples. Depending on the FPXRF instrument used, these calculations may be done manually or by the instrument software.

11.0 PROCEDURE

11.1 Operation of the various FPXRF instruments will vary according to the manufacturers' protocols. Before operating any FPXRF instrument, one should consult the manufacturer's manual. Most manufacturers recommend that their instruments be allowed to warm up for 15 to 30 minutes before analysis of samples. This will help alleviate drift or energy calibration problems later on in analysis.

11.2 Each FPXRF instrument should be operated according to the manufacturer's recommendations. There are two modes in which FPXRF instruments can be operated: in situ and intrusive. The in situ mode involves analysis of an undisturbed soil sediment or sample. Intrusive analysis involves collection and preparation of a soil or sediment sample before analysis. Some FPXRF instruments can operate in both modes of analysis, while others are designed to operate in only one mode. The two modes of analysis are discussed below.

11.3 For in situ analysis, one requirement is that any large or nonrepresentative debris be removed from the soil surface before analysis. This debris includes rocks, pebbles, leaves, vegetation, roots, and concrete. Another requirement is that the soil surface be as smooth as possible so that the probe window will have good contact with the surface. This may require some leveling of the surface with a stainless-steel trowel. During the study conducted to provide data for this method, this modest amount of sample preparation was found to take less than 5 minutes per sample location. The last requirement is that the soil or sediment not be saturated with water. Manufacturers state that their FPXRF instruments will perform adequately for soils with moisture contents of 5 to 20 percent but will not perform well for saturated soils, especially if ponded water exists on the surface. Another recommended technique for *in situ* analysis is to tamp the soil to increase soil density and compactness for better repeatability and representativeness. This condition is especially important for heavy element analysis, such as barium. Source count times for in situ analysis usually range from 30 to 120 seconds, but source count times will vary among instruments and depending on required detection limits.

11.4 For intrusive analysis of surface or sediment, it is recommended that a sample be collected from a 4- by 4-inch square that is 1 inch deep. This will produce a soil sample of approximately 375 grams or 250 cm^3, which is enough soil to fill an 8-ounce jar. The sample should be homogenized, dried, and ground before analysis. The sample can be homogenized before or after drying. The homogenization technique to be used after drying is discussed in Section 4.2. If the sample is homogenized before drying, it should be thoroughly mixed in a beaker or similar container, or if the sample is moist and has a high clay content, it can be kneaded in a plastic bag. One way to monitor homogenization when the sample is kneaded in a plastic bag is to add sodium fluorescein dye to the sample. After the moist sample has been homogenized, it is examined under an ultraviolet light to assess the distribution of sodium fluorescein throughout the sample. If the fluorescent dye is evenly distributed in the sample, homogenization is considered complete; if the dye is not evenly distributed, mixing should continue until the sample has been thoroughly homogenized. During the study conducted to provide data for this method, the homogenization procedure using the fluorescein dye required 3 to 5 minutes per sample. As demonstrated in Sections 13.5 and 13.7, homogenization has the greatest impact on the reduction of sampling variability. It produces little or no contamination. Often, it can be used without the more labor intensive steps of drying, grinding, and sieving given in Sections 11.5 and 11.6. Of course, to achieve the best data quality possible all four steps must be followed.

11.5 Once the soil or sediment sample has been homogenized, it should be dried. This can be accomplished with a toaster oven or convection oven. A small aliquot of the sample (20 to 50 grams) is placed in a suitable container for drying. The sample should be dried for 2 to 4 hours in the convection or toaster oven at a temperature not greater than 150°C. Microwave drying is not a recommended procedure. Field studies have shown that microwave drying can increase variability between the FPXRF data and confirmatory analysis. High levels of metals in a sample can cause arcing in the microwave oven, and sometimes slag forms in the sample. Microwave oven drying can also melt plastic containers used to hold the sample.

11.6 The homogenized dried sample material should be ground with a mortar and pestle and passed through a 60-mesh sieve to achieve a uniform particle size. Sample grinding should continue until at least 90 percent of the original sample passes through the sieve. The grinding step normally takes an average of 10 minutes per sample. An aliquot of the sieved sample should then be placed in a 31.0-mm polyethylene sample cup (or equivalent) for analysis. The sample cup should be one-half to three-quarters full at a minimum. The sample cup should be covered with a 2.5 µm Mylar (or equivalent) film for analysis. The rest of the soil sample should be placed in a jar, labeled, and archived for possible confirmation analysis. All equipment including the mortar, pestle, and sieves must be thoroughly cleaned so that any cross-contamination is below the MDLs of the procedure or DQOs of the analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

Most FPXRF instruments have software capable of storing all analytical results and spectra. The results are displayed in parts per million and can be downloaded to a PC, which can provide a hard copy printout. Individual measurements that are smaller than three times their associated SD should not be used for quantitation.

13.0 METHOD PERFORMANCE

13.1 This section discusses four performance factors, field-based method detection limits, precision, accuracy, and comparability to EPA-approved methods. The numbers presented in Tables 4 through 9 were generated from data obtained from six FPXRF instruments. The soil samples analyzed by the six FPXRF instruments were collected from two sites in the United States.

The soil samples contained several of the target analytes at concentrations ranging from nondetect to tens of thousands of mg/kg.

13.2 The six FPXRF instruments included the TN 9000 and TN Lead Analyzer manufactured by TN Spectrace; the X-MET 920 with a SiLi detector and X-MET 920 with a gas-filled proportional detector manufactured by Metorex, Inc.; the XL Spectrum Analyzer manufactured by Niton; and the MAP Spectrum Analyzer manufactured by Scitec. The TN 9000 and TN Lead Analyzer both have a HgI_2 detector. The TN 9000 utilized an Fe-55, Cd-109, and Am-241 source. The TN Lead Analyzer had only a Cd-109 source. The X-Met 920 with the SiLi detector had a Cd-109 and Am-241 source. The X-MET 920 with the gas-filled proportional detector had only a Cd-109 source. The XL Spectrum Analyzer utilized a silicon pin-diode detector and a Cd-109 source. The MAP Spectrum Analyzer utilized a solid-state silicon detector and a Cd-109 source.

13.3 All data presented in Tables 4 through 9 were generated using the following calibrations and source count times. The TN 9000 and TN Lead Analyzer were calibrated using fundamental parameters using NIST SRM 2710 as a calibration check sample. The TN 9000 was operated using 100, 60, and 60 second count times for the Cd-109, Fe-55, and Am-241 sources, respectively. The TN Lead analyzer was operated using a 60 second count time for the Cd-109 source. The X-MET 920 with the Si(Li) detector was calibrated using fundamental parameters and one well characterized site-specific soil standard as a calibration check. It used 140 and 100 second count times for the Cd-109 and Am-241 sources, respectively. The X-MET 920 with the gas-filled proportional detector was calibrated empirically using between 10 and 20 well characterized site-specific soil standards. It used 120 second times for the Cd-109 source. The XL Spectrum Analyzer utilized NIST SRM 2710 for calibration and the Compton peak normalization procedure for quantitation based on 60 second count times for the Cd-109 source. The MAP Spectrum Analyzer was internally calibrated by the manufacturer. The calibration was checked using a well-characterized site-specific soil standard. It used 240 second times for the Cd-109 source.

13.4 Field-Based Method Detection Limits: The field-based method detection limits are presented in Table 4. The field-based method detection limits were determined by collecting ten replicate measurements on site-specific soil samples with metals concentrations 2 to 5 times the expected method detection limits. Based on these ten replicate measurements, a standard deviation on the replicate analysis was calculated. The method detection limits presented in Table 4 are defined as 3 times the standard deviation for each analyte.

The field-based method detection limits were generated by using the count times discussed earlier in this section. All the field-based method detection limits were calculated for soil samples that had been dried and ground and placed in a sample cup with the exception of the MAP Spectrum Analyzer. This instrument can only be operated in the in situ mode, meaning the samples were moist and not ground.

Some of the analytes such as cadmium, mercury, silver, selenium, and thorium were not detected or only detected at very low concentrations such that a field-based method detection limit could not be determined. These analytes are not presented in Table 4. Other analytes such as calcium, iron, potassium, and titanium were only found at high concentrations (thousands of mg/kg) so that reasonable method detection limits could not be calculated. These analytes also are not presented in Table 4.

13.5 Precision Measurements: The precision data is presented in Table 5. Each of the six FPXRF instruments performed 10 replicate measurements on 12 soil samples that had analyte concentrations ranging from nondetects to thousands of mg/kg. Each of the 12 soil samples underwent 4 different preparation techniques from in situ (no preparation) to dried and ground in a

sample cup. Therefore, there were 48 precision data points for five of the instruments and 24 precision points for the MAP Spectrum Analyzer. The replicate measurements were taken using the source count times discussed at the beginning of this section.

For each detectable analyte in each precision sample a mean concentration, standard deviation, and RSD was calculated for each analyte. The data presented in Table 5 is an average RSD for the precision samples that had analyte concentrations at 5 to 10 times the MDL for that analyte for each instrument. Some analytes such as mercury, selenium, silver, and thorium were not detected in any of the precision samples so these analytes are not listed in Table 5. Some analytes such as cadmium, nickel, and tin were only detected at concentrations near the MDLs so that an RSD value calculated at 5 to 10 times the MDL was not possible.

One FPXRF instrument collected replicate measurements on an additional nine soil samples to provide a better assessment of the effect of sample preparation on precision. Table 6 shows these results. The additional nine soil samples were comprised of three from each texture and had analyte concentrations ranging from near the detection limit of the FPXRF analyzer to thousands of mg/kg. The FPXRF analyzer only collected replicate measurements from three of the preparation methods; no measurements were collected from the *in situ* homogenized samples. The FPXRF analyzer conducted five replicate measurements of the in situ field samples by taking measurements at five different points within the 4-inch by 4-inch sample square. Ten replicate measurements were collected for both the intrusive undried and unground and intrusive dried and ground samples contained in cups. The cups were shaken between each replicate measurement.

Table 6 shows that the precision dramatically improved from the in situ to the intrusive measurements. In general there was a slight improvement in precision when the sample was dried and ground. Two factors caused the precision for the in situ measurements to be poorer. The major factor is soil heterogeneity. By moving the probe within the 4-inch by 4-inch square, measurements of different soil samples were actually taking place within the square. Table 6 illustrates the dominant effect of soil heterogeneity. It overwhelmed instrument precision when the FPXRF analyzer was used in this mode. The second factor that caused the RSD values to be higher for the in situ measurements is the fact that only five versus ten replicates were taken. A lesser number of measurements caused the standard deviation to be larger which in turn elevated the RSD values.

13.6 Accuracy Measurements: Five of the FPXRF instruments (not including the MAP Spectrum Analyzer) analyzed 18 SRMs using the source count times and calibration methods given at the beginning of this section. The 18 SRMs included 9 soil SRMs, 4 stream or river sediment SRMs, 2 sludge SRMs, and 3 ash SRMs. Each of the SRMs contained known concentrations of certain target analytes. A percent recovery was calculated for each analyte in each SRM for each FPXRF instrument. Table 7 presents a summary of this data. With the exception of cadmium, chromium, and nickel, the values presented in Table 7 were generated from the 13 soil and sediment SRMs only. The 2 sludge and 3 ash SRMs were included for cadmium, chromium, and nickel because of the low or nondetectable concentrations of these three analytes in the soil and sediment SRMs.

Only 12 analytes are presented in Table 7. These are the analytes that are of environmental concern and provided a significant number of detections in the SRMs for an accuracy assessment. No data is presented for the X-MET 920 with the gas-filled proportional detector. This FPXRF instrument was calibrated empirically using site-specific soil samples. The percent recovery values from this instrument were very sporadic and the data did not lend itself to presentation in Table 7.

Table 8 provides a more detailed summary of accuracy data for one FPXRF instrument (TN 9000) for the 9 soil SRMs and 4 sediment SRMs. Table 8 shows the certified value, measured

value, and percent recovery for five analytes. These analytes were chosen because they are of environmental concern and were most prevalently certified for in the SRM and detected by the FPXRF instrument. The first nine SRMs are soil and the last 4 SRMs are sediment. Percent recoveries for the four NIST SRMs were often between 90 and 110 percent for all analytes.

13.7 Comparability: Comparability refers to the confidence with which one data set can be compared to another. In this case, FPXRF data generated from a large study of six FPXRF instruments was compared to SW-846 Methods 3050 and 6010 which are the standard soil extraction for metals and analysis by inductively coupled plasma. An evaluation of comparability was conducted by using linear regression analysis. Three factors were determined using the linear regression. These factors were the y-intercept, the slope of the line, and the coefficient of determination (r^2).

As part of the comparability assessment, the effects of soil type and preparation methods were studied. Three soil types (textures) and four preparation methods were examined during the study. The preparation methods evaluated the cumulative effect of particle size, moisture, and homogenization on comparability. Due to the large volume of data produced during this study, linear regression data for six analytes from only one FPXRF instrument is presented in Table 9. Similar trends in the data were seen for all instruments.

Table 9 shows the regression parameters for the whole data set, broken out by soil type, and by preparation method. The soil types are as follows: soil 1–sand; soil 2–loam; and soil 3–silty clay. The preparation methods are as follows: preparation 1–in situ in the field; preparation 2–in situ, sample collected and homogenized; preparation 3–intrusive, with sample in a sample cup but sample still wet and not ground; and preparation 4–sample dried, ground, passed through a 40-mesh sieve, and placed in sample cup.

For arsenic, copper, lead, and zinc, the comparability to the confirmatory laboratory was excellent with r^2 values ranging from 0.80 to 0.99 for all six FPXRF instruments. The slopes of the regression lines for arsenic, copper, lead, and zinc, were generally between 0.90 and 1.00 indicating the data would need to be corrected very little or not at all to match the confirmatory laboratory data. The r^2 values and slopes of the regression lines for barium and chromium were not as good as for the other for analytes, indicating the data would have to be corrected to match the confirmatory laboratory.

Table 9 demonstrates that there was little effect of soil type on the regression parameters for any of the six analytes. The only exceptions were for barium in soil 1 and copper in soil 3. In both of these cases, however, it is actually a concentration effect and not a soil effect causing the poorer comparability. All barium and copper concentrations in soil 1 and 3, respectively, were less than 350 mg/kg.

Table 9 shows there was a preparation effect on the regression parameters for all six analytes. With the exception of chromium, the regression parameters were primarily improved going from preparation 1 to preparation 2. In this step, the sample was removed from the soil surface, all large debris was removed, and the sample was thoroughly homogenized. The additional two preparation methods did little to improve the regression parameters. This data indicates that homogenization is the most critical factor when comparing the results. It is essential that the sample sent to the confirmatory laboratory match the FPXRF sample as closely as possible.

Section 11.0 of this method discusses the time necessary for each of the sample preparation techniques. Based on the data quality objectives for the project, an analyst must decide if it is worth the extra time required to dry and grind the sample for small improvements in comparability.

Homogenization requires 3 to 5 minutes. Drying the sample requires one to two hours. Grinding and sieving requires another 10 to 15 minutes per sample. Lastly, when grinding and sieving is conducted, time must be allotted to decontaminate the mortars, pestles, and sieves. Drying and grinding the samples and decontamination procedures will often dictate that an extra person be on site so that the analyst can keep up with the sample collection crew. The cost of requiring an extra person on site to prepare samples must be balanced with the gain in data quality and sample throughput.

13.8 The following documents may provide additional guidance and insight on this method and technique:

13.8.1 Hewitt, A.D. 1994. "Screening for Metals by X-ray Fluorescence Spectrometry/Response Factor/Compton K_α Peak Normalization Analysis." *American Environmental Laboratory.* Pages 24-32.

13.8.2 Piorek, S., and J.R. Pasmore. 1993. "Standardless, In Situ Analysis of Metallic Contaminants in the Natural Environment With a PC-Based, High Resolution Portable X-Ray Analyzer." *Third International Symposium on Field Screening Methods for Hazardous Waste and Toxic Chemicals.* Las Vegas, Nevada. February 24-26, 1993. Volume 2, Pages 1135-1151.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/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.

14.2 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 Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits 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 Sec. 14.2.

16.0 REFERENCES

1. Metorex. X-MET 920 User's Manual.

2. Spectrace Instruments. 1994. Energy Dispersive X-ray Fluorescence Spectrometry: An Introduction.

3. TN Spectrace. Spectrace 9000 Field Portable/Benchtop XRF Training and Applications Manual.

4. Unpublished SITE data, recieved from PRC Environment Management, Inc.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain Tables 1 through 9 and a method procedure flow diagram.

TABLE 1
INTERFERENCE FREE DETECTION LIMITS

Analyte	Chemical Abstract Series Number	Detection Limit in Quartz Sand (milligrams per kilogram)
Antimony (Sb)	7440-36-0	40
Arsenic (As)	7440-38-0	40
Barium (Ba)	7440-39-3	20
Cadmium (Cd)	7440-43-9	100
Calcium (Ca)	7440-70-2	70
Chromium (Cr)	7440-47-3	150
Cobalt (Co)	7440-48-4	60
Copper (Cu)	7440-50-8	50
Iron (Fe)	7439-89-6	60
Lead (Pb)	7439-92-1	20
Manganese (Mn)	7439-96-5	70
Mercury (Hg)	7439-97-6	30
Molybdenum (Mo)	7439-93-7	10
Nickel (Ni)	7440-02-0	50
Potassium (K)	7440-09-7	200
Rubidium (Rb)	7440-17-7	10
Selenium (Se)	7782-49-2	40
Silver (Ag)	7440-22-4	70
Strontium (Sr)	7440-24-6	10
Thallium (Tl)	7440-28-0	20
Thorium (Th)	7440-29-1	10
Tin (Sn)	7440-31-5	60
Titanium (Ti)	7440-32-6	50
Vanadium (V)	7440-62-2	50
Zinc (Zn)	7440-66-6	50
Zirconium (Zr)	7440-67-7	10

Source: References 1, 2, and 3

TABLE 2
SUMMARY OF RADIOISOTOPE SOURCE CHARACTERISTICS

Source	Activity (mCi)	Half-Life (Years)	Excitation Energy (keV)	Elemental Analysis Range	
Fe-55	20-50	2.7	5.9	Sulfur to Chromium Molybdenum to Barium	K Lines L Lines
Cd-109	5-30	1.3	22.1 and 87.9	Calcium to Rhodium Tantalum to Lead Barium to Uranium	K Lines K Lines L Lines
Am-241	5-30	458	26.4 and 59.6	Copper to Thulium Tungsten to Uranium	K Lines L Lines
Cm-244	60-100	17.8	14.2	Titanium to Selenium Lanthanum to Lead	K Lines L Lines

Source: Reference 1, 2, and 3

TABLE 3
SUMMARY OF X-RAY TUBE SOURCE CHARACTERISTICS

Anode Material	Recommended Voltage Range (kV)	K-alpha Emission (keV)	Elemental Analysis Range	
Cu	18-22	8.04	Potassium to Cobalt Silver to Gadolinium	K Lines L Lines
Mo	40-50	17.4	Cobalt to Yttrium Europium to Radon	K Lines L Lines
Ag	50-65	22.1	Zinc to Technicium Ytterbium to Neptunium	K Lines L Lines

Source: Reference 4

Notes: The sample elements excited are chosen by taking as the lower limit the same ratio of excitation line energy to element absorption edge as in Table 2 (approximately 0.45) and the requirement that the excitation line energy be above the element absorption edge as the upper limit (L2 edges used for L lines). K-beta excitation lines were ignored.

TABLE 4
FIELD-BASED METHOD DETECTION LIMITS (mg/kg)[a]

Analyte	Instrument					
	TN 9000	TN Lead Analyzer	X-MET 920 (SiLi Detector)	X-MET 920 (Gas-Filled Detector)	XL Spectrum Analyzer	MAP Spectrum Analyzer
Antimony	55	NR	NR	NR	NR	NR
Arsenic	60	50	55	50	110	225
Barium	60	NR	30	400	NR	NR
Chromium	200	460	210	110	900	NR
Cobalt	330	NR	NR	NR	NR	NR
Copper	85	115	75	100	125	525
Lead	45	40	45	100	75	165
Manganese	240	340	NR	NR	NR	NR
Molybdenum	25	NR	NR	NR	30	NR
Nickel	100	NR	NA	NA	NA	NR
Rubidium	30	NR	NR	NR	45	NR
Strontium	35	NR	NR	NR	40	NR
Tin	85	NR	NR	NR	NR	NR
Zinc	80	95	70	NA	110	NA
Zirconium	40	NR	NR	NR	25	NR

Source: Reference 4

[a] MDLs are related to the total number of counts taken. See Section 13.3 for count times used to generate this table.

NR Not reported.

NA Not applicable; analyte was reported but was not at high enough concentrations for method detection limit to be determined.

TABLE 5
PRECISION

Analyte	Average Relative Standard Deviation for Each Instrument at 5 to 10 Times the MDL					
	TN 9000	TN Lead Analyzer	X-MET 920 (SiLi Detector)	X-MET 920 (Gas-Filled Detector)	XL Spectrum Analyzer	MAP Spectrum Analyzer
Antimony	6.54	NR	NR	NR	NR	NR
Arsenic	5.33	4.11	3.23	1.91	12.47	6.68
Barium	4.02	NR	3.31	5.91	NR	NR
Cadmium	29.84[a]	NR	24.80[a]	NR	NR	NR
Calcium	2.16	NR	NR	NR	NR	NR
Chromium	22.25	25.78	22.72	3.91	30.25	NR
Cobalt	33.90	NR	NR	NR	NR	NR
Copper	7.03	9.11	8.49	9.12	12.77	14.86
Iron	1.78	1.67	1.55	NR	2.30	NR
Lead	6.45	5.93	5.05	7.56	6.97	12.16
Manganese	27.04	24.75	NR	NR	NR	NR
Molybdenum	6.95	NR	NR	NR	12.60	NR
Nickel	30.85[a]	NR	24.92[a]	20.92[a]	NA	NR
Potassium	3.90	NR	NR	NR	NR	NR
Rubidium	13.06	NR	NR	NR	32.69[a]	NR
Strontium	4.28	NR	NR	NR	8.86	NR
Tin	24.32[a]	NR	NR	NR	NR	NR
Titanium	4.87	NR	NR	NR	NR	NR
Zinc	7.27	7.48	4.26	2.28	10.95	0.83
Zirconium	3.58	NR	NR	NR	6.49	NR

Source: Reference 4

[a] These values are biased high because the concentration of these analytes in the soil samples was near the detection limit for that particular FPXRF instrument.
NR Not reported.
NA Not applicable; analyte was reported but was below the method detection limit.

TABLE 6
PRECISION AS AFFECTED BY SAMPLE PREPARATION

Analyte	Average Relative Standard Deviation for Each Preparation Method		
	In Situ-Field	Intrusive-Undried and Unground	Intrusive-Dried and Ground
Antimony	30.1	15.0	14.4
Arsenic	22.5	5.36	3.76
Barium	17.3	3.38	2.90
Cadmium[a]	41.2	30.8	28.3
Calcium	17.5	1.68	1.24
Chromium	17.6	28.5	21.9
Cobalt	28.4	31.1	28.4
Copper	26.4	10.2	7.90
Iron	10.3	1.67	1.57
Lead	25.1	8.55	6.03
Manganese	40.5	12.3	13.0
Mercury	ND	ND	ND
Molybdenum	21.6	20.1	19.2
Nickel[a]	29.8	20.4	18.2
Potassium	18.6	3.04	2.57
Rubidium	29.8	16.2	18.9
Selenium	ND	20.2	19.5
Silver[a]	31.9	31.0	29.2
Strontium	15.2	3.38	3.98
Thallium	39.0	16.0	19.5
Thorium	NR	NR	NR
Tin	ND	14.1	15.3
Titanium	13.3	4.15	3.74
Vanadium	NR	NR	NR
Zinc	26.6	13.3	11.1
Zirconium	20.2	5.63	5.18

Source: Reference 4

[a] These values may be biased high because the concentration of these analytes in the soil samples was near the detection limit.
ND Not detected.
NR Not reported.

TABLE 7
ACCURACY

Analyte	Instrument															
	TN 9000				TN Lead Analyzer				X-MET 920 (SiLi Detector)				XL Spectrum Analyzer			
	n	Range of % Rec.	Mean % Rec.	SD	n	Range of % Rec.	Mean % Rec.	SD	n	Range of % Rec.	Mean % Rec	SD	n	Range of % Rec.	Mean % Rec.	SD
Sb	2	100-149	124.3	NA	--	--	--	--	--	--	--	--	--	--	--	--
As	5	68-115	92.8	17.3	5	44-105	83.4	23.2	4	9.7-91	47.7	39.7	5	38-535	189.8	206
Ba	9	98-198	135.3	36.9	--	--	--	--	9	18-848	168.2	262	--	--	--	--
Cd	2	99-129	114.3	NA	--	--	--	--	6	81-202	110.5	45.7	--	--	--	--
Cr	2	99-178	138.4	NA	--	--	--	--	7	22-273	143.1	93.8	3	98-625	279.2	300
Cu	8	61-140	95.0	28.8	6	38-107	79.1	27.0	11	10-210	111.8	72.1	8	95-480	203.0	147
Fe	6	78-155	103.7	26.1	6	89-159	102.3	28.6	6	48-94	80.4	16.2	6	26-187	108.6	52.9
Pb	11	66-138	98.9	19.2	11	68-131	97.4	18.4	12	23-94	72.7	20.9	13	80-234	107.3	39.9
Mn	4	81-104	93.1	9.70	3	92-152	113.1	33.8	--	--	--	--	--	--	--	--
Ni	3	99-122	109.8	12.0	--	--	--	--	--	--	--	--	3	57-123	87.5	33.5
Sr	8	110-178	132.6	23.8	--	--	--	--	--	--	--	--	7	86-209	125.1	39.5
Zn	11	41-130	94.3	24.0	10	81-133	100.0	19.7	12	46-181	106.6	34.7	11	31-199	94.6	42.5

Source: Reference 4

n Number of samples that contained a certified value for the analyte and produced a detectable concentration from the FPXRF instrument.
SD Standard deviation.
NA Not applicable; only two data points, therefore, a SD was not calculated.
%Rec. Percent recovery.
-- No data.

TABLE 8
ACCURACY FOR TN 9000[a]

Standard Reference Material	Arsenic			Barium			Copper			Lead			Zinc		
	Cert. Conc.	Meas. Conc.	%Rec.	Cert. Conc.	Meas. Conc.	%Rec.	Cert. Conc.	Meas. Conc.	%Rec.	Cert. Conc.	Meas. Conc.	%Rec.	Cert. Conc.	Meas. Conc.	%Rec.
RTC CRM-021	24.8	ND	NA	586	1135	193.5	4792	2908	60.7	144742	149947	103.6	546	224	40.9
RTC CRM-020	397	429	92.5	22.3	ND	NA	753	583	77.4	5195	3444	66.3	3022	3916	129.6
BCR CRM 143R	--	--	--	--	--	--	131	105	80.5	180	206	114.8	1055	1043	99.0
BCR CRM 141	--	--	--	--	--	--	32.6	ND	NA	29.4	ND	NA	81.3	ND	NA
USGS GXR-2	25.0	ND	NA	2240	2946	131.5	76.0	106	140.2	690	742	107.6	530	596	112.4
USGS GXR-6	330	294	88.9	1300	2581	198.5	66.0	ND	NA	101	80.9	80.1	118	ND	NA
NIST 2711	105	104	99.3	726	801	110.3	114	ND	NA	1162	1172	100.9	350	333	94.9
NIST 2710	626	722	115.4	707	782	110.6	2950	2834	96.1	5532	5420	98.0	6952	6476	93.2
NIST 2709	17.7	ND	NA	968	950	98.1	34.6	ND	NA	18.9	ND	NA	106	98.5	93.0
NIST 2704	23.4	ND	NA	414	443	107.0	98.6	105	106.2	161	167	103.5	438	427	97.4
CNRC PACS-1	211	143	67.7	--	772	NA	452	302	66.9	404	332	82.3	824	611	74.2
SARM-51	--	--	--	335	466	139.1	268	373	139.2	5200	7199	138.4	2200	2676	121.6
SARM-52	--	--	--	410	527	128.5	219	193	88.1	1200	1107	92.2	264	215	81.4

Source: Reference 4

[a] All concentrations in milligrams per kilogram.
%Rec. Percent recovery.
ND Not detected.
NA Not applicable.
-- No data.

TABLE 9
REGRESSION PARAMETERS FOR COMPARABILITY[1]

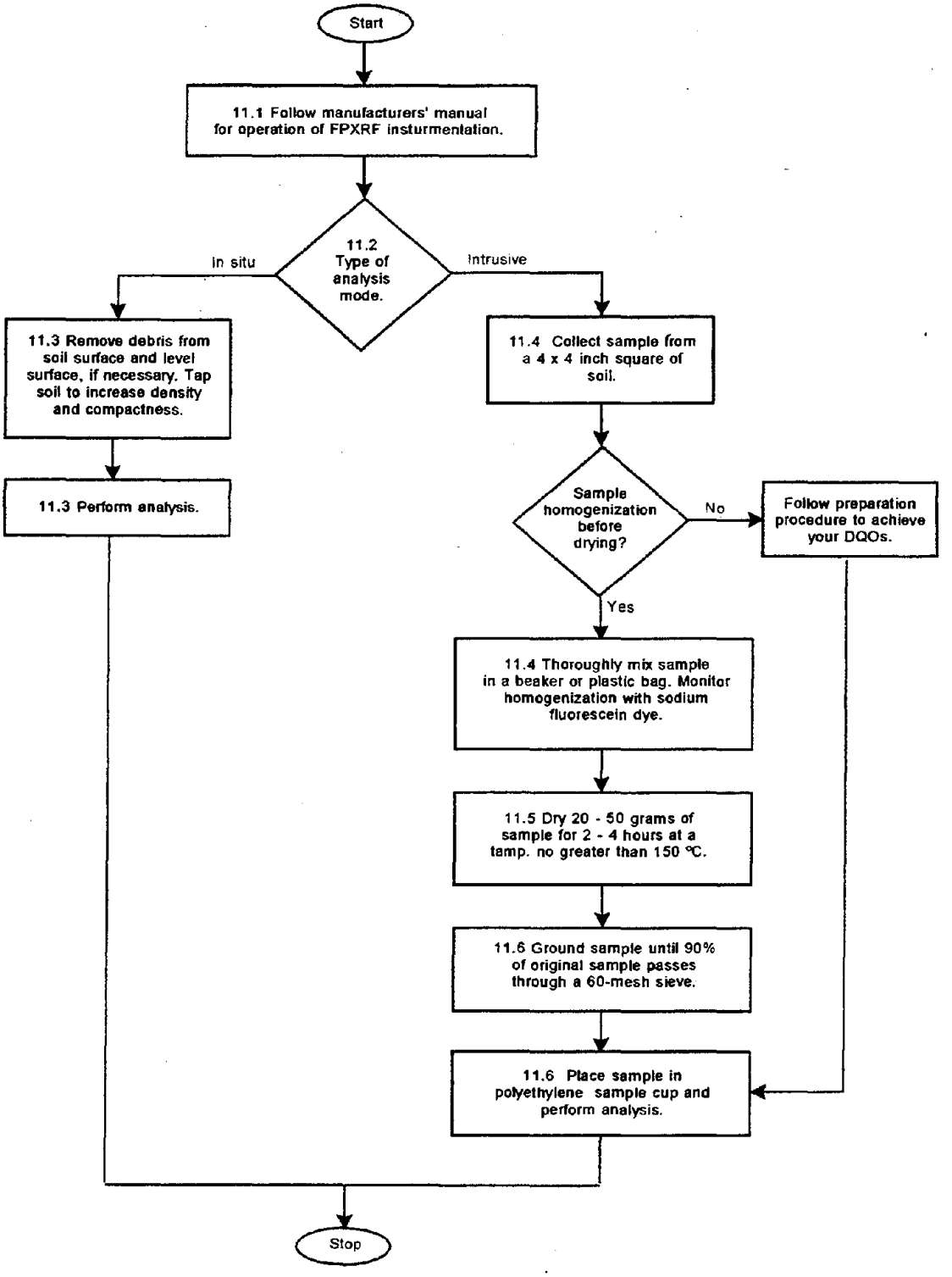
	Arsenic				Barium				Copper			
	n	r^2	Int.	Slope	n	r^2	Int.	Slope	n	r^2	Int.	Slope
All Data	824	0.94	1.62	0.94	1255	0.71	60.3	0.54	984	0.93	2.19	0.93
Soil 1	368	0.96	1.41	0.95	393	0.05	42.6	0.11	385	0.94	1.26	0.99
Soil 2	453	0.94	1.51	0.96	462	0.56	30.2	0.66	463	0.92	2.09	0.95
Soil 3	—	—	—	—	400	0.85	44.7	0.59	136	0.46	16.60	0.57
Prep 1	207	0.87	2.69	0.85	312	0.64	53.7	0.55	256	0.87	3.89	0.87
Prep 2	208	0.97	1.38	0.95	315	0.67	64.6	0.52	246	0.96	2.04	0.93
Prep 3	204	0.96	1.20	0.99	315	0.78	64.6	0.53	236	0.97	1.45	0.99
Prep 4	205	0.96	1.45	0.98	313	0.81	58.9	0.55	246	0.96	1.99	0.96

	Lead				Zinc				Chromium			
	n	r^2	Int.	Slope	n	r^2	Int.	Slope	n	r^2	Int.	Slope
All Data	1205	0.92	1.66	0.95	1103	0.89	1.86	0.95	280	0.70	64.6	0.42
Soil 1	357	0.94	1.41	0.96	329	0.93	1.78	0.93	—	—	—	—
Soil 2	451	0.93	1.62	0.97	423	0.85	2.57	0.90	—	—	—	—
Soil 3	397	0.90	2.40	0.90	351	0.90	1.70	0.98	186	0.66	38.9	0.50
Prep 1	305	0.80	2.88	0.86	286	0.79	3.16	0.87	105	0.80	66.1	0.43
Prep 2	298	0.97	1.41	0.96	272	0.95	1.86	0.93	77	0.51	81.3	0.36
Prep 3	302	0.98	1.26	0.99	274	0.93	1.32	1.00	49	0.73	53.7	0.45
Prep 4	300	0.96	1.38	1.00	271	0.94	1.41	1.01	49	0.75	31.6	0.56

Source: Reference 4

[1] Log-transformed data
n Number of data points
r^2 Coefficient of determination
Int. Y-intercept
— No applicable data

FIELD PORTABLE X-RAY FLUORESCENCE SPECTROMETRY FOR THE DETERMINATION OF ELEMENTAL CONCENTRATIONS IN SOIL AND SEDIMENT



METHOD 6500

DISSOLVED INORGANIC ANIONS IN AQUEOUS MATRICES
BY CAPILLARY ION ELECTROPHORESIS

1.0 SCOPE AND APPLICATION

1.1 This test method is applicable for determination of the dissolved inorganic anions; fluoride, bromide, chloride, nitrite, nitrate, ortho-phosphate, and sulfate in aqueous matrices using capillary ion electrophoresis with indirect UV detection.

1.2 This test method is applicable to drinking water, wastewater and ground water for the analysis of inorganic anions in the concentration range of 0.1 to 50 mg/L, except for fluoride, which has a range of 0.1 to 25 mg/L. It is the user's responsibility to ensure the applicability of this test method for other anion concentration ranges and other aqueous sample matrices.

1.3 Capillary ion electrophoresis provides a simultaneous separation and determination of several inorganic anions using nanoliters of sample in a single injection. Only 500 µL of sample is required to fill the analysis vial. Analysis time is less than 5 minutes.

2.0 SUMMARY OF METHOD

2.1 Capillary ion electrophoresis (Figs. 1 - 4) is a free zone electrophoretic technique optimized for the analysis of anions with molecular weights less than 200. The anions migrate and are separated according to their mobility in the electrolyte when an electrical field is applied through the open tubular fused silica capillary. The electrolyte's electroosmotic flow (EOF) modifier dynamically coats the inner wall of the capillary, changing the surface to a net positive charge. This reversal of wall charge reverses the natural EOF. The modified EOF in combination with a negative power supply augments the mobility of the analyte anions towards the anode and detector achieving rapid analysis times. Cations migrate in the opposite direction towards the cathode and are removed from the sample during analysis. Water and other neutral species move toward the detector at the same rate as the EOF. The neutral species migrate slower than the analyte anions and do not interfere with anion analysis (Figs. 2 and 3).

2.2 The sample is introduced into the capillary using hydrostatic sampling. The inlet of the capillary, containing electrolyte, is immersed in the sample and the sample raised 10 cm for 30 seconds where 36 nanoliter volumes are siphoned into the capillary. After sample loading, the capillary is immediately immersed back into the electrolyte. The voltage is applied initiating the separation process. Pressure injection may also be used as long as the performance specifications of this method are achievable.

2.3 Anion detection is based upon the principles of indirect UV detection. The UV absorbing electrolyte anion is displaced charge-for-charge by the separated analyte anion. The analyte anion zone has a net decrease in background absorbance. This decrease in UV absorbance is quantitatively proportional to analyte anion concentration (Fig. 4). Detector output polarity is reversed to provide positive mV response to the data system, and to make the negative absorbance peaks appear positive.

2.4 The analysis is complete once the last anion of interest is detected. The capillary is then vacuum purged by the system of any remaining sample, and replenished with fresh electrolyte. The system is now ready for the next analysis.

3.0 DEFINITIONS

3.1 Capillary Ion Electrophoresis: An electrophoretic technique in which an UV absorbing electrolyte is placed in a 75 µm fused silica capillary. Voltage is applied through the capillary causing electrolyte and anions to migrate towards the anode and through the capillary's UV detector window. Anions are separated based upon the anion's differential rates of migration in the electrical field which is directly related to the anion's equivalent ionic conductance. Anion detection and quantitation are based upon the principles of indirect UV detection.

3.2 Electrolyte: A combination of a UV absorbing salt and an electroosmotic flow modifier placed inside the capillary, used as a carrier for the analytes, and for anion detection and quantitation. The UV absorbing portion of the salt must be anionic and have an electrophoretic mobility similar to the analyte anions of interest.

3.3 Electroosmotic Flow (EOF): The direction and velocity of electrolyte solution flow within the capillary under an applied electrical potential (voltage); the velocity and direction of flow is determined by electrolyte chemistry, power supply polarity and applied voltage.

3.4 Electroosmotic Flow Modifier (OFM): A cationic amine in the electrolyte that dynamically coats the negatively charged silica wall reversing the direction of the electrolyte's natural electroosmotic flow and directing it towards the anode and detector. This modifier augments anion migration and enhances speed of analysis (Fig. 2).

3.5 Electrophoretic Mobility: The specific velocity of a charged analyte in the electrolyte under specific electroosmotic flow conditions. The mobility of an analyte is directly related to the analyte's equivalent ionic conductance and applied voltage, and is the primary mechanism of separation.

3.6 Electropherogram: A graphical presentation of UV detector response versus time of analysis; the x axis is the migration time which is used to qualitatively identify the anion, and the y axis is the UV response which can be converted to time corrected peak area for quantification.

3.7 Hydrostatic Sampling: A sample introduction technique in which the capillary with electrolyte is immersed in the sample, and both are elevated to a specific height, typically 10 cm, above the receiving electrolyte reservoir for a preset amount of time, typically less than 60 seconds. Nanoliters of sample are siphoned into the capillary by differential head pressure and gravity.

3.8 Indirect UV Detection: A form of UV detection in which the analyte displaces an equivalent net charge amount of the highly UV absorbing component of the electrolyte causing a net decrease in background absorbance. The magnitude of the decreased absorbance is directly proportional to analyte concentration. Detector output polarity is switched in order to obtain a positive mV response.

3.9 Migration Time: The time required for a specific analyte to migrate through the capillary to the detector. The migration time in capillary ion electrophoresis is analogous to retention time in chromatography.

3.10 Time Corrected Peak Area (normalized peak area): Peak area divided by migration time. CIE principles state that peak area is dependant on migration time, i.e. for same concentration of analyte, as migration time increases (decreases) peak area increases (decreases). Timed corrected peak area accounts for these changes.

3.11 Midpoint of Peak Width - CIE peaks are typically asymmetrical with the peak apex shifting with increasing concentration, and peak apex may not be indicative of true analyte migration time. Midpoint of peak width is the midpoint between the analyte peak's start and stop integration.

4.0 INTERFERENCES

4.1 The most difficult quantitation and possible comigration occurs when one anion is in significant excess to other anions in close proximity. For two closely adjacent peaks reliable quantitation can be achieved when the concentration differential is less than 100:1. As the resolution between two anion peaks increase so does the tolerated concentration differential.

4.2 Dissolved carbonate, as HCO_3^{-1}, is an anion present in all aqueous environmental samples, especially alkaline samples. Under the defined analysis conditions, carbonate at less than 1000:1 concentration differential to the anions will not interfere with the quantitation of the anions listed in Section 1.1.

4.3 Most monovalent organic acids and neutral organics commonly found in wastewater and groundwater migrate later in the electropherogram, after carbonate, and do not interfere with the anions listed in Section 1.1. Formate, a common organic acid found in environmental samples, migrates shortly after fluoride but before phosphate. At high formate concentrations the quantification of fluoride may be incorrectly identified. Include 5 mg/L formate into the mixed anion working solution to aid with fluoride identification and quantitation (Fig. 5).

4.4 Other inorganic or organic anions present in the sample will be separated and detected yielding an anionic profile of the sample. Other matrix anions commonly found in drinking water or wastewater do not interfere with the analysis of anions given in Section 1.1. However, unknown matrix anions may co-migrate or be a direct interferant with the analyte anions of interest.

4.5 Divalent organic acids usually found in wastewater migrate after phosphate. At concentrations greater than 10 mg/L, they may interfere with phosphate identification and quantitation.

4.6 Chlorate also migrates in the phosphate region but does not interfere with phosphate identification or quantitation at concentrations less than 3 mg/L. For chlorate concentrations greater than 3 mg/L, add 5 mg/L chlorate to the mixed anion working solution to aid in identification of phosphate and chlorate.

4.7 As the concentration of analyte increases the analyte peak shape becomes asymmetrical. If adjacent analyte peaks are not baseline resolved, the data system will drop a perpendicular line between them to the baseline. This causes a decrease in peak area for both analyte peaks and a low bias for analyte amounts. For optimal quantitation, ensure that adjacent peaks are fully resolved, if they are not, dilute the sample 1:1 with reagent water.

5.0 SAFETY

5.1 Refer to Chapter Three for additional guidance on safety protocols.

5.2 It is the responsibility of the user to prepare, handle, and dispose of electrolyte solutions in accordance with all applicable federal, state, and local regulations.

WARNING -- This capillary electrophoresis method uses high voltage as a means for separating the analyte anions, and can be hazardous if not used properly. Use only those instruments with the appropriate safety features.

6.0 EQUIPMENT AND SUPPLIES

6.1 Capillary Ion Electrophoresis System: Consists of the following components, as shown in Fig. 1, or equivalent.

6.1.1 High Voltage Power Supply: Capable of generating voltage potential between 0 and minus 30 kV relative to ground.

6.1.2 Covered Sample Carousel: To prevent environmental contamination of the samples during a multi-sample analysis.

6.1.3 Sample Introduction Mechanism: Capable of hydrostatic or pressure sampling techniques.

6.1.4 Capillary Purge Mechanism: To automatically purge the capillary after every analysis to eliminate any cross contamination from the previous sample matrix and to replenish the capillary with fresh electrolyte, or clean the capillary with other reagents, such as sodium hydroxide.

6.1.5 UV Detector: Capable of monitoring 254 nm with a time constant of 0.1 s.

6.1.6 Fused Silica Capillary: A 75 µm (inner diameter) x 375 µm (outer diameter) x 60 cm (length) having a polymer coating for flexibility, and a non-coated section to act as the cell window for UV detection.

6.1.7 Constant Temperature Compartment: To keep the samples, capillary and electrolytes at constant temperature.

6.2 Data System: Computer system capable of acquiring data at 20 points per second, ability to express migration time or relative migration time in minutes to 3 decimal places, use midpoint of the analyte peak width to determine the migration time of the analyte, use reference peaks and normalized migration time relative to the reference peak for qualitative identification, report time corrected peak area, and express results in concentration units.

6.3 Anion exchange cartridge, hydroxide form or equivalent.

6.4 Plastic syringes, 20 mL disposable.

6.5 Vacuum filtration apparatus using a 0.45 µm aqueous compatible filter.

7.0 REAGENTS AND STANDARDS

7.1 Purity of Reagents: Unless otherwise indicated, it is intended that all reagents shall conform to the reagent grade specification of the Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficient high purity to permit its use without lessening the

performance or accuracy of the determination. Detection limits of this method are limited by the purity of the reagents.

7.2 Reagent Water: All references to water in this method refer to reagent water unless otherwise specified. Reagent water will be interference free. Refer to Chapter One for a definition of reagent water.

7.3 Individual Anion Solution, Stock Standard (1000 mg/L Anion): Individual stock solution may be purchased from an appropriate vendor or may be prepared in the laboratory. Recommend use of certified 1000 ppm stock standards.

NOTE: All weights given are for anhydrous or dried salts.

7.3.1 Bromide Solution, Standard: Dry approximately 0.5 g of sodium bromide (NaBr) for 6 hours at 150°C and cool in a desiccator. In 100 mL volumetric flask dissolve 0.128 g of the dry salt with water, and fill to mark with water.

7.3.2 Chloride Solution, Standard: Dry approximately 0.5 g of sodium chloride (NaCl) for 1 hour at 100°C and cool in a desiccator. In 100 mL volumetric flask dissolve 0.165 g of the dry salt with water, and fill to mark with water.

7.3.3 Fluoride Solution, Standard: Dry approximately 0.5 g of sodium fluoride (NaF) for 1 hour at 100°C and cool in a desiccator. In 100 mL volumetric flask dissolve 0.221 g of the dry salt with water, and fill to mark with water.

7.3.4 Formate Solution, Standard: Dissolve 0.151 g of sodium formate in a 100 mL volumetric flask with water, and make to volume.

7.3.5 Nitrate Solution, Standard: Dry approximately 0.5 g of sodium nitrate ($NaNO_3$) for 48 hours at 105°C and cool in a desiccator. In 100 mL volumetric flask dissolve 0.137 g of the dry salt with water, and fill to mark with water (1000 mg/L NO_3 = 225.8 mg/L N-NO_3).

7.3.6 Nitrite Solution, Standard: Dry approximately 0.5 g of sodium nitrite ($NaNO_2$) for 24 hours in a desiccator containing concentrated sulfuric acid. In 100 mL volumetric flask dissolve 0.150 g of the dry salt with water, and fill to mark with water. Store in a sterilized glass bottle. Refrigerate and prepare monthly. (1000 mg/L NO_2 = 304.3 mg/L N-NO_2)

NOTE: Nitrite is easily oxidized, especially in the presence of moisture. Use only fresh reagent.

NOTE: Prepare sterile bottles for storing nitrite solutions by heating for 1 hour at 170°C in an air oven.

7.3.7 Ortho-Phosphate Solution, Standard: In 100 mL volumetric flask dissolve 0.150 g of anhydrous dibasic sodium phosphate (Na_2HPO_4) with water, and fill to mark with water (1000 mg/L PO_4 = 326.1 mg/L P-PO_4).

7.3.8 Sulfate Solution, Standard: Dry approximately 0.5 g of sodium sulfate (Na_2SO_4) for 1 hour at 105°C and cool in a desiccator. In a 100 mL volumetric flask dissolve 0.148 g of the dry salt with water, and fill to mark with water.

7.4 Mixed Anion Solution, Working: Prepare a blank, and at least 3 different working standard concentrations for the analyte anion of interest within the desired range of analysis, typically between 0.1 and 50 mg/L. To a pre-rinsed 100 mL volumetric flask add an appropriate aliquot of individual anion stock standard solution (Section 7.3) and dilute with water. Add 5 mg/L formate to all standards.

NOTE: Use 0.1 mL of individual anion stock standard solution (Section 7.3) per 100 mL for 1 mg/L anion.

NOTE: Anions of no interest may be omitted.

NOTE: The mid-range mixed anion working solution (Section 7.4) may be used for the determination of migration times and resolution described in Section 10.1, and for quality control evaluation described in Section 9.0.

7.5 Electrolyte Reagents: Although any electrolyte meeting the performance criteria of this method may be used. This method has been validated using a chromate-based electrolyte.

7.5.1 Chromate Concentrate: (100 mM Chromate)- In a 1 L volumetric flask dissolve 23.41 g of sodium chromate tetrahydrate ($Na_2CrO_4 \cdot 4H_2O$) in 500 mL of water, and dilute to 1L with water. This concentrate may be stored in a capped glass or plastic container for up to 1 year.

7.5.2 Electroosmotic Flow Modifier Concentrate: (100 mM Tetradecyltrimethyl ammonium bromide, TTABr) - In a 100 mL volumetric flask dissolve 3.365 g of tetradecyltrimethyl ammonium bromide (TTABr) in 70 mL of water, and dilute to 100 mL with water.

NOTE: TTABr needs to be converted to the hydroxide form using the anion exchange cartridge. TTAOH is commercially available from Waters Corp. (sole source).

7.5.3 Buffer Solution: (100 mM CHES/1mM Calcium Gluconate) - In a 1 L volumetric flask dissolve 20.73 g of CHES (2-[N-Cyclohexylamino]-Ethane Sulfonic Acid) and 0.43 g of calcium gluconate in 500 mL of water, and dilute to 1 L with water. This concentrate may be stored in a capped glass or plastic container for up to one year.

7.5.4 Sodium Hydroxide Solution: (500 mM Sodium Hydroxide) - In a 100 mL volumetric flask dissolve 2 g of sodium hydroxide in 50 mL of water and dilute to 100 m L with water.

7.5.5 Electrolyte Solution, Working: (4.7 mM Chromate/4 mM TTAOH/10mM CHES/0.1mM Calcium Gluconate) -Wash the anion exchange cartridge in the hydroxide form using the 20 mL plastic syringe with 10 mL of 500 mM NaOH followed by 10 mL of water. Discard the washings. Slowly pass 4 mL of the 100 mM OFM Concentrate Solution through the cartridge into a 100 mL volumetric flask. Rinse the cartridge with 20 mL of water, adding the washing to the volumetric flask.

NOTE: The above procedure is used to convert the TTABr to TTAOH which is used in the electrolyte. If using commercially available 100 mM TTAOH, this step is not necessary.

Into the 100 mL volumetric flask add 4.7 mL of chromate concentrate solution and 10 mL buffer solution. Mix and dilute to 100 mL with water. The natural pH of the electrolyte should be 9.0 ± 0.1. Filter and degass using the vacuum filtration apparatus. Store the remaining electrolyte in a capped glass or plastic container at ambient temperature. The electrolyte is stable for one year. This electrolyte is commercially available from Waters Corp.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Sample collection procedures should address the considerations described in Chapter Nine of this manual.

8.2 See the introductory material in Chapter Three, Inorganic Analytes, for information on sample handling and preservation.

8.3 Rinse sampling containers with the sample and discard to eliminate any contamination from the container, fill to overflowing, and cap to exclude air.

8.4 Analyze samples as soon as possible after collection. For nitrite, nitrate, and phosphate refrigerate the sample at 4°C after collection and warm to room temperature before dilution and analysis. Determine nitrite and nitrate within 48 hours.

8.5 Filter samples containing suspended solids through a pre-rinsed 0.45 µm aqueous compatible membrane filter before transferring the sample to the analysis vial.

8.6 If sample dilution is required, dilute with reagent water only.

9.0 QUALITY CONTROL

9.1 All quality control data should be maintained and available for easy reference or inspection.

9.2 For each batch of samples processed, method blanks must be carried throughout the entire sample preparation and analytical process according to the frequency described in Chapter One. These blanks will be useful in determining if samples are being contaminated. Refer to Chapter One for the proper protocol when analyzing blanks.

9.3 Matrix Spike/Matrix Spike Duplicates (MS/MSDs): MS/MSDs are intralaboratory split samples spiked with identical concentrations of target analytes. The spiking occurs prior to sample preparation and analysis. An MS/MSD is used to document the bias and precision of a method in a given sample matrix. MS/MSDs are to be analyzed at the frequency of one per analytical batch as described in Chapter One. Refer to the definitions of bias and precision, in Chapter One, for the proper data reduction protocols. Each laboratory should calculate its own acceptance criteria based on its historical data for each matrix type. Refer to Chapter One for guidance.

9.4 A laboratory control sample shall also be processed with each sample batch. Refer to Chapter One for more information.

9.5 Recalibrate after 15 analyses to account for any changes in migration time or response. Use the single mixed anion working solution (Sec. 7.4). Replace the new calibration results with the previous calibration results.

9.6 The new calibration curve is validated if the single point calibration response factor of new recalibration generated in Section 9.5 is ± 5% of the previous calibration response factor, and if analyte migration time is + 5% of previous migration time determined in Section 10.1.

9.7 If the calibration curve is not validated then discard the spent electrolyte and replace with fresh electrolyte. Calibrate as described in Section 10.1.

NOTE: Replace the electrolyte working solution in the instrument daily.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Determination of migration times - The migration time of an anion is dependent upon the electrolyte compositions, pH, capillary surface and length, applied voltage, the ionic strength of the sample, and temperature. For every fresh electrolyte determine the analyte migration time in minutes, to the third decimal place, of the mid-range mixed anion standard working solution (Section 7.4), using the analysis scheme described in (Section 11.0). Use mid-point of analyte peak width as the determinant of analyte migration time (Fig. 5 and Table 2).

NOTE: Analyte peak apex may be used as the migration time determinant, but potential analyte misidentification may result with asymmetrical shape at high analyte concentrations.

10.2 For each anion concentration (X-axis) plot time corrected peak area response (Y-axis). Determine the best linear calibration line through the data points, or use the linear regression calibration routine available in the data systems. Do not force the line through zero.

10.3 After verification of linear multiple calibration, a single point calibration can be used between 0.1 and 50 mg/L anion. This single point calibration solution can be used for subsequent recalibration.

11.0 PROCEDURE

11.1 Set up the capillary electrophoresis system according to the manufacturer's instructions. Fill the electrolyte reservoirs with fresh electrolyte. Transfer the blank, standard, or sample into a prerinsed plastic sample analysis vial and place in the covered sample carousel.

11.2 Program the system according to manufacturer's instructions using the following instrument settings as guidelines for analysis of standards, and samples.

11.2.1 Condition a new 75 µm i.d. x 375 µm o.d. x 60 cm capillary with 100 mM NaOH for 5 minutes followed by working chromate electrolyte solution A for 5 minutes.

NOTE: This conditioning step should be repeated weekly in order to regenerate the capillary surface for optimum reproducibility.

Program the system for at least a one minute purge of the capillary with electrolyte between each standard or sample. Using a 15 psi vacuum purge mechanism, one 60 cm capillary volume can be displaced in 30 seconds.

11.2.2 Program the system for the hydrostatic sampling technique for 30 seconds. Different sampling times may be used provided that samples and standards are analyzed identically. Approximately 1.2 nL of sample per second is siphoned into a 75 µm capillary.

11.2.3 Program the system for constant current 14 μA and a run time of 5 minutes; if an anionic profile of the sample is of interest set the time to 7 minutes. Using a capillary 60 cm in length, the field strength at 15 μv applied voltage is 250 V/cm.

11.2.4 Program the integrator or computer for data acquisition rate of 20 points per second with a run time designated in Section 11.2.3. Set up data processing method according to manufacturers instructions.

11.2.5 Monitor UV response at 254 nm. Since detector ranges are variable, the range setting required for analysis will depend on the concentration of anions in the sample and should be chosen accordingly.

11.2.6 The electropherogram of the working calibration standards (Section 7.4) should be similar to the inorganic anion electropherogram shown in Fig. 5.

11.3 Analyze all standards (Section 7.4) and samples as described in Section 11.2. Refer to Figs. 5-9 for representative anion standard, 0.1 mg/L anion standard, drinking water, and waste water (municipal and industrial).

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Relate the time corrected peak area for each sample anion with the calibration curve generated in Section 10.2 to determine mg/L concentration of anion. If the sample was diluted prior to analysis, then multiply mg/L anion by the dilution factor to obtain the original sample concentration.

Original Sample mg/L Anion = (A x SF)

where:

A = mg/L anion determined from the calibration curve
SF = scale or dilution factor

13.0 METHOD PERFORMANCE

13.1 Figures 6-12 display representative examples of electropherograms and linearity of calibration curves.

13.2 Tables 1-10 provide collaborative design, migration time reproducibility, comparison of CIE with other approved EPA methods, and interlaboratory reproducibility and precision for the capillary ion electrophoresis technique.

13.3 Table 11 is entitled "Capillary Ion Electrophoresis Anion Analysis Round Robin Using Chromate Electrolyte (mg/L)" and provides precision data in some common environmental matrices.

13.4 The following documents may provide additional information regarding this method and technique:

13.4.1 Romano, J., Krol, J, "Capillary Ion Electrophoresis, An Environmental Method for the Determination of Anions in Water", J. of Chromatography, Vol. 640, 1993, p. 403.

13.4.2 Romano, J., "Capillary Ion Analysis: A Method for Determining Ions in Water and Solid Waste Leachates", Amer. Lab., May 1993, p. 48.

13.4.3 Jones, W., "Method Development Approaches for Ion Electrophoresis", J. of Chromatography, Vol. 640, 1993, p. 387.

13.4.4 Jones, W., Jandik, P., "Various Approaches to Analysis of Difficult Sample Matrices for Anions using Capillary Electrophoresis", J. of Chromatography, Vol. 608, 1992, p. 385.

13.4.5 Bondoux, G., Jandik, P., Jones, W., "New Approaches to the Analysis of Low Level of Anions in Water", J. of Chromatography, Vol. 602, 1992, p. 79.

13.4.6 Jandik, P., Jones, W., Weston, A., Brown, P.,"Electrophoretic Capillary Ion Analysis: Origins, Principles, and Applications", LC GC, Vol. 9, Number 9, 1991, p. 634.

13.4.7 Romano, J., Jackson, P., "Optimization of Inorganic Capillary Electrophoresis for the Analysis of Anionic Solutes in Real Samples", J. of Chromatography, Vol. 546, 1991, p. 411.

13.4.8 Jandik, P., Jones, W., "Optimization of Detection Sensitivity in the Capillary Electrophoresis of Inorganic Anions", J of Chromatography, Vol. 546, 1991, p. 431.

13.4.9 Jandik, P., Jones, W., "Controlled Changes of Selectivity in the Separation of Ions by Capillary Electrophoresis", J. of Chromatography, Vol. 546, 1991, p 445.

13.4.10 Foret, R., et.al., "Indirect Photometric Detection in Capillary Zone Electrophoresis", J. of Chromatography, Vol. 470, 1989, p. 299.

13.4.11 Hjerte'n, S. et. al.,"Carrier-free Zone Electrophoresis, Displacement Electrophoresis and Isoelectric Focusing in an Electrophoresis Apparatus", J. of Chromatography, Vol. 403, 1987, p. 47

13.4.12 Jandik, P., Bonn, G., "Capillary Electrophoresis of Small Molecules and Ions", VCH Publishers, 1993.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/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.

14.2 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, Department of Government Relations and Science Policy, 1155 16th Street, NW, Washington, DC, 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits 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 Section 14.2.

16.0 REFERENCES

1. Waters Chromatography, "Innovative Methods for Ion Analysis", Method N-601b, 1992.

2. Waters Chromatography, Validation Data for Method 6500, Millipore Corporation Waters Chromatography Division, Ion Analysis Group; Milford, Massachusetts.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain Table 1 through 11, Figures 1 through 12, and a flow diagram of method procdures.

TABLE 1

COLLABORATIVE DESIGN AS FOUR YOUDEN PAIR SETS[1]

Individual Youden Pair Standards, in mg/L

Analyte Anion	1	2	3	4	5	6	7	8
Cl	0.7	2.0	3.0	15.0	40.0	20.0	50.0	0.5
Br	2.0	3.0	15.0	40.0	20.0	50.0	0.7	0.5
NO_2	3.0	40.0	20.0	15.0	50.0	0.5	2.0	0.7
SO_4	40.0	50.0	0.5	0.7	2.0	3.0	15.0	20.0
NO_3	15.0	20.0	40.0	50.0	0.5	0.7	2.0	3.0
F	2.0	0.7	0.5	3.0	10.0	7.0	20.0	25.0
PO_4	50.0	40.0	20.0	0.5	3.0	2.0	0.7	15.0

Source: Reference 2

[1]The collaborative design is intended to demonstrate performance between 0.1 and 50 mg/L anion, except for Fluoride between 0.1 and 25 mg/L. The concentrations among anions is varied as not to have any one standard at all lowor all high anion concentrations.

TABLE 2

ANION MIGRATION TIME REPRODUCIBILITY FROM YOUDEN PAIR STANDARDS USING CHROMATE ELECTROLYTE AND CONSTANT CURRENT

Analyte Mid-Point Migration Time, Ave of Triplicate Samplings

Youden Standards	Analyte	Cl	Br	NO_2	SO_4	NO_3	F	PO_4
	1	3.132	3.226	3.275	3.405	3.502	3.761	3.906
	2	3.147	3.239	3.298	3.431	3.517	3.779	3.931
	3	3.138	3.231	3.283	3.411	3.497	3.771	3.925
	4	3.158	3.244	3.307	3.434	3.510	3.781	3.963
	5	3.184	3.271	3.331	3.435	3.551	3.787	3.981
	6	3.171	3.260	3.312	3.418	3.537	3.776	3.964
	7	3.191	3.272	3.315	3.437	3.544	3.773	3.978
	8	3.152	3.248	3.294	3.418	3.526	3.739	3.954
	Std Dev	0.021	0.015	0.018	0.012	0.20	03015	0.027
	%RSD	0.67%	0.46%	0.55%	0.36%	0.56%	0.40%	0.68%

Ave Migration Time Std Dev = 0.018 min = 1.1 sec Ave %RSD = 0.53%

TABLE 3

COMPARISON OF CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE AND APPROVED METHODS USING A PERFORMANCE EVALUATION STANDARD

	Analyte	Cl	NO_2	SO_4	NO_3	F	PO_4
Performance Evaluation Standard[1]	True Value in mg/L	43.00	1.77	37.20	15.37	2.69	6.29
Official Anion Methods Wet Chem & IC	Measured Mean[2]	43.20	1.77	37.00	15.42	2.75	6.38
	Measured Std Dev	3.09	0.07	2.24	1.15	0.26	0.21
CIE Using Chromate Electrolyte[3]	Ave CIE n=18	42.51	1.78	37.34	14.06	2.63	6.34
	CIE/Mean CIE/True Value	0.984 0.989	1.006 1.006	1.009 1.003	0.911 0.945	0.956 0.978	0.994 1.008

Source: Reference 2

[1]The performance evaluation standard was purchased from APG Laboratories and diluted 1:100 with Type I DI water.
[2]The measured result is the average from numerous laboratories using Approved Standard Methods and EPA wet chemistry and ion chromatography methods
[3]The CIE results were determined using the proposed EPA and ASTM method, and are the average from 4 laboratories using the Youden Pair Standards for quantitation.

TABLE 4

CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE
INTERLABORATORY REPRODUCIBILITY AND PRECISION[1]

Analyte[2]	Cl	NO_2	SO_4	NO_3	F
Lab 1 n = 5	43.22 ± 0.22	1.58 ± 0.09	36.39 ± 0.33	14.57 ± 0.12	2.54 ± 0.10
Lab 2 n=5	43.68 ± 0.61	1.58 ± 0.08	37.01± 0.37	13.94 ± 0.09	2.69 ± 0.02
Lab 3 n=5	43.93 ± 0.39	1.60 ± 0.06	37.68 ± 0.24	15.05 ± 0.11	2.69 ± 0.03
Lab 4 n=3	42.51 ± 0.22	1.78 ± 0.06	37.34 ± 0.19	14.06 ± 0.07	2.69 ± 0.02
Average Mean ± Std Dev	43.34 ± 0.36	1.64 ± 0.07	37.11 ± 0.28	14.41 ± 0.10	2.64 ± 0.04
% RSD	0.83%	4.5%	0.77%	0.67%	1.61%

[1]Results from 4 laboratories analyzing the performance evaluation standard using the Youden Pair Standards for quantitation. Results expressed as mg/L.
[2]Only 1 lab reported results for PO_4 as 6.34 ± 0.02 mg/L on triplicate samplings yielding an %RSD of 0.07%

TABLE 5

CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE KNOWN ADDITION RECOVERY AND PRECISION USING PERFORMANCE EVALUATION STANDARD WITH DRINKING WATER

Analyte	Cl	NO_2	SO_4	NO_3	F	PO_4
Milford Drinking Water n=3, as ppm	24.27 ± 0.18	Not Detected	7.99 ± 0.07	0.36 ± 0.05	Not Detected	Not Detected
%RSD	0.73%		0.91%	13.3%		
Performance Evaluation Std[1]	43.00	1.77	37.20	15.37	2.69	6.29
MDW + PES n=3, as ppm	66.57 ± 0.34	1.74 ± 0.03	45.19 ± 0.17	15.42 ± 0.12	2.62 ± 0.07	5.55 ± 0.31
%RSD	0.51	1.85	0.38	0.79	2.69	5.52
% Recovery	97.9%	98.3%	100.2%	98.1%	97.4%	88.2%

Source: Reference 2.

[1]The performance evaluation standard was diluted 1:100 with Drinking Water.

TABLE 6

COMPARISON OF APPROVED METHOD AND CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE FOR THE DETERMINATION OF CHLORIDE
Data given as mg/L

Analyte	Sample #	Titration[1]	IC[2]	CIE
Effluent	1	--[3]	149	147
	2	--	162	161
	3	--	153	152
	4	--	139	140
	5	--	111	110
	6	--	109	107
	7	--	3.6	3.5
Drinking Water	1	5.5	5.1	5.0
	2	5.5	5.0	4.9
	3	5.3	5.2	5.1
	4	5.5	5.1	5.1
	5	5.3	5.0	5.0
	6	5.3	4.9	4.9
	7	5.5	4.9	4.9
Landfill Leachate	1	0.1	<0.1	ND
	2	230	245	240

Source: Reference 2.

[1] Chloride determined using 4500 Cl C, Iodometric Method
[2] Chloride determined using 4110 C, Single Column Ion Chromatography Using Direct Conductivity Detection
[3] A dash line indicates test not performed. ND indicates anion not detected

TABLE 7

COMPARISON OF APPROVED METHOD AND CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE FOR THE DETERMINATION OF FLUORIDE

Analyte	Sample #	Electrode[1]	IC[2]	CIE
Effluent	1	1.7	1.2	1.5
	2	0.9	0.6	0.6
	3	0.8	0.5	0.6
	4	0.8	0.4	0.7
	5	0.9	0.5	0.8
	6	0.9	0.5	0.7
	7	<0.1	ND	<0.1
Drinking Water	1	1.2	0.9	0.9
	2	1.3	0.9	0.9
	3	1.3	0.9	0.9
	4	1.3	0.9	0.9
	5	1.3	0.9	0.9
	6	0.9	0.6	0.6
	7	1.3	0.9	0.9
Landfill Leachate	1	<0.2	ND	ND
	2	16	10.6	10.9

Source: Reference 2.

[1] Fluoride determined using 4500-F C, Ion Selecteive Electrode Method
[2] Fluoride determined using 4110 C, Single Column Ion Chromatography Using Direct Conductivity Detection

TABLE 8

COMPARISON OF APPROVED METHOD AND CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE FOR THE DETERMINATION OF SULFATE
Data given as mg/L

Analyte	Sample #	Turbidimetric[1]	IC[2]	CIE
Effluent	1	98	87.5	98.0
	2	110	95.3	95.9
	3	130	118	115
	4	130	139	136
	5	110	113	110
	6	100	107	106
	7	6	5.6	5.8
Drinking Water	1	6	5.8	6.0
	2	6	5.8	6.0
	3	6	5.9	6.1
	4	6	5.9	6.1
	5	5	5.8	6.2
	6	4	3.0	3.4
	7	5	5.8	6.1
Landfill Leachate	1	<1	ND	ND
	2	190	211	201

Source: Reference 2.

[1] Sulfate determined using 4500 SO_4 E, Turbidimetric Method
[2] Sulfate determined using 4110 C, Single Column Ion Chromatography Using Direct Conductivity Detection

TABLE 9

COMPARISON OF APPROVED METHOD AND CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE FOR THE DETERMINATION OF NITRITE + NITRATE[3]
Data given as mg/L

Analyte	Sample #	Cd Red'n[1]	IC[2]	CIE
Effluent	1	0.3	ND	ND
	2	--	ND	ND
	3	--	ND	ND
	4	--	ND	0.5
	5	--	2.1	2.4
	6	2.4	1.9	2.2
	7	0.7	0.3	0.4
Drinking Water	1	0.6	0.3	0.4
	2	0.6	0.3	4.4
	3	0.4	0.3	4.4
	4	0.6	0.3	0.3
	5	0.6	0.3	0.4
	6	0.3	0.1	0.1
	7	0.5	0.3	0.4
Landfill Leachate	1	--	ND	ND
	2	--	ND	ND

Source: Reference 2.

[1] Total nitrite + nitrate determined using 4500-NO3 F, Cadmium Reduction Method
[2] Nitrite + nitrate determined using 4110 C, Single Column Ion chromatorgraphy Using Direct Conductivity Detection
[3] Each technique gave separte nitrate and nitrate values; because of their liability results were added for comparison purposes

TABLE 10

COMPARISON OF APPROVED METHOD AND CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE FOR THE DETERMINATION OF ORTHO-PHOSPHATE
Data given as mg/L

Analyte	Sample #	Ascorbic Acid[1]	IC[2]	CIE
Effluent	1	3.4	ND	2.8
	2	4.9	ND	4.4
	3	4.7	ND	4.5
	4	5.3	ND	4.2
	5	3.0	ND	3.0
	6	2.9	ND	2.3
	7	<0.1	ND	<0.1
Drinking Water	1	<0.1	ND	ND
	2	<0.1	ND	ND
	3	--	ND	ND
	4	<0.1	ND	ND
	5	<0.1	ND	ND
	6	--	ND	ND
	7	--	ND	ND
Landfill Leachate	1	<0.1	ND	<0.1
	2	2.2	1.6	1.4

Source: Reference 2.

[1] Phosphate determined using 4500 PO_4 E, Ascorbic Acid Method
[2] Phosphate determined using 4110 C, Single Column Ion Chromatography Using Direct Conductivity Detection

TABLE 11

CAPILLARY ION ELECTROPHORESIS ANION ANALYSIS ROUND ROBIN[1]
USING CHROMATE ELECTROLYTE (mg/L)

Sample	Chloride	Bromide	Nitrite	Sulfate	Nitrate	Fluoride	Phosphate
1. Bleachwaste	<0.046	<0.046	<0.072	0.30±0.37	<0.84	<0.020	<0.041
2. Creekwater	3.06±0.27	<0.046	<0.072	3.00±0.30	0.37±0.19	0.11±0.09	<0.061
3. Wastewater	24.6±0.62	<0.046	<0.072	2.02±0.56	<0.084	0.08±0.08	3.74±0.75
4. Wastewater	59.7±2.9	0.85±0.52	<0.072	109±4.4	44.9±1.6	0.988±0.21	4.94±1.32
5. Wastewater	63.8±2.0	0.68±0.52	<0.072	115±3.9	44.3±1.06	1.04±0.17	4.78±1.55
6. Wastewater	72.0±5.4	0.05±0.01	<0.072	144±11.8	5.38±2.57	0.57±0.21	1.18±1.01
7. Wastewater	139±10.0	<0.046	4.0±1.3	584±35	353±25.5	3.01±0.80	9.34±5.17
8. Wastewater	51.4±7.7	<0.046	<0.072	40.2±6.1	39.9±7.9	1.17±0.24	6.99±1.31
9. Wastewater	29.9±4.3	<0.046	2.14±1.35	217±19	13.9±4.9	1.33±0.28	9.95±5.04
10. Wastewater	766±44	<0.046	<0.072	489±46	12.9±6.9	<0.020	41.3±8.5
11. Surfacewater	3.71±0.39	<0.046	<0.072	2.70±0.39	0.23±0.20	0.11±0.097	<0.041
12. Wastewater	22.1±0.62	8.47±0.30	<0.072	133±4.4	<0.084	0.76±0.11	<0.041
13. Drinking Water	5.15±0.35	<0.046	<0.072	2.64±0.26	0.50±0.27	0.59±0.097	<0.041
14. Drinking Water	4.95±0.24	<0.046	<0.072	2.62±0.21	0.54±0.25	0.56±0.09	<0.041

Source: Reference 2.

[1] Five laboratory interlaboratory precision.

FIGURE 1

HARDWARE SCHEMATIC OF A CAPILLARY
ION ELECTROPHORESIS SYSTEM

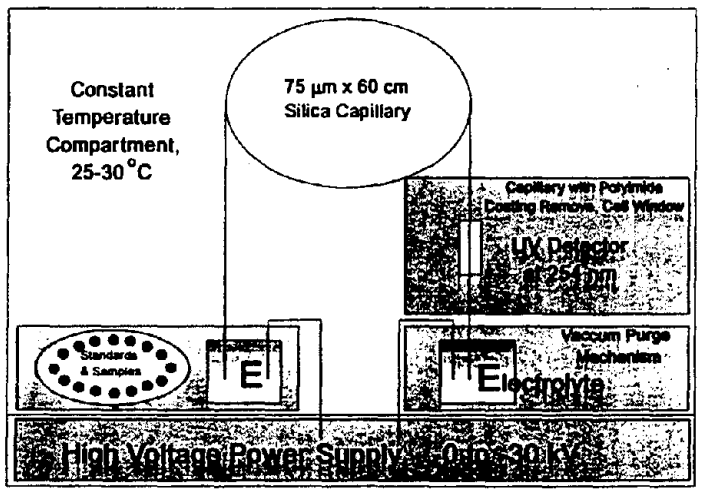


FIGURE 2

PICTORIAL DIAGRAM OF ANION MOBILITY AND
ELECTROOSMATIC FLOW MODIFIER

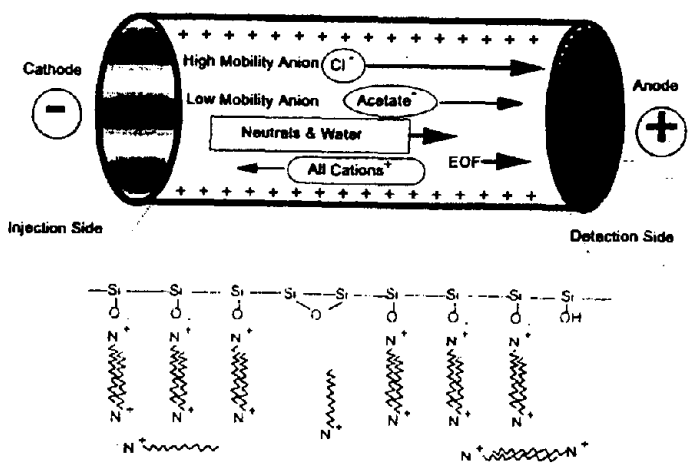


FIGURE 3

SELECTIVITY DIAGRAM OF ANION MOBILITY
USING CAPILLARY ION ELECTROPHORESIS

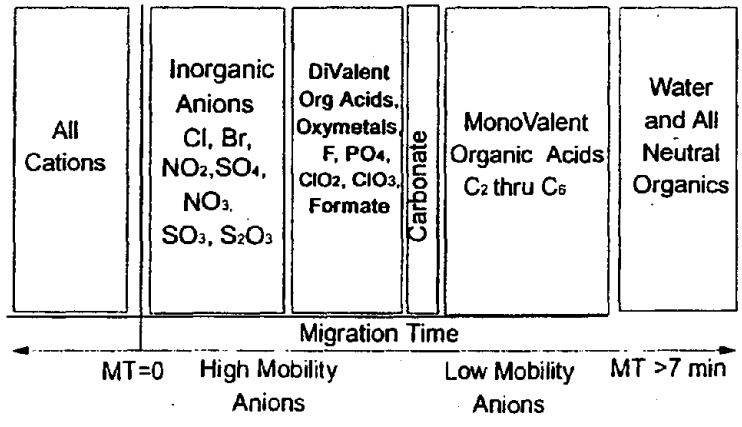


FIGURE 4

PICTORIAL DIAGRAM OF INDIRECT UV DETECTION

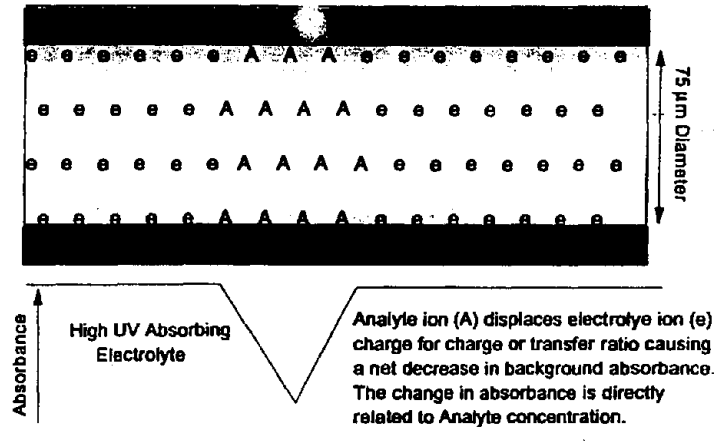
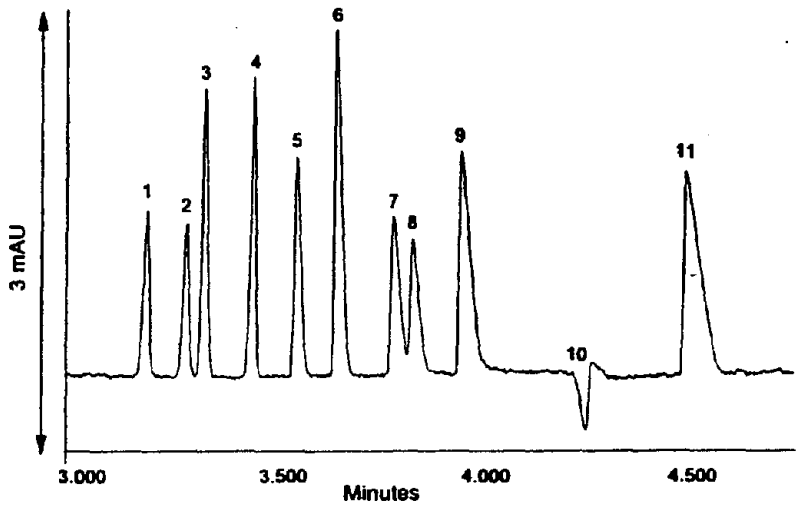


FIGURE 5

ELECTROPHEROGRAM OF THE INORGANIC ANIONS AND TYPICALLY FOUND ORGANIC ACIDS USING CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE



Electrolyte: 4.7 mM Na_2CrO_4/4.0 mM TTAOH / 10 mM CHES / 0.1 mM Calcium Gluconate
Capillary: 75 µm (id) x 375 µm x 60 cm (length), Uncoated Silica
Voltage: 15 kV using a Negative Power Supply
Current: 14 ± µA, Constant Current
Sampling: Hydrostatic at 10 cm for 30 seconds
Detection: Indirect UV using a Hg Lamp and 254 nm Filter

Anion	Conc. Mg/L	Migration Time in Mintues	Migration Time Ratio to Cl	Peak Area	Time Corrected Peak Area
1. Chloride	2.0	3.200	1.000	1204	376.04
2. Bromide	4.0	3.296	1.030	1147	348.05
3. Nitrite	4.0	3.343	1.045	2012	601.72
4. Sulfate	4.0	3.465	1.083	1948	562.05
5. Nitrate	4.0	3.583	1.120	1805	503.69
6. Oxalate	5.0	3.684	1.151	3102	842.14
7. Fluoride	1.0	3.823	1.195	1708	446.65
8. Formate	5.0	3.873	1.210	1420	366.61
9. O-Phosphate	4.0	4.004	1.251	2924	730.25
10. Carbonate	–	4.281	1.338	–	–
11. Acetate	5.0	4.560	1.425	3958	868.01

FIGURE 6

ELECTROPHEROGRAM OF 0.1 MG/L INORGANIC ANIONS
MINIMUM DETECTION LIMIT WITH CHROMATE ELECTROLYTE

Seven replicates of the 0.1 mg/L inorganic anion standard was used to calculate the minimum detection limits, as mg/L, using analytical protocol described in Standard Methods 1030 E.

Chloride = 0.046	Bromide = 0.090	Nitrite = 0.072	Sulfate = 0.032
Nitrate = 0.084	Fluoride = 0.020	phosphate = 0.041	

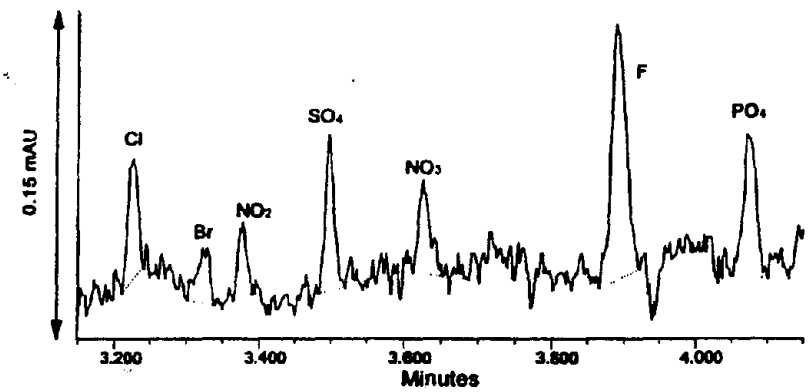


FIGURE 7

ELECTROPHEROGRAM OF TYPICAL DRINKING WATER
USING CHROMATE ELECTROLYTE

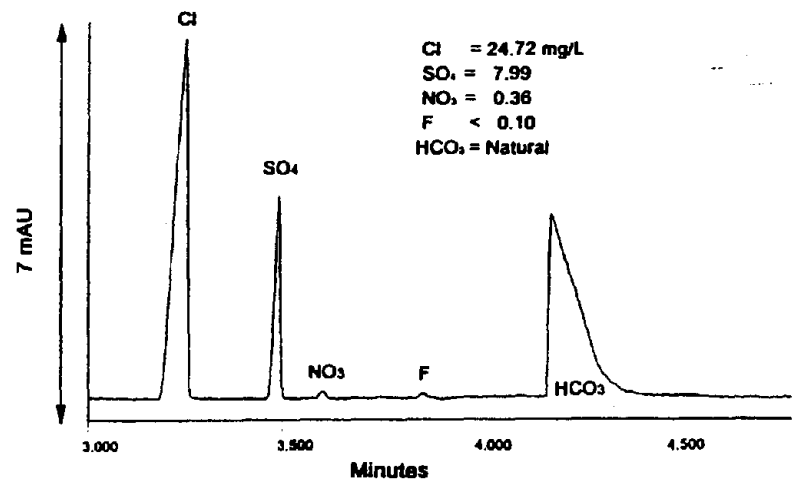


FIGURE 8

ELECTROPHEROGRAM OF TYPICAL MUNICIPAL WASTEWATER DISCHARGE
USING CHROMATE ELECTROLYTE

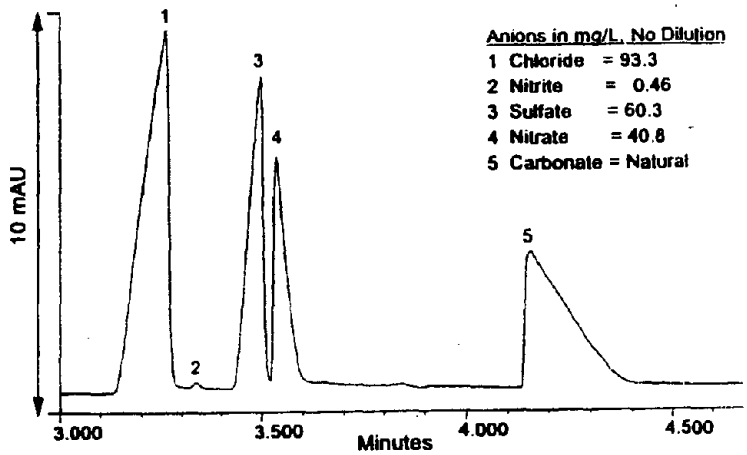


FIGURE 9

ELECTROPHEROGRAM OF TYPICAL INDUSTRIAL WASTEWATER DISCHARGE
USING CHROMATE ELECTROLYTE

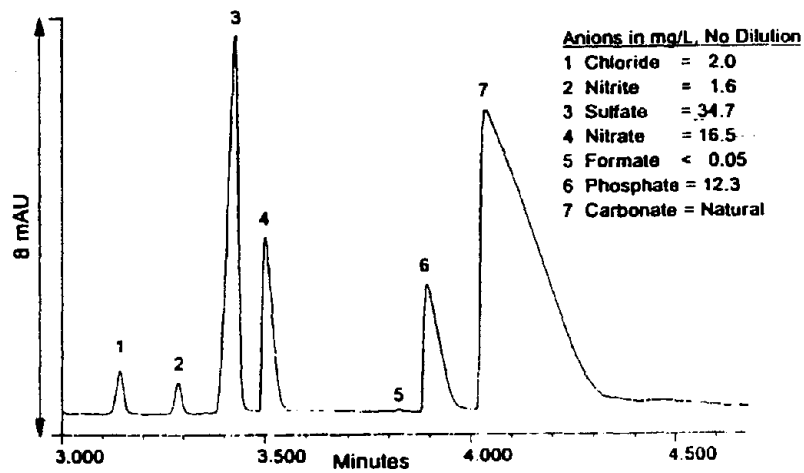


FIGURE 10

LINEARTY CALIBRATION CURVE FOR CHLORIDE, BROMIDE, AND SULFATE USING CHROMATE ELECTROLYTE

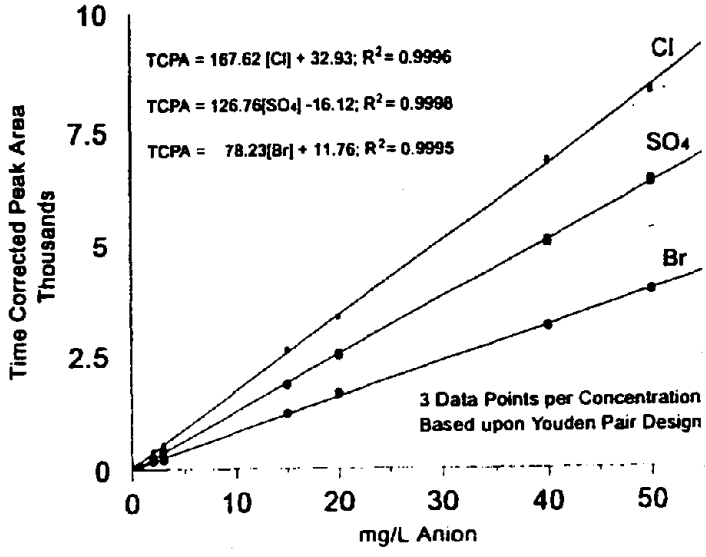


FIGURE 11

LINEARTY CALIBRATION CURVE FOR FLUORIDE AND O-PHOSPHATE USING CHROMATE ELECTROLYTE

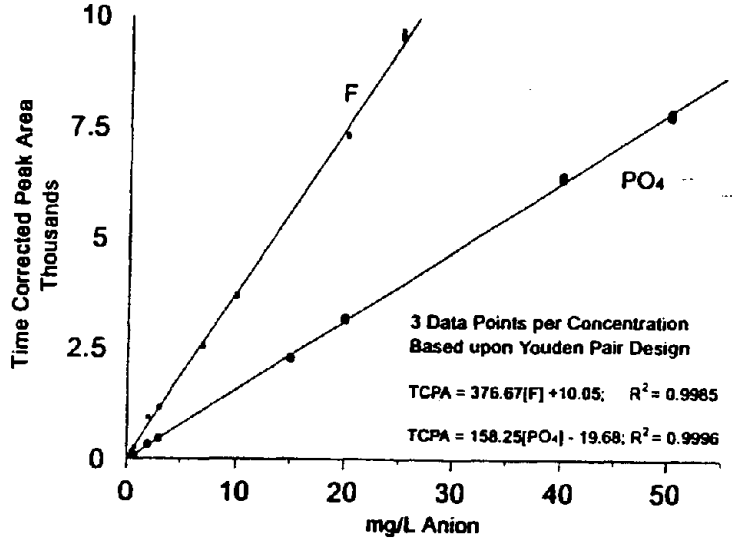
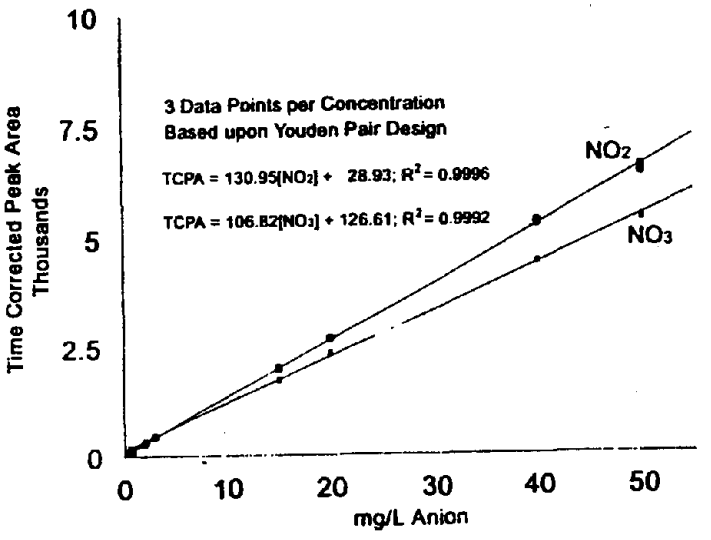
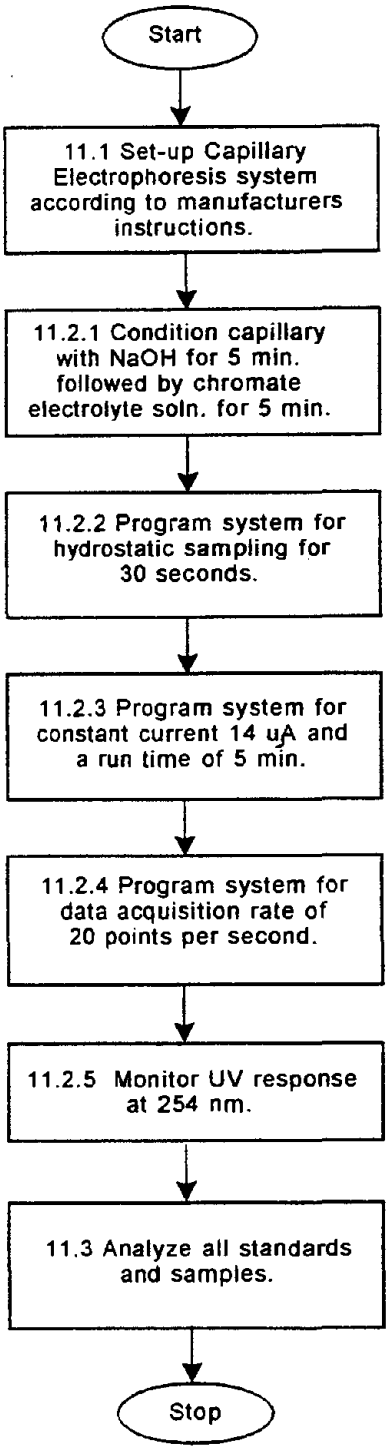


FIGURE 12

LINEARITY CALIBRATION CURVE FOR NITRITE AND NITRATE
USING CHROMATE ELECTROLYTE



DISSOLVED INORGANIC ANIONS IN AQUEOUS MATRICES BY CAPILLARY ION ELECTROPHORESIS



METHOD 6800

ELEMENTAL AND SPECIATED ISOTOPE DILUTION MASS SPECTROMETRY

1.0 SCOPE AND APPLICATION

1.1 This method consists of two approaches: isotope dilution mass spectrometry (IDMS) for the determination of total metals and speciated isotope dilution mass spectrometry (SIDMS) for the determination of elemental species. This method is applicable to the determination of total metals and metal species at sub µg/L levels in water samples or in waste extracts or digests. In general, elements that have more than one available stable isotope can be analyzed by IDMS. SIDMS may require more isotopes of an element, depending on the number of interconvertable species. The current method is applicable to the following elements.

Element		CASRN[a]
Antimony	(Sb)	7440-36-0
Boron	(B)	7440-42-8
Barium	(Ba)	7440-39-3
Cadmium	(Cd)	7440-43-9
Calcium	(Ca)	7440-70-2
Chromium	(Cr)	7440-47-3
Copper	(Cu)	7440-50-8
Iron	(Fe)	7439-89-6
Lead	(Pb)	7439-92-1
Magnesium	(Mg)	7439-95-4
Mercury	(Hg)	7439-97-6
Molybdenum	(Mo)	7439-98-7
Nickel	(Ni)	7440-02-0
Potassium	(K)	7440-09-7
Selenium	(Se)	7782-49-2
Silver	(Ag)	7440-22-4
Strontium	(Sr)	7440-24-6
Thallium	(Tl)	7440-28-0
Vanadium	(V)	7440-62-2
Zinc	(Zn)	7440-66-6

[a] Chemical Abstracts Service Registry Number

Other elements and species may be analyzed by this method if appropriate performance is demonstrated for the analyte of interest, in the matrices of interest, at the concentration levels of interest (see Section 9.0).

1.2 Isotope dilution is based on the addition of a known amount of enriched isotope to a sample. Equilibration of the spike isotope with the natural element/species in the sample alters the isotope ratio that is measured. With the known isotopic abundance of both spike and sample, the amount of the spike added to the sample, and the altered isotope ratio, the concentration of the element/species in the sample can be calculated.

1.3 IDMS has proven to be a technique of high accuracy for the determination of total metals in various matrices (Reference 1). IDMS has several advantages over conventional calibration methodologies. Partial loss of the analyte after equilibration of the spike and the sample will not influence the accuracy of the determination. Fewer physical and chemical interferences influence the determination as they have similar effects on each isotope of the same element. The isotope ratio to be measured for quantification in IDMS can be measured with very high precision, typically RSD≤0.25%.

1.4 SIDMS takes a unique approach to speciated analysis that differs from traditional methods. Traditional speciation methods attempt to hold each species static while making the measurement. Unfortunately, speciation extraction and analysis methods inherently measure the species after species conversions have occurred. SIDMS has been developed to address the correction for the species conversions. In SIDMS, each species is "labeled" with a different isotope-enriched spike in the corresponding species form. Thus, the interconversions that occur after spiking are traceable and can be corrected. While SIDMS maintains the advantages of IDMS, it is capable of correcting the degradation of the species or the interconversion between the species (Reference 2). SIDMS is also a diagnostic tool that permits the evaluation of species-altering procedures and permits evaluation and validation of other more traditional speciation analysis methods.

1.5 Both IDMS and SIDMS require the equilibration of the spike isotope(s) and the natural isotopes. For IDMS, the spike and sample can be in different chemical forms; only total elemental concentrations will result. In general, equilibration of the spike and sample isotopes occurs as a result of decomposition, which also destroys all species-specific information when the isotopes of an element are all oxidized or reduced to the same oxidation state. For SIDMS, spikes and samples must be in the same speciated form. This requires the chemical conversion of the elements in spikes. For solution samples, spiking and equilibration procedures can be as simple as mixing the known amount of the sample and the spikes. Efforts are taken to keep the species in their original species forms after spiking. While drinking water, ground water, and other aqueous samples may be directly spiked, soils, sludges, sediments, and other solid wastes require extraction or digestion prior to analysis to solubilize the elemental species.

1.6 Detection limits, sensitivity, and optimum ranges of the elements will vary with the matrix, separation method, and isotope ratio measurement methods. With the popularity of chromatography and ICP-MS, it is convenient to separate elemental species and to measure the isotope ratios. Although this method is not restricted to chromatography as the separation method of the species and the ICP-MS as the isotope ratio measurement method, this method will use these two techniques as examples in describing the procedures. Other species separation methods, such as extraction, precipitation, and solid phase chelation, and other isotope ratio measurement techniques, such as thermal ionization mass spectrometry (TIMS), can also be used.

2.0 SUMMARY OF METHOD

2.1 IDMS method:

2.1.1 Samples may require a variety of sample preparation procedures, depending on sample matrices and the isotope ratio measurement methods. One primary purpose of sample preparation is to solubilize the analyte of interest and equilibrate the spike isotopes with sample isotopes. Solids, slurries, and suspended material must be subjected to digestion after spiking using appropriate sample preparation methods (such as Method 3052). Water samples may not require digestion when ICP-MS is used as a detection method because ICP can destroy elemental species and thus many species are indistinguishable for ICP-MS.

2.1.2 A representative measured sample is thoroughly mixed with a measured amount of the isotopic spike. If a digestion procedure is required, the spiked sample is then digested to equilibrate the spikes and samples. The sample solutions are then measured with mass spectrometry such as ICP-MS to obtain the altered isotope ratios. Method 6020 can be used as a reference method for ICP-MS detection. In addition to Method 6020, dead time correction and mass bias correction must be included in the measurement protocol. The equations described in Section 12.1 are used to calculate the concentrations. Figure 2 shows an example of an IDMS determination of vanadium in crude oil (Reference 1).

2.2 SIDMS method:

2.2.1 Speciated samples generally require sample preparation specific to the sample matrices, species, and the isotope ratio measurement method. The purpose of sample preparation is to solubilize the species of interest and to equilibrate the natural and spiked species, creating a homogeneous solution. Solids, slurries, and suspended material must be subjected to extraction before spiking, using appropriate sample preparation methods (such as Method 3060 for the determination of Cr(VI) in soils). Water samples may not need extraction. In contrast to total element analysis, efforts must be taken to avoid the destruction of the species in SIDMS.

2.2.2 Although SIDMS is a general method applicable to many elements in various species forms, environmental samples, such as water samples or soil extracts, containing chromium species Cr(III) and Cr(VI) will be used for demonstration purposes. Two isotopic spikes are prepared and characterized: ^{50}Cr(III) spike enriched in ^{50}Cr and ^{53}Cr(VI) enriched in ^{53}Cr. The dominant natural isotope for Cr is ^{52}Cr, at 83.79% (^{50}Cr, 4.35%; ^{53}Cr, 9.50%; ^{54}Cr, 2.36%). A measured amount of a representative aqueous sample is mixed well with an appropriate amount of ^{50}Cr(III) and ^{53}Cr(VI) spike solutions. The spiked sample is then separated into Cr(III) and Cr(VI) using chromatography or other separation method. Four isotope ratios are measured: ^{50}Cr(III)/^{52}Cr(III), ^{53}Cr(III)/^{52}Cr(III), ^{50}Cr(VI)/^{52}Cr(VI), and ^{53}Cr(VI)/^{52}Cr(VI). The concentrations of the species are determined from speciated isotope dilution calculations. Figure 4 and Figure 5 show an example of the SIDMS for the determination of chromium species in an aqueous sample. Any transformation from Cr(VI) to Cr(III) or from Cr(III) to Cr(VI) are mathematically corrected, as described in Section 12.2.

3.0 Definitions

3.1 Isotope dilution mass spectrometry (IDMS): A quantitative method for total concentration of an analyte based on the measurement of the isotope ratio of a nuclide using mass spectrometry after isotope dilution.

3.2 Isotope dilution: Mixing of a given nuclide with one or more of its isotopes. The isotope usually has an enriched isotopic abundance different from that occurring naturally.

3.3 Speciated isotope dilution mass spectrometry (SIDMS). A quantitative method for determining elemental species based on the measurement of isotope ratio(s) in each species of a nuclide using mass spectrometry after speciated isotope dilution. Samples are mixed with one or more isotopic spikes which have different isotopic abundance and are artificially converted to chemical forms corresponding to the species to be analyzed. The spiked samples are then subjected to the separation of the species and the measurement of the altered isotope ratios in each species. Both species concentrations and species conversions can be mathematically deconvoluted.

3.4 Isotopic abundance: The relative number of atoms of a particular isotope in a mixture of the isotopes of an element, expressed as a fraction of all the atoms of the element.

3.5 Isotopes: Nuclides having the same atomic number but different mass numbers.

3.6 Species: Chemical forms in which an element exists.

3.7 Natural isotopic abundance: Isotopic abundance of elements from natural sources. Most elements (except lithium, lead and uranium) found in nature have a constant isotope abundance.

3.8 Isotope ratio: Ratio of the isotopic abundances of two isotopes.

3.9 Speciation (or speciated) analysis: Quantification of elements in specific chemical forms.

3.10 Isotope-enriched material: Material containing elements artificially enriched in minor isotopes.

3.11 Isotopic spike (Isotope-enriched spike): Standards prepared from isotope-enriched materials.

3.12 Dead time: The interval during which the detector and its associated counting electronics are unable to resolve successive pulses. The measured counts are lower than the true counts if no correction is performed.

3.13 Gain loss: The loss of gain in detector caused by the inability of the multiplier's dynode string to supply enough current to maintain constant dynode voltage drops. The measured counts are lower than the true counts, and cannot be mathematically corrected if gain loss occurs.

3.14 Mass Bias: The deviation of the measured isotope ratio from the true value caused by the differential sensitivity of the instrument to mass. This effect may occur in the ionization process or from differential transmission/detection by the mass spectrometer.

3.15 Mass bias factor: A number used to correct the mass bias of the measured isotope ratios. Mass bias factor is measured by employing an isotopically certified standard.

3.16 Isotopic-abundance-certified standard (Isotopically certified standard): Standard material with certified isotopic abundance.

3.17 Inverse isotope dilution: Analysis method to determine the concentrations of isotopic spikes. A known quantity and isotopic abundance of an isotopic spike is mixed with a known amount and isotopic abundance (usually tabulated natural isotopic abundance or certified isotopic abundance) of standard(s), and the altered isotope ratio(s) is(are) measured and used in the calculation to find the concentration of the isotopic spike. Usually, a natural material is used to calibrate and determine the concentration of the separated isotopic spike solution using this method. Only in the case of such elements as uranium, lead, and lithium are the natural isotopic abundances not constant in terrestrial materials.

3.18 Single spiking: Addition of one isotopic spike to the sample.

3.19 Double spiking: Addition of two isotopic spikes to the sample. The two isotopic spikes are enriched in different isotopes, and are prepared in different chemical forms, each of which corresponds to a species form.

3.20 Unidirectional conversion: One directional transformation occurring between two species. One species can convert to the other; the reverse transformation does not occur.

3.21 Interconversion: Bi-directional transformation occurring between two species. Species convert back and forth between the two chemical forms.

3.22 Time resolved analysis (TRA): A data collection mode in which the data can be acquired at specified intervals for a continuously aspirated sample, over a user-defined period of time.

4.0 INTERFERENCES

4.1 Sample preparation

4.1.1 Because this method requires the equilibration of the spike isotope(s) and the natural isotopes, the sample must be digested, dissolved or extracted into a solution. If the analyte of interest does not completely dissolve, if the spike or sample isotopes are selectively lost before equilibration, or if contamination occurs in the sample preparation process, the measured isotope ratio will not reflect the accurate ratio of added spike atoms to sample atoms for that element or species (Reference 1).

4.1.2 In general, SIDMS incorporates the assumption that all the converted species can be found in other species that are monitored. As an example, in the interconversion between Cr(III) and Cr(VI), the lost Cr in one species must be found in the other species. Thus, efforts should be made to keep all species in solution.

4.1.3 Preservation of the species is required in SIDMS since the interconversion degrades the precision of the determination. The complete conversion of the species will disable the deconvolution of the species concentration. Thus, digestion methods used for total metals are inappropriate for SIDMS. However, the altered isotope ratios will indicate the conversion that has occurred and will not lead to an incorrect answer, but to a situation where the concentration cannot be determined. Approaches that have been developed to maintain the species are applicable to SIDMS.

4.2 Isotope ratio measurement

4.2.1 Discussions about isobaric interference, doubly-charged ion interference, and memory interference in Method 6020 are applicable to this method. The discussion about the physical interference, suggesting the addition of an internal standard, does not apply. The internal standard is unnecessary because the isotope ratio measurement is free from physical interferences. (General considerations for isotope ratio measurement can be found in the document of Section 13.3.1).

4.2.2 Dead time measurement must be performed daily. At high count rates, two effects cause pulse counting systems to count less events than actually occur (Section 13.3.2). The first is dead time (τ), the interval during which the detector and its associated counting electronics are unable to resolve successive pulses. If the true rate, n, is much less than $1/\tau$, then:

$$m \approx n(1-n\tau)$$

where m is the observed rate. The second effect is the loss of gain at high rates caused by the inability of the multiplier's dynode string to supply enough current to maintain constant dynode voltage drops. This effect is indicated by a sharp increase in apparent dead time at high count rates. Both effects cause the measured isotope ratios to diverge from the true isotope ratios with increasing count rate. While the dead time can be mathematically corrected, the gain loss cannot.

A series of solutions with different concentrations can be prepared from isotopically certified standards for the determination of dead time. The concentrations may not be accurate, but the concentrations should spread out evenly, covering the blank to the highest count rate that may be used in measurements. The isotope pairs that are monitored should have large differences between their isotopic abundances, since the major isotopes suffer dead time effects much more seriously than minor isotopes; this makes the dead time correction significant. The sum of the dead-time-corrected counts is used for calculating the isotope ratios after background subtraction.

$$R_m = \frac{{}^{Isotope1}S_{sample/standard} - {}^{Isotope1}S_{background}}{{}^{Isotope2}S_{sample/standard} - {}^{Isotope2}S_{background}}$$

- R_m is the dead-time-corrected isotope ratio;
- ${}^{Isotope1}S_{sample/standard}$ and ${}^{Isotope2}S_{sample/standard}$ and are the integrated dead-time-corrected-counts for the sample or standard of Isotope1 and Isotope2, respectively;
- ${}^{Isotope1}S_{background}$ and ${}^{Isotope2}S_{background}$ are the integrated dead-time-corrected-counts for the background of Isotope1 and Isotope2, respectively.

As shown in Figure 1, which displays the $^{50}Cr/^{52}Cr$ ratios for SRM 979 ($Cr(NO_3)_3 \bullet 9H_2O$) as a function of the count rate, the isotope ratios are highly dependent on the number used for dead time correction. When the dead time is set to 43.5 ns, the isotope ratios are approximately constant up to the count rate of 5.8×10^5. At higher count rates, gain loss will occur and cannot be mathematically corrected. Therefore, the solutions must be diluted in the case where the count rate is higher than this value.

NOTE: Dead time correction is performed before mass bias correction, so the dead-time-corrected isotope ratios may be different from the certified isotope ratios. Although it is unnecessary to use isotopically certified material for the determination of dead time, the certified material is still required for the measurement of mass bias factors. Thus, it is convenient to use the same certified material for both dead time and mass bias factor measurement.

NOTE: It has been observed that using different isotope pairs for dead time measurement may obtain different dead times. Thus, it is required to do the dead time measurement for each isotope pair that will be used. The dead time must be determined daily.

4.2.3 Instrumental discrimination/fraction effects are changes induced in the "true" isotope ratios from the ionization process or from differential transmission/detection by the mass spectrometer. This effect can bias the ratios either positively or negatively. To correct the mass bias, mass bias factors should be determined with isotopically certified materials.

$$\text{mass bias factor} = R_t / R_m$$

where:

- R_t and R_m are the certified isotope ratio and the measured dead-time-corrected-isotope-ratios of the standard material.

The dead-time-corrected isotope ratios of the samples can be corrected using:

$$R_c = \text{mass bias factor} \times R_m$$

where:

- R_c and R_m are the corrected isotope ratio and the measured dead-time-corrected-isotope-ratios of the sample, respectively.

Mass discrimination is a time-dependent instrumental effect, so the mass bias factors must be determined periodically during the measurement of the samples. Samples are run with the assumption that mass bias factors remain constant. In general, the mass bias factors are stable over several hours for ICP-MS measurements.

NOTE: Some previous work observed the following relationship between the measured and the true isotope ratios for ICP-MS: $R_m=R_t(1+an)$, where a is the bias per mass unit, n is the mass difference between isotopes. This enables the calculation of the mass bias factors of other isotope pairs based on the measurement of one pair of isotopes. However, this must be verified experimentally. Otherwise, the mass bias factor for each isotope pair must be determined.

5.0 SAFETY

5.1 Refer to Chapter Three for a discussion on safety related references and issues.

5.2 Many chromium compounds are highly toxic if swallowed, inhaled, or absorbed through the skin. Extreme care must be exercised in the handling of hexavalent chromium reagents. Hexavalent chromium reagents should only be handled by analysts who are knowledgeable of their risks and of safe handling procedures.

6.0 EQUIPMENT AND SUPPLIES

6.1 Inductively coupled plasma-mass spectrometer (ICP-MS) or other mass spectrometer systems capable of base line (at least 1 amu) resolution are required. The data system should allow for corrections of isobaric interferences, dead time and mass bias, or the raw data may be exported to a computer for further processing. For quadrupole mass spectrometers, the dwell time should be adjustable since proper settings of dwell time can significantly improve the precision of the isotope ratio measurement. Both scan mode and peak jump mode can be used, depending on the instrumentation. The use of a mass-flow controller for the nebulizer argon and a peristaltic pump for the sample solution are recommended. When chromatography is coupled to ICP-MS for on-line

detection, the ICP-MS data system must be capable of correcting interferences, dead time and mass bias, and calculating the isotope ratios in time resolved analysis mode (TRA), or the raw data can be exported for off-line processing. Other mass spectrometers may also be used, providing a precision of 0.5% or better can be obtained for the isotope ratio measurement.

6.2 Chromatography or other separation methods are used to isolate species prior to isotope ratio measurement. Chromatography, such as ion exchange chromatography, may be used to separate the species on-line in SIDMS (Figure 3). Chromatography components should be chemically inert based on the specific reagents and analytes. The eluent components and the flow rate of the chromatography system must be compatible with ICP-MS. An interface between the chromatography and ICP-MS may be required for compatibility reasons. Alternatively, any appropriate separation methods, including extraction, chelation, and precipitation, can be used after validation.

7.0 REAGENTS AND STANDARDS

7.1 All reagents should be of appropriate purity to minimize the blank levels due to contamination. Whenever possible, acids should be sub-boiling distilled. All references to water in the method refer to high purity reagent water. Other reagent grades may be used if it is first ascertained that the reagent is of sufficient purity to permit its use without lessening the accuracy of the determination. If the purity of a reagent is questionable, analyze the reagent to determine the level of impurity.

7.2 For higher precision, solutions may be prepared by weight. For IDMS, standard stock solutions with natural isotopic abundance may be purchased or prepared from ultra-high purity grade chemicals or metals. See Method 6010 for instruction on preparing standard solutions from solids. Generally, the same procedures are applicable to isotope-enriched materials. However, when a limited amount of the isotope-enriched material is used (usually due to cost considerations) to prepare the stock solutions, the solutions require calibration with inverse isotope dilution (Section 7.4.1). Isotope-enriched materials with known enrichment can be purchased from several suppliers, such as the Oak Ridge National Laboratory Electromagnetic Isotope Enrichment Facility (ORNL-EMIEF).

7.3 Currently, few standard stock solutions made for speciation analysis are commercially available. Thus, in addition to the dissolution of the standard solid, the chemical conversion of the element into the desired species is usually required for SIDMS. The preparation of Cr(VI) and Cr(III) stock standards for SIDMS will be illustrated as an example. For other elements and species, procedures must be specifically developed.

7.3.1 There are five standards to be prepared for the simultaneous analysis of Cr(VI) and Cr(III), including ^{nat}Cr(VI) and ^{nat}Cr(III) with natural abundance, ^{53}Cr(VI) enriched in ^{53}Cr, ^{50}Cr(III) enriched in ^{50}Cr, and isotopic-abundance-certified Cr standard solution.

7.3.2 1 mg/mL Cr(VI) and Cr(III) standards are commercially available. ^{nat}Cr(VI) and ^{nat}Cr(III) can also be prepared from $K_2Cr_2O_7$ and Cr metal, respectively.

7.3.2.1 ^{nat}Cr(VI) standard solution, stock, 1 g = 1 mg Cr: Dissolve 0.2829 grams of $K_2Cr_2O_7$ in about 80 mL of reagent water and dilute to 100 g with reagent water.

7.3.2.2 $^{nat}Cr(III)$ standard solution, stock, 1 g = 1 mg Cr: Dissolve 0.1 g Cr metal in a minimum amount of 6M HCl and dilute the solution with 1% HNO_3 to 100 grams.

7.3.3 $^{53}Cr(VI)$ standard solution, 1 g ≈ 10 µg Cr: The following procedure describes chromium oxide as the source material. A 150 mL glass or quartz beaker is used for the dissolution. Weigh 5.8 mg (the exact amount should be calculated based on the content of Cr in the material) ^{53}Cr-enriched oxide into the beaker and add 8 mL concentrated $HClO_4$. Slowly heat the beaker on a hot plate until bubbles form on the bottom; the solution should not boil. Keep heating the solution for up to 6 hours until all solids are dissolved and only 1 to 2 mL of the solution remains. Turn off the hot plate and wait until the beaker cools down. Rinse the beaker and watch glass with 10 mL reagent water; the solution should turn intense yellow. Add 50 µL of 30% H_2O_2 and 4.5 mL of concentrated NH_4OH. Slowly heat the vessel until the solution gently boils to oxidize all Cr to Cr(VI). Allow the solution to boil for at least 15 minutes to remove the excessive H_2O_2. Transfer the solution to a 500 mL polymeric (Teflon, polyethylene, polypropylene, etc.) bottle and dilute the solution to 400 g. The exact concentration of the $^{53}Cr(VI)$ spike must be calibrated with $^{nat}Cr(VI)$ standard as described in Section 7.4.

NOTE: The procedure may be simpler when the isotope-enriched materials are available in other forms. For example, when $K_2Cr_2O_7$ enriched in ^{53}Cr is available, the solid can be dissolved in reagent water without further conversion; when Cr metal is available, the metal can be dissolved in 6M HCl as described in Section 7.3.2.2, followed by the addition of H_2O_2 and NH_4OH to oxidize Cr(III) to Cr(VI) as described above.

CAUTION: Concentrated $HClO_4$ is a very strong oxidizer. Safety protocols require this reagent only be used in a perchloric acid hood or equivalent solution and vapor handling system.

7.3.4 $^{50}Cr(III)$ standard solution, 1 g ≈ 10 µg Cr: The following procedure describes chromium metal as the source material. Weigh 4 mg of the metal into a 30 mL Teflon vessel. Add 4 mL of 6M HCl and gently heat the solution but do not boil it until the solid is dissolved. Continue to heat the solution until only 1 to 2 mL of the solution remains. The solution is then cooled and transferred to a 500 mL polymeric bottle. Dilute the solution with 1% HNO_3 to 400 grams. The exact concentration of the $^{50}Cr(III)$ spike must be calibrated with ^{nat}Cr standard as described in Section 7.4.

NOTE: The procedure depends on the form of the material. For example, when $K_2Cr_2O_7$ enriched in ^{50}Cr is available, the solid can be dissolved in 1% HNO_3, followed by the addition of H_2O_2 to reduce Cr(VI) to Cr(III). The excessive H_2O_2 can be removed by boiling the solution.

7.3.5 Isotopic-abundance-certified standard solution, stock, 1 g ≈ 10 µg Cr: Weigh 31 mg $Cr(NO_3)_3 \cdot 9H_2O$ (SRM 979) into a 500 mL polymeric container. Dissolve the solid and dilute it with 1% HNO_3 to 400 g.

7.4 The isotope-enriched spikes require characterization since a limited amount of material is usually weighed, complex treatment is involved, or the purity of the source material is limited (frequently <99%). For the SIDMS method, in addition to the total concentration of the standard, the distribution of the species must be determined before it can be used. Inverse IDMS and inverse

SIDMS measurement is used to calibrate the isotope-enriched spike and to determine the species distribution. The characterization of $^{53}Cr(VI)$ spike solution will be illustrated as an example.

7.4.1 Calibration of total concentration of spike solution with natural material: Weigh the proper amount (W_X) of 10 µg/g ($C_{Standard}$) ^{nat}Cr standard and the proper amount (W_S) of the $^{53}Cr(VI)$ spike (nominal concentration is 10 µg/g) into a polymeric container, and dilute the mixture with 1% HNO_3 to a concentration suitable for isotope ratio measurement. Use direct aspiration mode to determine the isotope ratio of $^{53}Cr/^{52}Cr$ ($R_{53/52}$). The concentration of the spike, C_{Spike}, can be calculated using the following equations.

$$C_{Spike} = C_S M_S$$

$$C_S = \frac{C_X W_x}{W_S}\left(\frac{{}^{53}A_X - R_{53/52}\,{}^{52}A_X}{R_{53/52}\,{}^{52}A_S - {}^{53}A_S}\right)$$

$$C_X = C_{standard} / M_X$$

where, C_S and C_X are the concentrations of the isotope-enriched spike and the standard with natural isotopic abundance in mmole/g, respectively. M_S and M_X are the average atomic weights of the spike and the standard in g/mol, respectively. $^{53}A_S$ and $^{53}A_X$ are the atomic fractions of ^{53}Cr for the spike and standard, respectively. $^{52}A_S$ and $^{52}A_X$ are the atomic fractions of ^{52}Cr for the spike and standard, respectively.

NOTE: The same procedure is applicable to the calibration of the isotope-enriched spike solutions in IDMS. The same procedure is also applicable to the calibration of $^{50}Cr(III)$ by changing isotope ^{53}Cr to ^{50}Cr.

NOTE: Average atomic weight = Σ(atomic weight of the isotope x atomic fraction)

7.4.2 Calibration of the concentration of the Cr(VI) in the $^{53}Cr(VI)$ spike with $^{nat}Cr(VI)$: Weigh the proper amount (W_X) of 10 µg/g ($C^{VI}_{standard}$) $^{nat}Cr(VI)$ standard and the proper amount (W_S) of the $^{53}Cr(VI)$ spike (nominal concentration is 10 µg/g) into a polymeric container, and dilute the mixture with reagent water to a concentration suitable for measurement. Acidify the solution to pH 1.7-2.0 with concentrated HNO_3. Separate the Cr(VI) with chromatography or other separation methods and measure the isotope ratio of $^{53}Cr/^{52}Cr$ in Cr(VI) species ($R^{VI}_{53/52}$). The concentration of Cr(VI) in the spike, C^{VI}_{Spike}, can be calculated using the following equations.

$$C^{VI}_{Spike} = C^{VI}_{S} M_S$$

$$C^{VI}_{S} = \frac{C^{VI}_{X} W_x}{W_S} \left(\frac{{}^{53}A_X - R^{VI}_{53/52} {}^{52}A_X}{R^{VI}_{53/52} {}^{52}A_S - {}^{53}A_S} \right)$$

$$C^{VI}_{X} = C^{VI}_{Standard} / M_X$$

where, C^{VI}_{S} and C^{VI}_{X} are the concentrations of Cr(VI) in the isotope-enriched spike and standard with natural isotopic abundance in µmole/g, respectively. M_S and M_X are the average atomic weights of the spike and the standard in g/mol, respectively. ${}^{53}A_S$ and ${}^{53}A_X$ are the atomic fractions of ^{53}Cr for the spike and standard, respectively. ${}^{52}A_S$ and ${}^{52}A_X$ are the atomic fractions of ^{52}Cr for the spike and standard, respectively.

NOTE: This set of equations is similar to those used in the determination of total Cr in $^{53}Cr(VI)$ standard (Section 7.4.1). The general equations for inverse SIDMS are not so simple. However, for speciation of Cr(VI) and Cr(III) in standard solutions, because the matrix is so simplified, only the reduction of Cr(VI) to Cr(III) is observed at low pH. Thus, the existence of Cr(III) species will not influence the isotope ratio of Cr(VI), and the complex equations can be simplified to the equations shown above (Reference 3).

7.4.3 The distribution of Cr(III) and Cr(VI) in $^{53}Cr(VI)$ spike can be calculated as:

$$\text{percentage of Cr(VI)} = \frac{C^{VI}_{Spike}}{C_{Spike}} \times 100\%$$

$$\text{percentage of Cr(III)} = \left(1 - \frac{C^{VI}_{Spike}}{C_{Spike}} \right) \times 100\%$$

NOTE: No determination of the species distribution in $^{50}Cr(III)$ spike is required because only Cr(III) is present in this solution.

7.5 Blanks: Three types of blanks are required for the analysis: background blank for subtracting background in isotope ratio measurement, preparation blank for monitoring possible contamination resulting from the sample preparation procedures, and rinse blank for flushing the system between all samples and standards.

7.5.1 The background blank consists of the same concentration(s) of the acid(s) used to prepare the final dilution of the sample solution (often 1% HNO_3 (v/v) in reagent water).

7.5.2 The preparation (or reagent) blank must be carried through the complete preparation procedure and contain the same volumes of reagents as the sample solutions.

7.5.3 The rinse blank consists of 1 to 2 % HNO_3 (v/v) in reagent water. Prepare a sufficient quantity to flush the system between standards and samples. Refer to Method 6020 for interference check solution.

7.6 Refer to Method 6020 for preparing mass spectrometer tuning solution.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

8.2 Due to the possible degradation or interconversion of the species, samples collected for speciation analysis must be isotopically spiked as soon as possible. The measurement, however, can be carried out later provided that less than 80% degradation or interconversion occurs. The holding time prior to measurement depends on the preservation of the species.

8.3 Proper methods to retard the chemical activity of the species are applicable to SIDMS.

8.4 All sample containers must be prewashed with detergents, acids, and water. Polymeric containers should be used. See Chapter Three of this manual for further information on clean chemistry procedures to reduce blank effects in these measurements.

9.0 QUALITY CONTROL

9.1 All quality control data must be available for reference or inspection. This method is restricted to use by, or under supervision of, experienced analysts. Refer to the appropriate section of Chapter One for additional quality control guidance.

9.2 Duplicate samples should be processed on a routine basis. A duplicate sample is a sample processed through the entire sample preparation and analytical procedure. A duplicate sample should be processed with each analytical batch or every 20 samples, whichever is the greater number. A duplicate sample should be prepared for each matrix type (i.e., soil, sludge, etc.).

9.3 Spiked samples and/or standard reference materials should be included with each group of samples processed or every 20 samples, whichever is the greater number. A spiked sample should also be included whenever a new sample matrix is being analyzed. For SIDMS, because the species may degrade or convert to other species when they are spiked into samples, low recovery may be observed. Thus, the low recovery does not immediately invalidate this method. For example, if Cr(III) is spiked into a basic solution, due to the hydrolysis of Cr(III) and the limited solubility of chromium hydroxide, low recovery of Cr(III) will be obtained. Low recovery may indicate an unfavorable matrix for preserving the corresponding species (Reference 4).

9.4 Blank samples should be prepared using the same reagents and quantities used in sample preparation, placed in vessels of the same type, and processed with the samples.

10.0 CALIBRATION AND STANDARDIZATION

10.1 IDMS calibration:

10.1.1 Follow the appropriate sections in Method 6020 to set up and tune the ICP-MS. The determination is performed in direct aspiration mode. The following procedure is illustrated with the measurement of $^{50}Cr/^{52}Cr$ and $^{53}Cr/^{52}Cr$ isotope ratios.

10.1.2 Determine the dead time (Section 4.2.2). Solutions prepared from reference material SRM 979 ($Cr(NO_3)_3 \bullet 9H_2O$) are used in this determination. A range of solutions of different concentrations should be prepared, but do not need to be accurately known. Masses 50, 52 and 53, as well as masses which could affect data quality should be monitored. The raw count rates for each solution are measured and integrated. Assume a dead time and use the equation described in Section 4.2.2 to correct the integrated counts. The dead-time-corrected counts are then used for calculating the isotope ratios after background subtraction. By trial and error, the dead time is determined to bring the isotope ratios obtained from solutions of different concentrations to a constant (the relative standard deviation of the isotope ratios reach the minimum). The isotope ratios obtained from high counts may be excluded as gain loss may occur.

NOTE: The concentration range of the solutions may be adjusted depending on the sensitivity and dynamic range of the instrument.

NOTE: For direct aspiration mode, the dead time correction can be done either before or after the integration of the raw data. However, it is simpler to do the dead time correction after the integration.

10.1.3 Determine the mass bias factor (Section 4.2.3). The mean of isotope ratios obtained in Section 10.1.2 is used for calculating the mass bias factor. The equation is provided in Section 4.2.3. The measurement of the mass bias factor must be done periodically between sample measurements. The interval between these measurements depends on the mass bias stability of the instrument. The relative difference between two consecutive mass bias factors should not exceed 1%.

10.2 SIDMS calibration:

10.2.1 Follow the appropriate sections in Method 6020 to set up and tune the ICP-MS. Follow Section 10.1.2 to measure the dead time. If the calibration of the isotope-enriched spikes is required, the mass bias factors for direct aspiration mode and the altered isotope ratios for the spiked standards are measured at this step. The measured isotope ratios obtained at this step are used in the calibration of total concentrations.

10.2.2 Determine the mass bias factor (Section 4.2.3). Connect the chromatography outlet to the nebulizer of the ICP-MS. Stabilize the entire system. Background blank and an isotopic abundance certified standard are used for the measurement of the mass bias factors for TRA mode. The raw data at each point are corrected for dead time using the equation described in Section 4.2.2 and then integrated by summing the data across each peak. The intervals between two consecutive injections must be long enough for the signal to return to baseline. The integrated counts are then used to calculate the isotope ratios with the equation shown in Section 4.2.2. Apply the equation in Section 4.2.3 to the calculation of the mass bias factors for each isotope pair by comparing the measured isotope ratios to the certified isotope ratios.

NOTE: For the TRA mode, the dead time correction must be done at each data point before the data integration.

11.0 PROCEDURE

11.1 IDMS

11.1.1 Closed-vessel microwave digestion is used as an example method to decompose, solubilize and stabilize the elements of interest. The following procedure is applicable to samples specified in Method 3052. Refer to Method 3052 for specification of the microwave apparatus.

11.1.2 Prepare the isotope-enriched spike and calibrate it with the inverse isotope dilution mass spectrometry procedure described in Sections 7.2 and 7.4.1.

11.1.3 Weigh a representative sample to the nearest 0.001 g into an appropriate microwave digestion vessel equipped with a pressure relief mechanism. Spike the sample with the calibrated isotope-enriched spike. The concentration of the spike should be high enough so that only a small volume of the solution is used. At least three significant figures should be maintained for the mass of the spike.

11.1.4 Digest the sample according to the procedure described in Method 3052.

NOTE: For filtered and acidified aqueous samples, digestion may not be required. Sample solutions can be directly analyzed with ICP-MS after spiking and equilibration.

11.1.5 Measurement of the isotope ratios can be carried out using ICP-MS or other appropriate mass spectrometers.

11.1.5.1 Determine the mass bias factor periodically as described in Section 10.1.3.

11.1.5.2 Measure the isotope ratio of each sample. Flush the system with the rinse blank. The ideal isotope ratio is 1:1. Isotope ratios must be within the range from 0.1:1 to 10:1, except for blanks and samples with extremely low concentrations. Samples must be diluted if too high a count rate is observed to avoid gain loss of the detector.

NOTE: For elements such as lithium, lead, and uranium, the unspiked solution is used to measure the isotopic abundance of all the isotopes because the isotopic abundances of these elements are not invariant in nature.

11.2 SIDMS:

11.2.1 SIDMS is currently applicable to the quantification of elemental species in various aqueous solutions. Solid samples require isolation and separation to solubilize the elemental species before spiking. Procedures for such extraction of the species from different matrices must be specifically designed. The following procedure is an illustration of the simultaneous determination of Cr(III) and Cr(VI) in water samples or soil or sediment extracts. Solids are extracted for Cr(VI) using Method 3060.

11.2.2 Prepare the isotope-enriched spikes in species forms and calibrate them with inverse isotope dilution mass spectrometry described in Section 7.4.

11.2.3 Extract the species from the samples such as soils and sludges. Proposed Method 3060 can be used to extract Cr(VI) from soils.

NOTE: For aqueous samples, extraction may not be required.

11.2.4 Weigh a proper amount of water sample or extract to the nearest 0.0001 g into a polymeric container. Spike the sample with 10 µg/g ^{53}Cr(VI) spike to a concentration so that the isotope ratio of $^{53}Cr/^{52}Cr$ in Cr(VI) will be approximately 1:1. Thoroughly mix the spike and the sample. The isotope ratios $^{53}Cr/^{52}Cr$ for samples must be within the range of 0.1:1 to 10:1, except for blanks or samples with extremely low concentrations.

11.2.5 Dilute the ^{53}Cr(VI)-spiked sample with reagent water. If the solution is strongly basic, neutralize the sample with concentrated HNO_3 to avoid the hydrolysis of Cr(III). Spike the diluted sample with 10 µg/g ^{50}Cr(III) spike to a concentration so that the isotope ratios of $^{50}Cr/^{52}Cr$ in Cr(III) will also be approximately 1:1 and the species concentrations are suitable for measurement. The measured isotope ratios $^{50}Cr/^{52}Cr$ for samples must be within the range of 0.1:1 to 10:1 except for blanks and samples with extremely low concentrations, or the sample should be respiked and analyzed. (Sections 11.2.4 and 11.2.5 should be completed as quickly as possible.)

NOTE: If only the Cr(VI) is of interest, the sample can be single spiked with ^{53}Cr(VI) instead of double-spiking with both ^{50}Cr(III) and ^{53}Cr(VI). However, this is based on the assumption that only unidirectional conversion, the reduction Cr(VI) to Cr(III), can occur after spiking. This is usually true if the sample is acidified to low pH after spiking, especially for matrices containing reducing agents.

11.2.6 Acidify the spiked samples to pH 1.7 to 2.0; under these conditions Cr is usually retained in the solutions, (although there might be interconversion between Cr(III) and Cr(VI)). The spiked samples can be stored at 4°C to retard the interconversion of the species. Other methods that can retard the transformation of the species are applicable as long as no interference with the isotope ratio measurement is introduced. For example, some soil extracts contain large concentrations of reducing agents that reduce Cr(VI) rapidly after acidification. To slow down the reduction, stoichiometric amounts of $KMnO_4$ can be added to the sample to compete with Cr(VI) in the oxidation of reducing matrices.

NOTE: Studies have shown that the lower the interconversion, the more precise the determination (Reference 3). Thus, efforts should be made to prevent interconversion between the species.

11.2.7 The measurement of the isotope ratios in each species can be carried out using ICP-MS or other equivalent mass spectrometers following the separation of the species using chromatography or other separation methods. An ion-exchange chromatograph coupled with ICP-MS will be illustrated as an example in the measurement of $^{50}Cr/^{52}Cr$ and $^{53}Cr/^{52}Cr$ isotope ratios in both Cr(III) and Cr(VI) species in samples.

11.2.7.1 Determine the mass bias factors periodically as described in Section 10.2.2.

11.2.7.2 Measure the isotope ratios of each sample. Flush the system with the eluent until the signal returns to the baseline. The ideal isotope ratios for $^{50}Cr/^{52}Cr$ in Cr(III) and $^{53}Cr/^{52}Cr$ in Cr(VI) are 1:1. Ratios between 0.1:1 to 10:1 are also acceptable. Samples may be respiked to achieve an isotope ratio close to 1:1. Samples must be diluted if excessively high count rates are observed to avoid gain loss of the detector.

NOTE: For elements such as lithium, lead, and uranium, the unspiked solution is used to measure the isotopic abundance of all the isotopes because the isotopic abundances are not invariant in nature.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 IDMS-Calculations: The quantitative values shall be reported in appropriate units, such as micrograms per liter (µg/L) for aqueous samples and milligrams per kilogram (mg/kg) for solid samples. If dilutions are performed, the appropriate corrections must be applied to the sample values.

12.1.1 Calculate the isotope ratios. Calculations should include appropriate interference corrections (see Section 4.2 for data integration, dead time correction, and mass bias correction).

12.1.2 The following equations are applied to the calculation of the concentration of the element, C_{Sample}(µg/g), in the final sample solutions.

$$C_{Sample} = C_X M_X$$

$$C_S = C_{Spike} / M_S$$

$$C_X = \frac{C_S W_S}{W_X} \left(\frac{{}^{53}A_S - R_{53/52}\,{}^{52}A_S}{R_{53/52}\,{}^{52}A_X - {}^{53}A_X} \right)$$

where, C_S and C_X are the concentrations of the isotope-enriched spike and the sample in mmole/g, respectively. M_S and M_X are the average atomic weights of the isotope-enriched spike and the sample in g/mole, respectively. $^{53}A_S$ and $^{53}A_X$ are the atomic fractions of ^{53}Cr for the isotope-enriched spike and sample, respectively. $^{52}A_S$ and $^{52}A_X$ are the atomic fractions of ^{52}Cr for the isotope-enriched spike and sample, respectively. C_{spike} is the concentration of the isotope-enriched spike in µg/g.

NOTE: When isotope ^{50}Cr is used, substitute 53 with 50 in the above equations.

12.1.3 If appropriate or required, calculate results for solids on a dry-weight basis as follows:

(1) A separate determination of percent solids must be performed.
(2) The concentrations determined in the digest are to be reported on the basis of the dry weight of the sample.

$$\text{Concentration (dry weight)(mg/kg)} = \frac{C_{Sample}}{S}$$

where, C_{Sample} = Concentration based on the wet sample (µg/g)

$$S = \frac{\%\ \text{Solids}}{100}$$

12.2 SIDMS-Calculations: The quantitative values shall be reported in appropriate units, such as micrograms per liter (µg/L) for aqueous samples and milligrams per kilogram (mg/kg) for solid samples. If dilutions are performed, the appropriate corrections must be applied to the sample values.

12.2.1 Calculate the isotope ratios. Calculations should include appropriate interference corrections, dead time correction, and mass bias correction (Section 4.2).

12.2.2 The following equations are used to deconvolute the concentrations of the species at the time of spiking, as well as the conversion of the species after spiking.

$$R^{III}_{50/52} = \frac{\left({}^{50}A_x C^{III}_x W_x + {}^{50}A^{III}_s C^{III}_s W^{III}_s\right)(1-\alpha) + \left({}^{50}A_x C^{VI}_x W_x + {}^{50}A^{VI}_s C^{VI}_s W^{VI}_s\right)\beta}{\left({}^{52}A_x C^{III}_x W_x + {}^{52}A^{III}_s C^{III}_s W^{III}_s\right)(1-\alpha) + \left({}^{52}A_x C^{VI}_x W_x + {}^{52}A^{VI}_s C^{VI}_s W^{VI}_s\right)\beta}$$

$$R^{III}_{53/52} = \frac{\left({}^{53}A_x C^{III}_x W_x + {}^{53}A^{III}_s C^{III}_s W^{III}_s\right)(1-\alpha) + \left({}^{53}A_x C^{VI}_x W_x + {}^{53}A^{VI}_s C^{VI}_s W^{VI}_s\right)\beta}{\left({}^{52}A_x C^{III}_x W_x + {}^{52}A^{III}_s C^{III}_s W^{III}_s\right)(1-\alpha) + \left({}^{52}A_x C^{VI}_x W_x + {}^{52}A^{VI}_s C^{VI}_s W^{VI}_s\right)\beta}$$

$$R^{VI}_{50/52} = \frac{\left({}^{50}A_x C^{III}_x W_x + {}^{50}A^{III}_s C^{III}_s W^{III}_s\right)\alpha + \left({}^{50}A_x C^{VI}_x W_x + {}^{50}A^{VI}_s C^{VI}_s W^{III}_s\right)(1-\beta)}{\left({}^{52}A_x C^{III}_x W_x + {}^{52}A^{III}_s C^{III}_s W^{III}_s\right)\alpha + \left({}^{52}A_x C^{VI}_x W_x + {}^{52}A^{VI}_s C^{VI}_s W^{VI}_s\right)(1-\beta)}$$

$$R^{VI}_{53/52} = \frac{\left({}^{53}A_x C^{III}_x W_x + {}^{53}A^{III}_s C^{III}_s W^{III}_s\right)\alpha + \left({}^{53}A_x C^{VI}_x W_x + {}^{53}A^{VI}_s C^{VI}_s W^{III}_s\right)(1-\beta)}{\left({}^{52}A_x C^{III}_x W_x + {}^{52}A^{III}_s C^{III}_s W^{III}_s\right)\alpha + \left({}^{52}A_x C^{VI}_x W_x + {}^{52}A^{VI}_s C^{VI}_s W^{VI}_s\right)(1-\beta)}$$

where,

$R^{III}_{50/52}$ is the measured isotope ratio of ^{50}Cr to ^{52}Cr of Cr(III) in the spiked sample

$^{50}A_X$ is the atomic fraction of ^{50}Cr in the sample (usually a constant in nature)

C^{III}_X is the concentration of Cr(III) in the sample (µmole/g, unknown)

W_X is the weight of the sample (g)

$^{50}A^{III}_S$ is the atomic fraction of ^{50}Cr in the ^{50}Cr(III) spike

C^{III}_S is the concentration of Cr(III) in the ^{50}Cr(III) spike (µmole/g)

W^{III}_S is the weight of the ^{50}Cr(III) spike (g)

C^{VI}_X is the concentration of Cr(VI) in the sample (µmole/g, unknown)

α is the percentage of Cr(III) oxidized to Cr(VI) after spiking (unknown)

β is the percentage of Cr(VI) reduced to Cr(III) after spiking (unknown)

NOTE: The unit of the concentrations shown above is µmole/g. The conversion factor from µmole/g to µg/g is: M, where M is the average atomic weight of the element in g/mole (Section 7.4.1). The following equation can be used to convert the unit of the concentration. Be aware that samples with different isotopic abundance have different average atomic weights.

Concentration (µmole/g)× M = Concentration (µg/g)

NOTE: Although the species distribution of the isotopic spike is determined (Section 7.4), the above equations assume that each isotope-enriched spike is only in one species form to simplify the equations. This has been validated for ^{50}Cr(III) and ^{53}Cr(VI) spikes prepared using the procedures described in Section 7.3. For other speciation analysis, this assumption must be verified experimentally, or the distribution of the species in the isotope-enriched spikes must be taken into account.

NOTE: For the quantification of the single-spiked samples, the following equations are used:

$$C^{VI}_{Sample} = C^{VI}_X M_X$$

$$C^{VI}_S = C^{VI}_{Spike}/M^{VI}_S$$

$$C_X^{VI} = \frac{C_S^{VI} W_S}{W_X} \left(\frac{{}^{53}A_S^{VI} - R_{53/52}^{VI}\, {}^{52}A_S^{VI}}{R_{53/52}^{VI}\, {}^{52}A_X - {}^{53}A_X} \right)$$

where, C_S^{VI} and C_X^{VI} are the concentrations of the isotope-enriched spike and the sample in µmole/g, respectively. M_S and M_X are the average atomic weight of the isotope-enriched spike and the sample in g/mole, respectively. ${}^{53}A_S$ and ${}^{53}A_X$ are the atomic fraction of ^{53}Cr for the isotope-enriched spike and sample, respectively. ${}^{52}A_S$ and ${}^{52}A_X$ are the atomic fractions of ^{52}Cr for the isotope-enriched spike and sample, respectively. C_{spike} is the concentration of the isotope-enriched spike in µg/g.

NOTE: When isotope ^{50}Cr is used, substitute 53 with 50 in the above equations.

12.2.3 A computer program such as a spreadsheet can be developed to solve this set of second power, four variable equations. Solutions of the values for, C_X^{III}, C_X^{VI}, α and β are required. The following mathematics is a way to solve the equations iteratively. To assist the analyst a spreadsheet file with these preprogrammed equations has been placed on the internet (Reference 10). Additional discussion and alternate equations are also available.

To make the expression simpler, assume

$$C_X^{III} W_X = N_X^{III},\ C_X^{VI} W_X = N_X^{VI},\ C_S^{III} W_S = N_S^{III},\ C_S^{VI} W_S = N_S^{VI}$$

At the beginning of the iteration, arbitrary values can be assigned to N_X^{VI} and α. For example, both of them are assigned as 0s. Now we need to know the expression of N_X^{III} and β. After careful derivation, we can get the following equations:

$$\begin{cases} (1-\alpha)\left(R_{50/52}^{III}\, {}^{52}A_X - {}^{50}A_X\right)N_X^{III} + \left[R_{50/52}^{III}\left({}^{52}A_X N_X^{VI} + {}^{52}A_S^{VI} N_S^{VI}\right) - \left({}^{50}A_X N_X^{VI} + {}^{50}A_S^{VI} N_S^{VI}\right)\right]\beta = \left(-R_{50/52}^{III}\, {}^{52}A_S^{III} + {}^{50}A_S^{III}\right)N_S^{III}(1-\alpha) \\ (1-\alpha)\left(R_{53/52}^{III}\, {}^{52}A_X - {}^{53}A_X\right)N_X^{III} + \left[R_{53/52}^{III}\left({}^{52}A_X N_X^{VI} + {}^{52}A_S^{VI} N_S^{VI}\right) - \left({}^{53}A_X N_X^{VI} + {}^{53}A_S^{VI} N_S^{VI}\right)\right]\beta = \left(-R_{53/52}^{III}\, {}^{52}A_S^{III} + {}^{53}A_S^{III}\right)N_S^{III}(1-\alpha) \end{cases}$$

These equations can be rewritten as:

$$\begin{cases} A_1 N_X^{III} + B_1\beta = C_1 \\ A_2 N_X^{III} + B_2\beta = C_2 \end{cases}$$

The solutions are

$$N_X^{III} = \frac{\begin{vmatrix} C_1 & B_1 \\ C_2 & B_2 \end{vmatrix}}{\begin{vmatrix} A_1 & B_1 \\ A_2 & B_2 \end{vmatrix}} \quad \text{and} \quad \beta = \frac{\begin{vmatrix} A_1 & C_1 \\ A_2 & C_2 \end{vmatrix}}{\begin{vmatrix} A_1 & B_1 \\ A_2 & B_2 \end{vmatrix}}$$

Use these two values in the following equations to solve N_X^{VI} and α

$$\begin{cases} (1-\beta)\left(R_{50/52}^{VI}\,{}^{52}A_X - {}^{50}A_X\right)N_X^{VI} + \left[R_{50/52}^{VI}\left({}^{52}A_X N_X^{III} + {}^{52}A_S^{III}N_S^{III}\right) - \left({}^{50}A_X N_X^{III} + {}^{50}A_S^{III}N_S^{III}\right)\right]\alpha = \left(-R_{50/52}^{VI}\,{}^{52}A_S^{VI} + {}^{50}A_S^{VI}\right)N_S^{VI}(1-\beta) \\ (1-\beta)\left(R_{53/52}^{VI}\,{}^{52}A_X - {}^{53}A_X\right)N_X^{VI} + \left[R_{53/52}^{VI}\left({}^{52}A_X N_X^{III} + {}^{52}A_S^{III}N_S^{III}\right) - \left({}^{53}A_X N_X^{III} + {}^{53}A_S^{III}N_S^{III}\right)\right]\alpha = \left(-R_{53/52}^{VI}\,{}^{52}A_S^{VI} + {}^{53}A_S^{VI}\right)N_S^{VI}(1-\beta) \end{cases}$$

Rewrite the equation as:

$$\begin{cases} A_3 N_X^{VI} + B_3\alpha = C_3 \\ A_4 N_X^{VI} + B_4\alpha = C_4 \end{cases}$$

again

$$N_X^{VI} = \frac{\begin{vmatrix} C_3 & B_3 \\ C_4 & B_4 \end{vmatrix}}{\begin{vmatrix} A_3 & B_3 \\ A_3 & B_3 \end{vmatrix}} \quad \text{and} \quad \alpha = \frac{\begin{vmatrix} A_3 & C_3 \\ A_4 & C_4 \end{vmatrix}}{\begin{vmatrix} A_3 & B_3 \\ A_4 & B_4 \end{vmatrix}}$$

Repeating the calculation, the variables N_X^{III}, N_X^{VI}, α and β will converge to constant values, and these values are the solution of the equations.

12.2.4 Results should be discarded when $\alpha + \beta > 80\%$ because the interconversion will be too extensive and cause inaccuracy and imprecision in the corrections. Samples should be respiked with the isotope-enriched spikes and analyzed, and the preservation should be improved to retard the conversion of the species.

12.2.5 If appropriate or required, calculate results for solids on a dry-weight basis as described in Section 12.1.3.

13.0 METHOD PERFORMANCE

13.1 Performance and use of IDMS as a definitive method in standard reference material certification has been well established in practice and in the literature. Review and discussion articles are referenced for performance criteria of this highly accurate method (References 1, 8, 9).

13.2 Accuracy, precision, and capability of SIDMS in correcting species interconversion are shown in Table 1. Table 2 and Table 3 compare data against Method 7196 analysis for Cr(VI) in chromium ore process residues and soil extracts. Table 1 demonstrates the ability of Method 6800 to correct for transformations of both Cr(VI) and Cr(III) in aqueous samples and also the magnitude of errors that may be expected when using other methods that are unable to determine the conversion of these species. Table 2 indicates a sample type where both the traditional 3060/7196 methods and 3060/6800 methods produced statistically similar data indicating confirmation between these two analytical methods. Table 3 demonstrates the correction necessary in some soil samples

where the sample matrix would cause a bias in more traditional methods. These bias corrections demonstrate the ability of Method 6800 to identify and correct for the degradation of a species during the measurement process.

13.3 The following documents may provide additional guidance and insight on this method and technique:

13.3.1 Javis, K. E.; Gray, A. L.; Houk, R. S. *Handbook of Inductively Coupled Plasma Mass Spectrometry*; Blackie: London, 1992.

13.3.2 Russ, G. P., III; Bazan, J. M. *Spectrochim. Acta, Part B* 1987, 42B, 49- 62.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quality and/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 the 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.

14.2 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.

15.0 Waste Management

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits 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.

16.0 References

1. Fasset, J. D.; Paulsen, P. J. Isotope Dilution Mass Spectrometry *Anal. Chem.* 1989, 61, 643A-649A.

2. Kingston, H. M. *Method of Speciated Isotope Dilution Mass Spectrometry*, US Patent Number: 5,414,259, 1995.

3. Kingston, H. M.; Huo, D.; Lu, Y. "Speciated Isotope Dilution Mass Spectrometry (SIDMS): The Accurate Determination of Reactive Species," *(submitted Anal. Chem.* 1998).

4. James, B. R.; Petura, J. C.; Vitale, R. J.; Mussoline, G. R. *Environ. Sci. Tech.* 1995, 29, 2377.

5. Kingston, H. M.; Huo, D.; Lu, Y.; Chalk, S. "Accuracy in Species Analysis. Speciated Isotope Dilution Mass Spectrometry (SIDMS) Exemplified by Evaluation of Chromium," *(Accepted) Spectrochim Acta.* 1998.
6. Kingston, H. M.; Huo, D.; Chalk, S.; Walter, P. The Accurate Determination of Species by Speciated Isotope Dilution Mass Spectrometry: Exemplified by the Evaluation of Chromium (VI) in Soil, The Twelfth Annual Waste Testing & Quality Assurance Symposium; Washington, DC, July 23-36 1996; 112-119.

7. Lu, Y.; Huo, D.; Kingston, H. M. "Determination of Analytical Biases and Chemical Mechanisms in the Analysis of Cr(VI) Using EPA Protocols, *(submitted Environ. Sci. Tech.,* 1998).

8. Bowers, G. N, Jr.; Fassett, J. D.; White, D. V. *Anal. Chem.* 1993, 65, 475R.

9. Moore, L. J.; Kingston, H. M.; Murphy, T. J. *Environ. Intern.* 1984, 10, 169.

10. Kingston, H. M.; Huo, D. (copywright 1998) SamplePrep Web™ [Homepage of SamplePrep Web™], [Online]. Available: http://www.sampleprep.duq.edu/sampleprep/ [1998, January 22].

17.0 Tables, Diagrams, Flowcharts, and Validation Data

The pages to follow contain Tables 1 through 3, Figures 1 through 5, and a method procedure flow diagram.

TABLE 1

ANALYSIS OF AN ARTIFICIALLY SYNTHESIZED WATER SAMPLE.
(Reference 6)

		Concentration (ng/g)		Conversion (%)	
Aliquot	Days after spiking	Cr(III)	Cr(VI)	Cr(III) to Cr(VI)	Cr(VI) to Cr(III)
1	1	69.8 ± 0.3	68.8 ± 0.3	4.87 ± 0.22	3.57 ± 0.03
	4	69.2 ± 0.6	69.4 ± 0.3	3.47 ± 0.11	11.9 ± 0.5
	13	70.5 ± 0.9	68.5 ± 0.4	2.80 ± 0.13	22.4 ± 0.2
2	1	69.6 ± 0.2	68.8 ± 0.4	17.6 ± 0.1	2.95 ± 0.02
	4	69.3 ± 0.7	69.6 ± 0.6	14.6 ± 1.3	11.4 ± 0.7
	13	70.7 ± 0.4	68.8 ± 0.3	12.8 ± 0.1	22.1 ± 0.3
3	1	69.8 ± 0.6	69.0 ± 0.2	23.8 ± 0.3	2.76 ± 0.08
	4	69.0 ± 0.8	69.6 ± 0.3	21.6 ± 0.2	10.2 ± 0.1
	13	70.4 ± 0.5	68.9 ± 0.8	17.6 ± 0.3	22.1 ± 0.1
True		69.67	68.63		

mean ± 95% confidence interval

Aliquots 1, 2 and 3 were from the same isotopically-spiked synthesized sample. These aliquots were treated in different ways to induce different degrees of interconversion between Cr(III) and Cr(VI). Measurements were done on different days to check the stability of the species during storage. Despite the different degrees of interconversion, the deconvoluted concentrations for both Cr(III) and Cr(VI) were always corrected successfully within experimental error to the true concentrations.

TABLE 2

CONCENTRATIONS OF CR(VI) IN COPR SAMPLES DETERMINED WITH METHOD 7196 AND SIDMS
(Reference 7)

	Method 7196		SIDMS	
sample	Conc. of Cr(VI) (µg/g)	Average (mean ±std)	Conc. of Cr(VI) (µg/g)	Average (mean ±std)
COPR1	1330	1410 ± 85	1373	1445 ± 70
	1410		1449	
	1500		1512	
COPR3	91.2	85.3 ± 5.2	93.9	88.8 ± 6.1
	81.5		82.1	
	83.1		90.4	
COPR4	408.9	407.8 ± 7.2	419.8	418.0 ± 9.2
	414.4		426.1	
	400.2		408.0	

COPR: chromite ore processing residue.
Method 3060 was used for Cr(VI) extraction.
Results obtained from SIDMS and Method 7196 are comparable for COPR samples.

TABLE 3

RECOVERY OF CR(VI) SPIKED INTO SOIL EXTRACTS
(Reference 7)

Sample	Mass of Soil (g)	Spiked ^{nat}Cr(VI)	Recovery (%)	
		(µg/g)	Method 7196	SIDMS
1	0	2.997	101 ± 0.4	100 ± 1.3
2	1.53	3.033	91.8 ± 1.7	100 ± 0.3
3	3.06	2.993	81.9 ± 1.1	101 ± 0.3
4	3.12	1.587	71.6 ± 2.5	99.3 ± 0.3

Results obtained from SIDMS and Method 7196 are incomparable for soil extracts due to the serious matrix effects resulting from the coexisting reducing agents in soil. Method 7196 is incapable of correcting conversion of Cr(VI) leading to low recoveries. Results are based on N = 3 with uncertainties expressed in standard deviation.

FIGURE 1

THE INFLUENCE OF THE DEAD TIME CORRECTION ON THE ISOTOPE RATIOS MEASURED WITH ICP-MS EQUIPPED WITH A CONTINUOUS DYNODE MULTIPLIER
Gain loss occurs when the count rate exceeds $5.8^{x}10^{5}$.

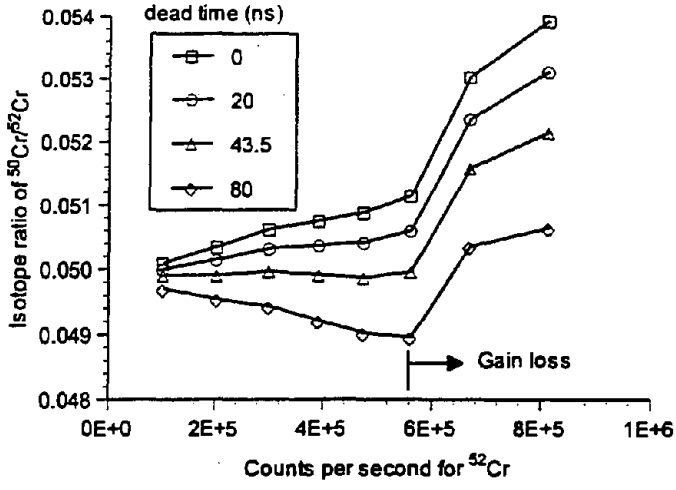


FIGURE 2

IDMS DETERMINATION OF VANADIUM IN CRUDE OIL. NUMBERS SHOWN ABOVE THE BARS ARE THE ATOMIC FRACTION
(Revised from Reference 1)

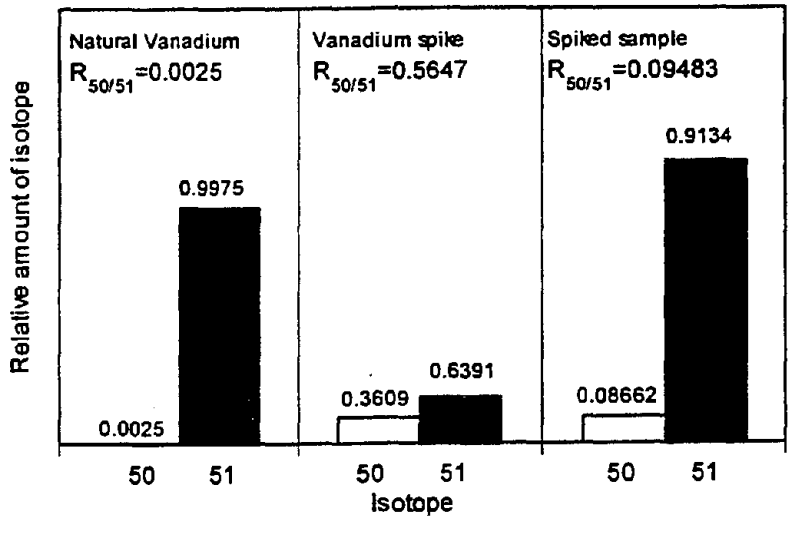


FIGURE 3

SEPARATION AND DETECTION OF CR(III) AND CR(VI) WITH ION-EXCHANGE CHROMATOGRAPHY COUPLED WITH AN ICP-MS
(Reference 5)

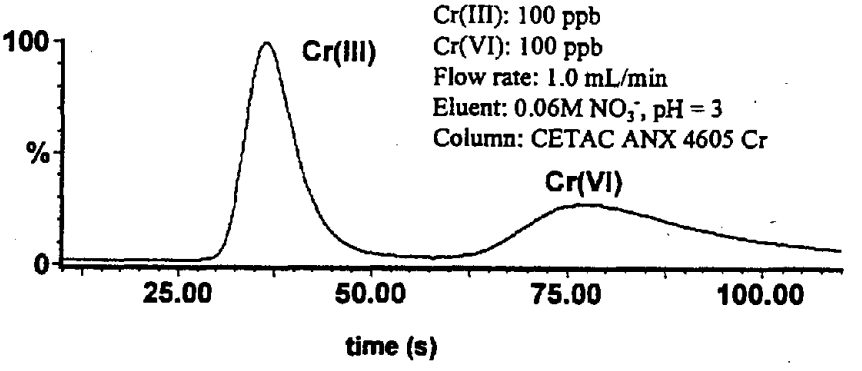


FIGURE 4
SEPARATION OF THE UNSPIKED SAMPLE AND ISOTOPICALLY SPIKED SAMPLE
(Reference 3)

(a): Chromatograms of a solution containing Cr(III) and Cr(VI) with natural isotopic abundance.
(b): Chromatograms of the same solution spiked with isotope-enriched spikes **^{50}Cr(III) and ^{53}Cr(VI)** .

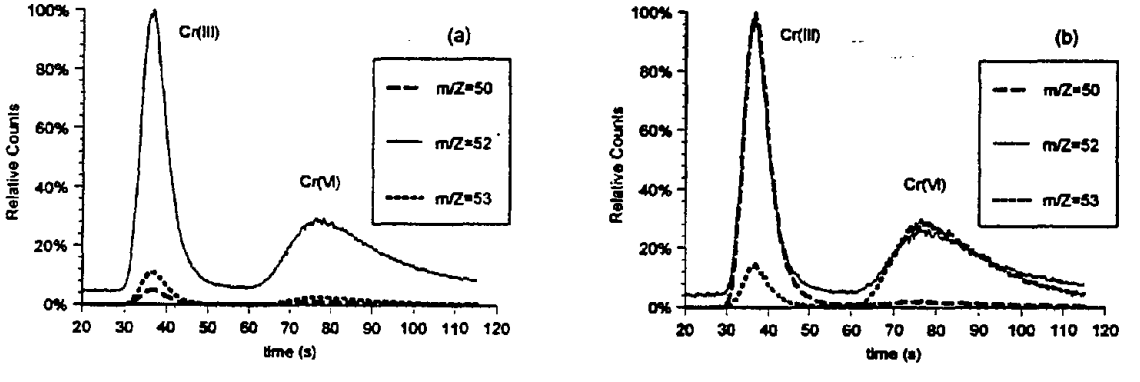
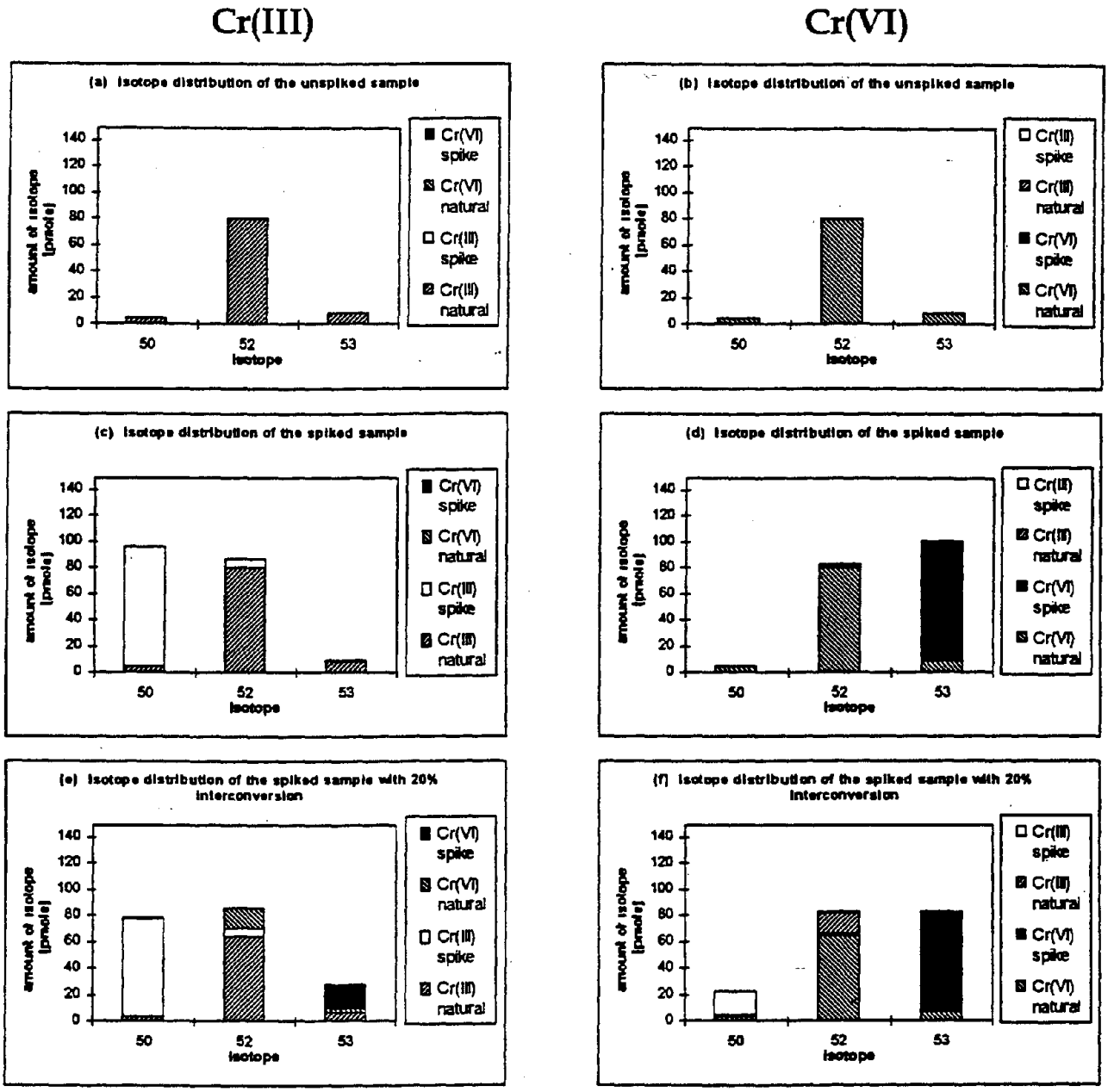


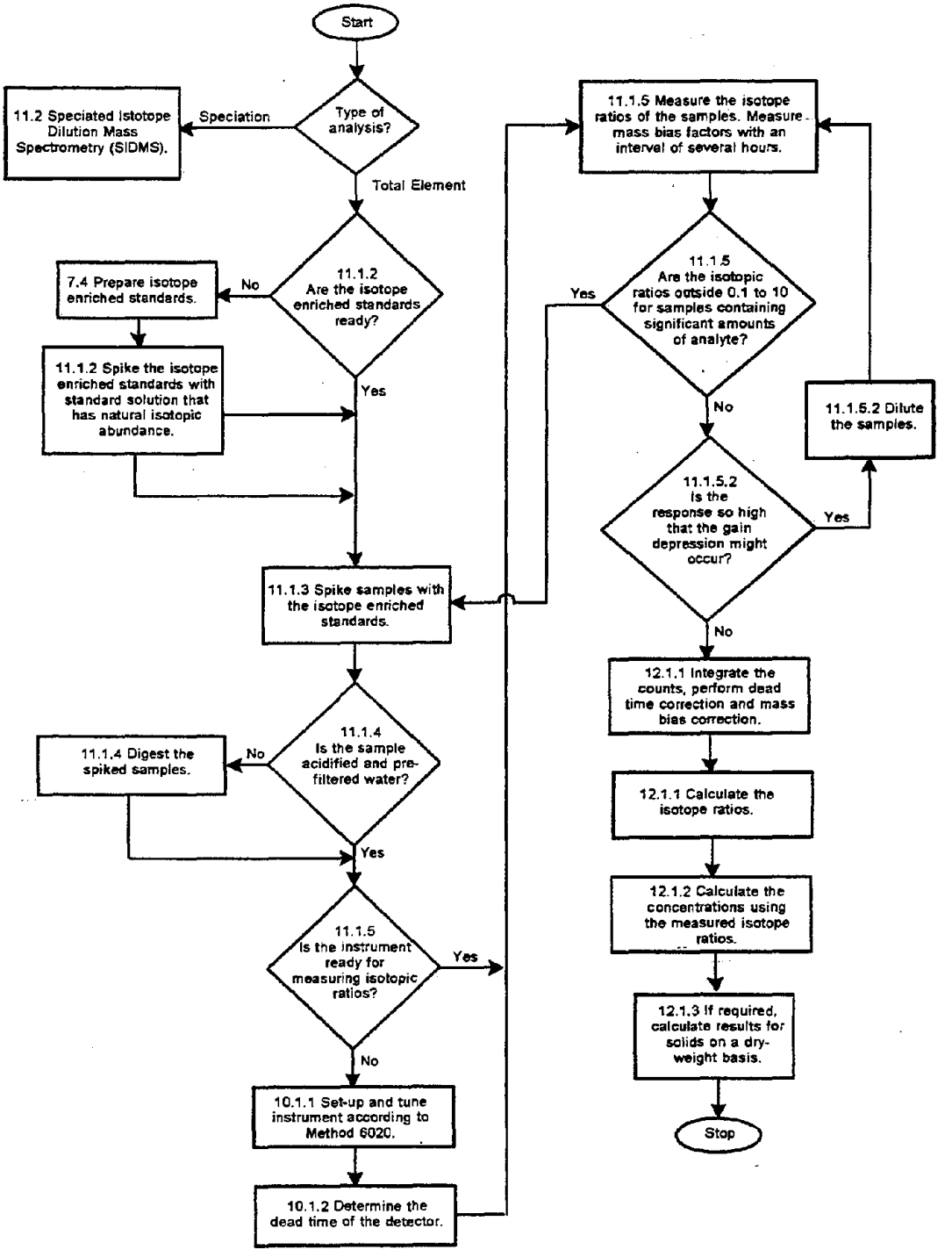
FIGURE 5

GRAPHIC CALCULATED ILLUSTRATION OF THE APPLICATION OF SIDMS TO THE SIMULTANEOUS DETERMINATION OF CR(III) AND CR(VI)

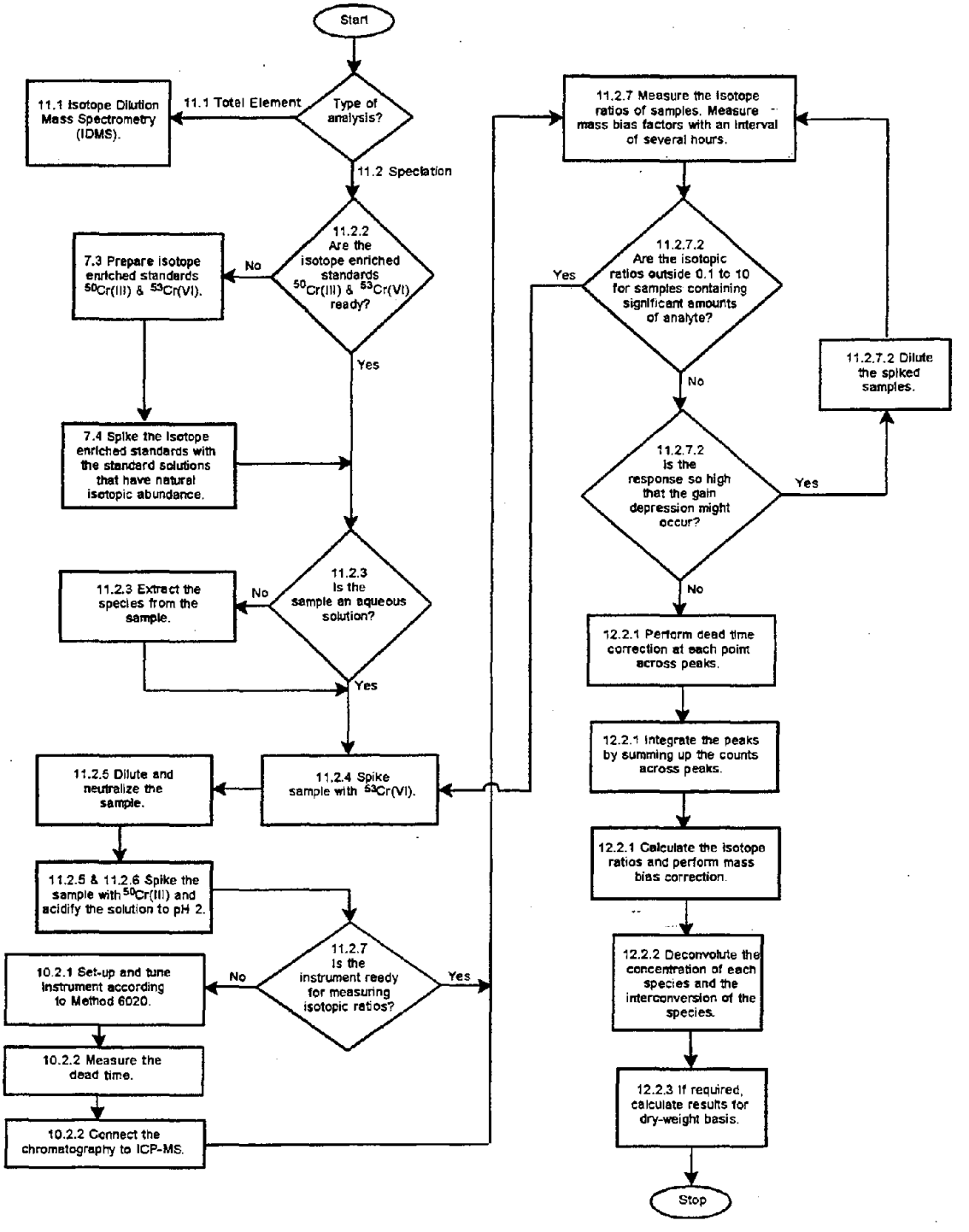


(a) and (b) show the initial natural isotopic abundance of species Cr(III) and Cr(VI) in a 50μl 200 ppb Cr solution in which the concentrations of both Cr(III) and Cr(VI) are 100 ppb. In (c) and (d), the sample is spiked with 100 ppb ^{50}Cr(III) (in which ^{50}Cr is enriched) and 100 ppb ^{53}Cr(VI) (in which ^{53}Cr is enriched), there is no interconversion between Cr(III) and Cr(VI). In (e) and (f), 20% of Cr(III) is converted to Cr(VI), and 20% of Cr(VI) is converted to Cr(III). Different degrees of interconversion results in different isotopic abundances, so the change of the relative isotopic abundance can be applied to the determination of the species and the degree of the interconversion.

ELEMENTAL AND SPECIATED ISOTOPE DILUTION MASS SPECTROMETRY: ISOTOPE DILUTION MASS SPECTROMETRY (IDMS)



ELEMENTAL AND SPECIATED ISOTOPE DILUTION MASS SPECTROMETRY: SPECIATED ISOTOPE DILUTION MASS SPECTROMETRY (SIDMS)



METHOD 7000B

FLAME ATOMIC ABSORPTION SPECTROPHOTOMETRY

1.0 SCOPE AND APPLICATION

1.1 Metals in solution may be readily determined by flame (direct aspiration) atomic absorption spectrophotometry. The method is simple, rapid, and applicable to a large number of environmental samples including, but not limited to, ground water, aqueous samples, extracts, industrial wastes, soils, sludges, sediments, and similar wastes. With the exception of the analyses for dissolved constituents, all samples require digestion prior to analysis (refer to Chapter Three). Analysis for dissolved elements does not require digestion if the sample has been filtered and then acidified.

Note: The analyst should be aware that organo-metallic species may not be detected if the sample is not digested.

This method is applicable to the following elements:

ELEMENT		CASRN[a]
Aluminum	(Al)	7429-90-5
Antimony	(Sb)	7440-36-0
Barium	(Ba)	7440-39-3
Beryllium	(Be)	7440-41-7
Cadmium	(Cd)	7440-43-9
Calcium	(Ca)	7440-70-2
Chromium	(Cr)	7440-47-3
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Iron	(Fe)	7439-89-6
Lead	(Pb)	7439-92-1
Lithium	(Li)	7439-93-2
Magnesium	(Mg)	7439-95-4
Manganese	(Mn)	7439-96-5
Molybdenum	(Mo)	7439-98-7
Nickel	(Ni)	7440-02-0
Osmium	(Os)	7440-04-2
Potassium	(K)	7440-09-7
Silver	(Ag)	7440-22-4
Sodium	(Na)	7440-23-5
Strontium	(Sr)	7440-24-6
Thallium	(Tl)	7440-28-0
Tin	(Sn)	7440-31-5
Vanadium	(V)	7440-62-2
Zinc	(Zn)	7440-66-6

[a] Chemical Abstract Service Registry Number

1.2 Method detection limits, sensitivity, and optimum ranges of the metals will vary with the matrices and models of atomic absorption spectrophotometers. The data shown in Table 1 provide some indication of the detection limits obtainable by the direct aspiration technique. For clean aqueous samples, the detection limits shown in the table by direct aspiration may be extended

downward with scale expansion and upward by using a less sensitive wavelength or by rotating the burner head. Detection limits by direct aspiration may also be extended through concentration of the sample and/or through solvent extraction techniques. Method detection limits (MDLs) must be established empirically for each matrix type analyzed (refer to Chapter One for guidance) and would be required for each preparatory/determinative method combination used. These MDLs must be documented and kept on file and should be updated when a change in operation or instrument conditions occurs. Refer to Chapter One for guidance.

1.3 Users of this method should state the data quality objectives prior to analysis and must document and have on file the required initial demonstration performance data described in the following sections prior to using the method for analysis.

1.4 Where direct-aspiration atomic absorption techniques do not provide adequate sensitivity, refer to specialized procedures such as graphite furnace atomic absorption (Method 7010) or the gaseous-hydride methods.

1.5 Other elements and matrices may be analyzed by this method as long as the method performance is demonstrated for these additional elements of interest, in the additional matrices of interest, at the concentration levels of interest in the same manner as the listed elements and matrices (see Sec. 9.0).

1.6 Use of this method is restricted to analysts who are knowledgeable in the chemical and physical interferences as described in this method.

2.0 SUMMARY OF METHOD

2.1 Although methods have been reported for the analysis of solids by atomic absorption spectrophotometry, the technique generally is limited to metals in solution or dissolved through some form of sample processing (refer to Chapter Three). Preliminary treatment of waste water, ground water, extracts, and industrial waste is always necessary because of the complexity and variability of sample matrix. Solids, slurries, and suspended material must be subjected to a solubilization process before analysis. This process may vary because of the metals to be determined and the nature of the sample being analyzed. Solubilization and digestion procedures are presented in Chapter Three.

2.2 In direct-aspiration atomic absorption spectrophotometry, a sample is aspirated and atomized in a flame. A light beam from a hollow cathode lamp or an electrodeless discharge lamp is directed through the flame into a monochromator, and onto a detector that measures the amount of absorbed light. Absorption depends upon the presence of free unexcited ground-state atoms in the flame. Because the wavelength of the light beam is characteristic of only the metal being determined, the light energy absorbed by the flame is a measure of the concentration of that metal in the sample. This principle is the basis of atomic absorption spectrophotometry.

3.0 DEFINITIONS

Refer to Chapter One and Chapter Three for a listing of applicable definitions.

4.0 INTERFERENCES

4.1 The most troublesome type of interference in atomic absorption spectrophotometry is usually termed "chemical" and is caused by lack of absorption of atoms bound in molecular combination in the flame. This phenomenon can occur when the flame is not sufficiently hot to

dissociate the molecule, as in the case of phosphate interference with magnesium, or when the dissociated atom is immediately oxidized to a compound that will not dissociate further at the temperature of the flame. The addition of lanthanum will overcome phosphate interference in magnesium, calcium, and barium determinations. Similarly, silica interference in the determination of manganese can be eliminated by the addition of calcium. A nitrous oxide/acetylene gas mixture may be used to help prevent interferences from refractory compounds.

4.2 Chemical interferences may also be eliminated by separating the metal from the interfering material. Although complexing agents are employed primarily to increase the sensitivity of the analysis, they may also be used to eliminate or reduce interferences.

4.3 The presence of high dissolved solids in the sample may result in an interference from non-atomic absorbance such as light scattering. In the absence of background correction, this can result in false positives and/or falsely elevated values. If background correction is not available, a non-absorbing wavelength should be checked. Signal contribution from uncorrected background can not be diagnosed through the analysis of spike recovery, nor is it compensated for by the application of the method of standard additions (MSA). If background correction is not available and the non-absorbing wavelength test indicates the presence of background interference, the sample digestates must be extracted (liquid-liquid or solid phase) prior to analysis, or another analytical method must be selected.

4.4 Ionization interferences occur when the flame temperature is sufficiently high to generate the removal of an electron from a neutral atom, giving a positively charged ion. This type of interference can generally be controlled by the addition, to both standard and sample solutions, of a large excess (1,000 mg/L) of an easily ionized element such as K, Na, Li or Cs. Each sample and standard should contain 2 mL KCl/100 mL of solution. Use 95 g of potassium chloride in 1 L of reagent water for the KCl solution.

4.5 Spectral interference can occur when an absorbing wavelength of an element present in the sample, but not being determined, falls within the width of the absorption line of the element of interest. The results of the determination will then be erroneously high, due to the contribution of the interfering element to the atomic absorption signal. Interference can also occur when resonant energy from another element in a multielement lamp, or from a metal impurity in the lamp cathode, falls within the bandpass of the slit setting when that other metal is present in the sample. This type of interference may sometimes be reduced by narrowing the slit width.

4.6 The analyst should be aware that viscosity differences and/or high dissolved or suspended solids may alter the aspiration rate.

4.7 All metals are not equally stable in the digestate, especially if it only contains nitric acid and not a combination of acids including hydrochloric acid. The addition of HCl helps stabilize Sn, Sb, Mo, Ba, and Ag in the digestate. The digestate should be analyzed as soon as possible, with preference given to these analytes. Refer to Chapter Three for the suggested decomposition methods.

4.8 Specific interference problems related to the individual analytes are located in this section.

4.8.1 Aluminum: Aluminum may be as much as 15% ionized in a nitrous-oxide/acetylene flame. Use of an ionization suppressor (1,000 ug/mL K as KCl) as described in Sec. 4.4 will eliminate this interference.

4.8.2 Antimony: In the presence of lead (1,000 mg/L), a spectral interference may occur at the 217.6-nm resonance line. In this case, the 231.1-nm resonance line should be used. Excess concentrations of copper and nickel (and potentially other elements), as well as acids, can interfere with antimony analyses. If the sample contains these matrix types, either matrices of the standards should be matched to those of the sample or the sample should be analyzed using a nitrous oxide/acetylene flame.

4.8.3 Barium: Barium undergoes significant ionization in the nitrous oxide/acetylene flame, resulting in a significant decrease in sensitivity. All samples and standards must contain 2 mL of the KCl ionization suppressant per 100 mL of solution (refer to Sec. 4.4). In addition, high hollow cathode current settings and a narrow spectral band pass must be used because both barium and calcium emit strongly at barium's analytical wavelength.

4.8.4 Beryllium: Concentrations of Al greater than 500 ppm may suppress beryllium absorbance. The addition of 0.1% fluoride has been found effective in eliminating this interference. High concentrations of magnesium and silicon cause similar problems and require the use of the method of standard additions.

4.8.5 Calcium: All elements forming stable oxyanions will complex calcium and interfere unless lanthanum is added. Addition of lanthanum to prepared samples rarely presents a problem because virtually all environmental samples contain sufficient calcium to require dilution to be within the linear range of the method.

4.8.6 Chromium: An ionization interference may occur if the samples have a significantly higher alkali metal content than the standards. If this interference is encountered, an ionization suppressant (KCl) should be added to both samples and standards (refer to Sec. 4.4).

4.8.7 Magnesium: All elements forming stable oxyanions (P, B, Si, Cr, S, V, Ti, Al, etc.) will complex magnesium and interfere unless lanthanum is added. Addition of lanthanum to prepared samples rarely presents a problem because virtually all environmental samples contain sufficient magnesium to require dilution.

4.8.8 Molybdenum: Interference in an air/acetylene flame from Ca, Sr, SO_4, and Fe are severe. These interferences are greatly reduced in the nitrous oxide flame and by the addition of 1,000 mg/L aluminum to samples and standards (refer to Sec. 7.7).

4.8.9 Nickel: High concentrations of iron, cobalt, or chromium may interfere, requiring either matrix matching or use of a nitrous-oxide/acetylene flame. A non-response line of Ni at 232.14 nm causes non-linear calibration curves at moderate to high nickel concentrations, requiring sample dilution or use of the 352.4 nm line.

4.8.10 Osmium: Due to the volatility of osmium, standards must be made on a daily basis, and the applicability of sample preparation techniques must be verified for the sample matrices of interest.

4.8.11 Potassium: In air/acetylene or other high temperature flames (>2800°C), potassium can experience partial ionization, which indirectly affects absorption sensitivity. The presence of other alkali salts in the sample can reduce ionization and thereby enhance analytical results. The ionization-suppressive effect of sodium is small if the ratio of Na to K is under 10. Any enhancement due to sodium can be stabilized by adding excess sodium

(1,000 ug/mL) to both sample and standard solutions. If more stringent control of ionization is required, the addition of cesium should be considered.

4.8.12 Silver: Since silver nitrate solutions are light sensitive and have the tendency to plate silver out on the container walls, they should be stored in dark-colored bottles. In addition, it is recommended that the stock standard concentrations be kept below 2 ppm and the chloride content increased to prevent precipitation. If precipitation is occurring, a 5%:2% $HCl:HNO_3$ stock solution may prevent precipitation. Daily standard preparation may also be needed to prevent precipitation of silver.

4.8.13 Strontium: Chemical interference caused by silicon, aluminum, and phosphate are controlled by adding lanthanum chloride. Potassium chloride is added to suppress the ionization of strontium. All samples and standards should contain 1 mL of lanthanum chloride/potassium chloride solution per 10 mL of solution (refer to Sec. 7.8).

4.8.14 Vanadium: High concentrations of aluminum or titanium, or the presence of Bi, Cr, Fe, acetic acid, phosphoric acid, surfactants, detergents, or alkali metals, may interfere. The interference can be controlled by adding 1,000 mg/L aluminum to samples and standards (refer to Sec. 7.7).

4.8.15 Zinc: High levels of silicon, copper, or phosphate may interfere. Addition of strontium (1,500 mg/L) removes the copper and phosphate interference.

5.0 SAFETY

5.1 Refer to the guidance in Chapter Three.

5.2 Concentrated nitric and hydrochloric acids are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection when working with these reagents.

5.3 Many metal salts, including those of osmium, are extremely toxic if inhaled or swallowed. Extreme care must be taken to ensure that samples and standards are handled properly and that all exhaust gases are properly vented. Wash hands thoroughly after handling.

5.4 Protective eyeware and/or flame shields should be used when conducting analyses by acetylene-nitrous oxide flame due to the emission of UV light.

6.0 EQUIPMENT AND SUPPLIES

6.1 Atomic absorption spectrophotometer - Single- or dual-channel, single- or double-beam instrument having a grating monochromator, photomultiplier detector, adjustable slits, a wavelength range of 190 to 800 nm, and provisions for a computer or graphical interface.

6.2 Burner - The burner recommended by the particular instrument manufacturer should be used. For certain elements the nitrous oxide burner is required. Under no circumstance should an acetylene-air burner head be used with an acetylene-nitrous oxide flame.

6.3 Hollow cathode lamps - Single-element lamps are preferred, but multielement lamps may be used. Electrodeless discharge lamps may also be used when available. Other types of lamps meeting the performance criteria of this method may be used.

6.4 Graphical display and recorder - A recorder is recommended for flame work so that there will be a permanent record and that any problems with the analysis such as drift, incomplete atomization, losses during charring, changes in sensitivity, peak signal, etc., can be easily recognized.

6.5 Pipets - Class A or microliter, with disposable tips. Sizes can range from 5 to 100 uL as required. Pipet tips should be checked as a possible source of contamination when contamination is suspected or when a new source or batch of pipet tips is received by the laboratory. . The accuracy of variable pipets must be verified daily. Class A pipets can be used for the measurement of volumes equal to or larger than 1 mL.

6.6 Pressure-reducing valves - The supplies of fuel and oxidant should be maintained at pressures somewhat higher than the controlled operating pressure of the instrument by suitable valves.

6.7 Glassware - All glassware, polypropylene, or fluorocarbon (PFA or TFM) containers, including sample bottles, flasks and pipets, should be washed in the following sequence: 1:1 hydrochloric acid, tap water, 1:1 nitric acid, tap water, detergent, tap water, and reagent water. (Chromic acid should not be used as a cleaning agent for glassware if chromium is to be included in the analytical scheme.) If it can be documented through an active analytical quality control program using spiked samples and method blanks that certain steps in the cleaning procedure are not required for routine samples, those steps may be eliminated from the procedure. Alternative cleaning procedures must also be documented.

6.8 Volumetric flasks of suitable precision and accuracy.

7.0 REAGENTS AND STANDARDS

7.1 Reagents: Analytical reagent grade or trace metals grade chemicals should be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. All reagents should be analyzed to demonstrate that the reagents do not contain target analytes at or above the MDL.

7.2 Reagent water: All references to water in this method refer to reagent water unless otherwise specified. Reagent grade water is defined in Chapter One.

7.3 Nitric acid, HNO_3: Use a spectrograde acid certified for AA use. Prepare a 1:1 dilution with water by adding the concentrated acid to an equal volume of water. If the method blank does not contain target analytes at or above the MDL, then the acid may be used.

7.4 Hydrochloric acid (1:1), HCl: Use a spectrograde acid certified for AA use. Prepare a 1:1 dilution with water by adding the concentrated acid to an equal volume of water. If the method blank does not contain target analytes at or above the MDL, then the acid may be used.

7.5 Fuel and oxidant: High purity acetylene is generally acceptable. Air may be supplied from a compressed air line, a laboratory compressor, or a cylinder of compressed air and should be clean and dry. Nitrous oxide is also required for certain determinations. A centrifuge filter on the compressed air lines is also recommended to remove particulates.

7.6 Stock standard metal solutions: Stock standard solutions are prepared from analytical reagent grade high purity metals, oxides, or nonhygroscopic salts using reagent water and redistilled nitric or hydrochloric acids. Sulfuric or phosphoric acids should be avoided as they produce an adverse effect on many elements. The stock solutions are prepared at concentrations of 1,000 mg of the metal per liter. Commercially available standard solutions may also be used. When using pure metals (especially wire) for standards preparation, cleaning procedures, as detailed in Chapter Three, should be used to ensure that the solutions are not compromised. Stability of standards will be verified through the use of the QC protocols as specified in this method. Comparison of the daily ICVs and CCVs with the calibration curve enables the standards to be prepared as needed.

7.6.1 Aluminum: Dissolve 1.000 g of aluminum metal in dilute HCl with gentle warming and dilute to 1 L with reagent water.

7.6.2 Antimony: Carefully weigh 2.743 g of antimony potassium tartrate, $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$, and dissolve in reagent water. Dilute to 1 L with reagent water.

7.6.3 Barium: Dissolve 1.779 g barium chloride, $BaCl_2 \cdot 2H_2O$, analytical grade and dilute to 1 L with reagent water.

7.6.4 Beryllium: Dissolve 11.659 g beryllium sulfate, $BeSO_4$, in reagent water containing 2 mL nitric acid (conc.) and dilute to 1 L with reagent water.

7.6.5 Cadmium: Dissolve 1.000 g cadmium metal in 20 mL of 1:1 HNO_3 and dilute to 1 L with reagent water.

7.6.6 Calcium: Suspend 2.500 g of calcium carbonate, $CaCO_3$, dried for 1 hour at 180°C in reagent water and dissolve by adding a minimum of dilute HCl. Dilute to 1 L with reagent water.

7.6.7 Chromium: Dissolve 1.923 g of chromium trioxide, CrO_3, in reagent water, acidify (to pH $\leq$ 2) with redistilled HNO_3 (conc.), and dilute to 1 L with reagent water.

7.6.8 Cobalt: Dissolve 1.000 g of cobalt metal in 20 mL of 1:1 HNO_3 and dilute to 1 L with reagent water. Chloride or nitrate salts of cobalt(II) may be used. Although numerous hydrated forms exist, they are not recommended unless the exact composition of the compound is known.

7.6.9 Copper: Dissolve 1.000 g of electrolytic copper in 5 mL of redistilled HNO_3 (conc.) and dilute to 1 L with reagent water.

7.6.10 Iron: Dissolve 1.000 g iron wire in 10 mL redistilled HNO_3 (conc.) and reagent water and dilute to 1 L with reagent water. Note that iron passivates in conc. HNO_3, and therefore some water should be present.

7.6.11 Lead: Dissolve 1.599 g of lead nitrate, $Pb(NO_3)_2$, in reagent water, acidify with 10 mL redistilled HNO_3 (conc.), and dilute to 1 L with reagent water.

7.6.12 Lithium: Dissolve 5.324 g lithium carbonate, Li_2CO_3, in a minimum volume of 1:1 HCl and dilute to 1 L with reagent water.

7.6.13 Magnesium: Dissolve 1.000 g of magnesium metal in 20 mL 1:1 HNO_3 and dilute to 1 L with reagent water.

7.6.14 Manganese: Dissolve 1.000 g manganese metal in 10 mL redistilled HNO_3 (conc.) and dilute to 1 L with reagent water.

7.6.15 Molybdenum: Dissolve 1.840 g of ammonium molybdate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, and dilute to 1 L with reagent water.

7.6.16 Nickel: Dissolve 1.000 g nickel metal or 4.953 g nickel nitrate, $Ni(NO_3)_2\cdot 6H_2O$, in 10 mL HNO_3 (conc.) and dilute to 1 L with reagent water.

7.6.17 Osmium: Procure a certified aqueous standard from a supplier and verify by comparison with a second standard. If necessary, standards can be made from osmium compounds. However, due to the toxicity of these compounds, this approach is not advised.

7.6.18 Potassium: Dissolve 1.907 g of potassium chloride, KCl , dried at 110°C, in reagent water and dilute to 1 L with reagent water.

7.6.19 Silver: Dissolve 1.575 g of anhydrous silver nitrate, $AgNO_3$, in reagent water. Add 10 mL of HNO_3 (conc.) and dilute to 1 L with reagent water. Store in a dark-colored glass bottle in a refrigerator.

7.6.20 Sodium: Dissolve 2.542 g sodium chloride, NaCl, in reagent water, acidify with 10 mL redistilled HNO_3 (conc.), and dilute to 1 L with reagent water.

7.6.21 Strontium: Dissolve 2.415 g of strontium nitrate, $Sr(NO_3)_2$, in 10 mL of conc. HCl and 700 mL of reagent water. Dilute to 1 L with reagent water.

7.6.22 Thallium: Dissolve 1.303 g thallium nitrate, $TlNO_3$, in reagent water, acidify (to pH $\leq$ 2) with 10 mL conc. HNO_3, and dilute to 1 L with reagent water.

7.6.23 Tin: Dissolve 1.000 g of tin metal in 100 mL conc. HCl and dilute to 1 L with reagent water.

7.6.24 Vanadium: Dissolve 1.785 g of vanadium pentoxide, V_2O_5 , in 10 mL of conc. HNO_3 and dilute to 1 L with reagent water.

7.6.25 Zinc: Dissolve 1.000 g zinc metal in 10 mL of conc. HNO_3 and dilute to 1 L with reagent water.

7.7 Aluminum nitrate solution: Dissolve 139 g aluminum nitrate, $Al(NO_3)_3\cdot 9H_2O$, in 150 mL reagent water and heat to effect solution. Allow to cool and make to 200 mL. Add 2 mL of this solution to a 100 mL volume of standards and samples.

7.8 Lanthanum chloride/potassium chloride solution: Dissolve 11.73 g of lanthanum oxide, La_2O_3, in a minimum amount (approximately 50 mL) of conc. HCl. Add 1.91 g of potassium chloride, KCl. Allow solution to cool to room temperature and dilute to 100 mL with reagent water. CAUTION - REACTION IS VIOLENT! Add acid slowly and in small portions to control the reaction rate upon mixing.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 See the introductory material in Chapter Three, Inorganic Analytes.

9.0 QUALITY CONTROL

9.1 All quality control data should be maintained and available for easy reference or inspection.

9.2 For each batch of samples processed, at least one method blank must be carried throughout the entire sample preparation and analytical process as described in Chapter One. A method blank is prepared by using a volume or weight of reagent water at the volume or weight specified in the preparation method and then carried through the appropriate steps of the analytical process. These steps may include but are not limited to digestion, dilution, filtering, and analysis. If the method blank does not contain target analytes at a level that interferes with the project-specific DQOs then the method blank would be considered acceptable. In the absence of project-specific DQOs, if the blank is less than the MDL or less than 10% of the lowest sample concentration for each analyte, whichever is greater, then the method blank would be considered acceptable. If the method blank cannot be considered acceptable, the method blank should be re-run once and if still unacceptable then all samples after the last acceptable method blank must be reprepped and reanalyzed along with the other appropriate batch QC samples. These blanks will be useful in determining if samples are being contaminated. Refer to Chapter One for the proper protocol when analyzing blanks.

9.3 For each batch of samples processed, at least one laboratory control samples must be carried throughout the entire sample preparation and analytical process as described in Chapter One. The laboratory control samples should be spiked with each analyte of interest at the project-specific action level or when lacking project-specific action levels, between the low and midlevel standards. Acceptance criteria should be set at a laboratory derived limit developed through the use of historical analyses. In the absence of historical data this limit should be set at ± 20% of the spiked value. After the determination of historical data, ±20% must still be the limit of maximum deviation to express acceptability. If the laboratory control sample cannot be considered acceptable, the laboratory control sample should be re-run once and if still unacceptable then all samples after the last acceptable laboratory control sample must be reprepped and reanalyzed. Refer to Chapter One for more information.

9.4 Matrix Spike/Matrix Spike Duplicates (MS/MSDs): At the laboratory's discretion, a separate spike sample and a separate duplicate sample may be analyzed in lieu of the MS/MSD. For each batch of samples processed, at least one MS/MSD sample must be carried throughout the entire sample preparation and analytical process as described in Chapter One. MS/MSDs are intralaboratory split samples spiked with identical concentrations of each analyte of interest. The spiking occurs prior to sample preparation and analysis. An MS/MSD is used to document the bias and precision of a method in a given sample matrix. . Refer to the definitions of bias and precision, in Chapter One, for the proper data reduction protocols. MS/MSD samples should be spiked at the same level as the corresponding laboratory control sample that is at the project-specific action level or, when lacking project-specific action levels, between the low and midlevel standards. Acceptance criteria should be set at a laboratory derived limit developed through the use of historical analyses. In the absence of historical data this limit should be set at ± 20% of the spiked value for precision and ≤ 20 relative percent difference (RPD). After the determination of historical data, 20% must still be the limit of maximum deviation for both percent recovery and relative percent difference to express acceptability. Refer to of Chapter One for guidance. If the bias and precision indicators are outside the laboratory control limits or if the percent recovery is less than 80% or greater than 120% or if the relative percent difference is greater than 20%, the interference test as discussed in Sec. 9.5.2 and 9.7 should be conducted.

9.5 Interference tests

9.5.1 Recovery test (post-digestion spike) - The recovery test must be done on all samples within a batch that fails that batch's MS/MSD. To conduct this test, withdraw an aliquot of the test sample and add a known amount of analyte to bring the concentration of the analyte to 2 to 5 times the original concentration. If spiking at 2-5 times would exceed the linear range of the instrument, a lesser spike may be used. If all of the samples in the batch have analyte concentrations below the detection limit, spike the selected sample at the project-specific action level or when lacking project-specific action levels, between the low and midlevel standards. Analyze the spiked sample and calculate the spike recovery. If the recovery is less than 85% or greater than 115%, the method of standard additions should be used for all samples in the batch.

9.5.2 Dilution test - The dilution test is to be conducted when interferences are suspected and the sample concentration is high enough to allow for proper interpretation of the results. To conduct this test, determine the apparent concentration in the undiluted sample. Dilute the sample by a minimum of five fold (1+4) and reanalyze. Agreement within a 10% difference (RPD) between the concentration for the undiluted sample and five times the concentration for the diluted sample indicates the absence of interferences, and such samples may be analyzed without using the method of standard additions. If agreement between the dilutions is greater than 10%, the MSA should be used for all samples in the batch.

9.6 Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition (MSA) is recommended (see Section 9.7 below). Other options including, the use of different matrix modifiers, different furnace conditions, different preparatory methods or different analytical methods may also be attempted to properly characterize a sample. Section 9.5 provides tests to determine the potential of an interference and evaluates the need for using the MSA.

9.7 Method of standard additions - The standard addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique attempts to compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The method of standard additions may be appropriate for analysis of extracts, on analyses submitted as part of a delisting petition, whenever a new sample matrix is being analyzed and on every batch that fails the recovery test.

9.7.1 The simplest version of this technique is the single-addition method, in which two identical aliquots of the sample solution, each of volume V_x, are taken. To the first (labeled A) is added a known volume V_S of a standard analyte solution of concentration C_S. To the second aliquot (labeled B) is added the same volume V_S of reagent water. The analytical signals of A and B are measured and corrected for non-analyte signals. The unknown sample concentration C_x is calculated:

$$C_x = \frac{S_B V_S C_S}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average,

avoiding excess dilution of the sample. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure.

9.7.2 Improved results can be obtained by employing a series of standard additions. To equal volumes of the sample are added a series of standard solutions containing different known quantities of the analyte, and all solutions are diluted to the same final volume. For example, addition 1 should be prepared so that the resulting concentration is approximately 50 percent of the expected absorbance from the indigenous analyte in the sample. Additions 2 and 3 should be prepared so that the concentrations are approximately 100 and 150 percent of the expected endogenous sample absorbance. The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated to zero absorbance, the point of interception of the abscissa is the endogenous concentration of the analyte in the sample. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate. An example of a plot so obtained is shown in Figure 1. A linear regression program may be used to obtain the intercept concentration.

9.7.3 For the results of this MSA technique to be valid, the following limitations must be taken into consideration:

1. The apparent concentrations from the calibration curve must be linear (0.995 or greater) over the concentration range of concern. For the best results, the slope of the MSA plot should be nearly the same as the slope of the standard curve.

2. The effect of the interference should not vary as the ratio of analyte concentration to sample matrix changes, and the standard addition should respond in a similar manner as the analyte.

3. The determination must be free of spectral interference and corrected for nonspecific background interference.

9.8 All quality control measures described in Chapter One should be followed.

9.9 Independent source laboratory control samples or Standard Reference Materials (SRMs) should be used to help assess the quality of the analysis scheme. Follow the directions provided by the SRM's manufacturer for use and acceptance criteria.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Calibration standards - For those instruments which do not read out directly in concentration, a calibration curve is prepared to cover the appropriate concentration range. Usually, this means the preparation of a blank and standards which produce an absorbance of 0.0 to 0.7. Calibration standards can prepared by diluting the stock metal solutions in the same acids and acid concentrtions as the samples.

10.1.1 Calibration standards can be prepared fresh each time a batch of samples is analyzed. If the ICV solution is prepared daily and the ICV is analyzed within the acceptance criteria, calibration standards do not need to be prepared daily and may be prepared and stored for as long as the calibration standard viability can be verified through the use of the ICV. If the ICV is outside of the acceptance criteria, the calibration standards must be prepared

fresh and the instrument recalibrated. Prepare a blank and at least three calibration standards in graduated amounts in the appropriate range of the linear part of the curve.

10.1.2 The calibration standards should be prepared using the same type of acid or combination of acids and at the same concentration as will result in the samples following processing.

10.1.3 Beginning with the blank and working toward the highest standard, aspirate the solutions and record the readings. Repeat the operation with both the calibration standards and the samples a sufficient number of times to secure an average reading for each solution. Calibration curves are always required.

10.2 A calibration curve must be prepared each day with a minimum of a calibration blank and three standards. The curve must be linear and have a correlation coefficient of at least 0.995.

10.2.1 After initial calibration, the calibration curve must be verified by use of an initial calibration blank (ICB) and an initial calibration verification (ICV) standard. The ICV standard must be made from an independent (second source) material at or near mid-range. The acceptance criteria for the ICV standard must be ±10% of its true value and the ICB must not contain target analytes at or above the MDL for the curve to be considered valid. If the calibration curve cannot be verified within the specified limits, the cause must be determined and the instrument recalibrated before samples are analyzed. The analysis data for the ICV must be kept on file with the sample analysis data.

10.2.2 The calibration curve must also be verified at the end of each analysis batch and/or after every 10 samples by use of a continuing calibration blank (CCB) and a continuing calibration verification (CCV) standard. The CCV standard should be made from the same material as the initial calibration standards at or near midrange. The acceptance criteria for the CCV standard must be ±10% of its true value and the CCB must not contain target analytes at or above the MDL for the curve to be considered valid. If the calibration cannot be verified within the specified limits, the sample analysis must be discontinued, the cause determined and the instrument recalibrated. All samples following the last acceptable CCV/CCB must be reanalyzed. The analysis data for the CCV/CCB must be kept on file with the sample analysis data.

10.3 It is recommended that each standard should be analyzed (injected) twice and an average value determined. Replicate standard values should be within ±10% RPD.

10.4 If conducting trace analysis, it is recommended that the lowest calibration standard be set at the laboratory's quantitation level. The laboratory can use a reporting limit that is below the quantitation level but all values reported below the low standard should be reported as estimated values.

11.0 PROCEDURE

11.1 Preliminary treatment of aqueous and solid wastes is always necessary because of the complexity and variability of sample matrices. Solids, slurries, and suspended material must be subjected to a solubilization process before analysis. This process may vary because of the metals to be determined and the nature of the sample being analyzed. Solubilization and digestion procedures are presented in Chapter Three. Samples which are to be analyzed for dissolved constituents need not be digested if they have been filtered and then acidified. See first note of Section 1.0.

11.2 All atomic absorption analyses must be performed using a suitable form of background correction. Refer to Chapter Two for a detailed discussion on background correction.

11.3 Differences between the various makes and models of satisfactory atomic absorption spectrophotometers prevent the formulation of detailed instructions applicable to every instrument. The analyst should follow the manufacturer's operating instructions for a particular instrument.

11.3.1 In general, after choosing the proper lamp for the analysis, allow the lamp to warm up for a minimum of 15 minutes.

11.3.2 During this period, align the instrument, position the monochromator at the correct wavelength, select the proper monochromator slit width, and adjust the current according to the manufacturer's recommendation.

11.3.3 Light the flame and regulate the flow of fuel and oxidant. Adjust the burner and nebulizer flow rate for maximum percent absorption and stability. Balance the photometer.

11.3.4 Run a series of standards of the element under analysis. Construct a calibration curve by plotting the concentrations of the standards against absorbances. Set the curve corrector of a direct reading instrument to read out the proper concentration.

11.3.5 Aspirate the samples and determine the concentrations either directly or from the calibration curve. Standards must be run each time a sample or series of samples is run.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 For determination of metal concentration, read the concentration from the calibration curve or directly from the read-out system of the instrument.

12.1.1 If dilution of the sample was required:

$$\mu g/L \text{ metal in sample} = \frac{A\ (C+B)}{C}$$

where:

A = µg/L of metal in diluted aliquot from calibration curve.
B = Starting sample volume, mL.
C = Final volume of sample, mL.

12.1.2 For solid samples, report all concentrations in consistent units based on weight. Ensure that if the dry weight was used for the analysis, percent solids should be reported to the client.

$$\text{mg metal/kg sample} = \frac{A \times V}{W}$$

where:

A = mg/L of metal in processed sample from calibration curve.
V = Final volume of the processed sample, L.
W = Weight of sample, Kg.

12.1.3 Different integration times must not be used for samples and standards. Instead, the sample should be diluted and the same integration time should be used for both samples and standards. If dilution of the sample was required:

$$\mu\text{/L of metal sample} = \frac{Z\,(C + B)}{C}$$

where:

Z = µg/L of metal read from calibration curve or read-out system.
B = Starting sample volume, mL.
C = Final volume of sample, mL.

13.0 METHOD PERFORMANCE

13.1 Refer to the individual applicable methods from reference 1.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/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.

14.2 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, Department of Government Relations and Science Policy, 1155 16th Street, NW, Washington, DC 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits 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 Section 14.2.

16.0 REFERENCES

1. Methods for Chemical Analysis of Water and Wastes; U.S. Environmental Protection Agency. Office of Research and Development. Environmental Monitoring and Support Laboratory. ORD Publication Offices of Center for Environmental Research Information: Cincinnati, OH, 1983; EPA-600/4-79-020.

2. Reagent Chemicals, American Chemical Society Specifications, Rohrbough, W.G.; et al. 7th ed.; American Chemical Society: Washington, DC, 1986.

3. 1985 Annual Book of ASTM Standards, Vol. 11.01; "Standard Specification for Reagent Water"; ASTM: Philadelphia, PA, 1985; D1193-77.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain Tables 1 and 2, Figure 1 and a flow diagram of the method procedures.

TABLE 1

ATOMIC ABSORPTION DETECTION LIMITS AND SENSITIVITY FOR ANALYTES IN REAGENT WATER

	Direct Aspiration	
Metal	Detection Limit (mg/L)	Sensitivity (mg/L)
Aluminum	0.1	1
Antimony	0.2	0.5
Barium	0.1	0.4
Beryllium	0.005	0.025
Cadmium	0.005	0.025
Calcium	0.01	0.08
Chromium	0.05	0.25
Cobalt	0.05	0.2
Copper	0.02	0.1
Iron	0.03	0.12
Lead	0.1	0.5
Lithium	0.002	0.04
Magnesium	0.001	0.007
Manganese	0.01	0.05
Molybdenum	0.1	0.4
Nickel	0.04	0.15
Osmium	0.03	1
Potassium	0.01	0.04
Silver	0.01	0.06
Sodium	0.002	0.015
Strontium	0.03	0.15
Thallium	0.1	0.5
Tin	0.8	4
Vanadium	0.2	0.8
Zinc	0.005	0.02

TABLE 2

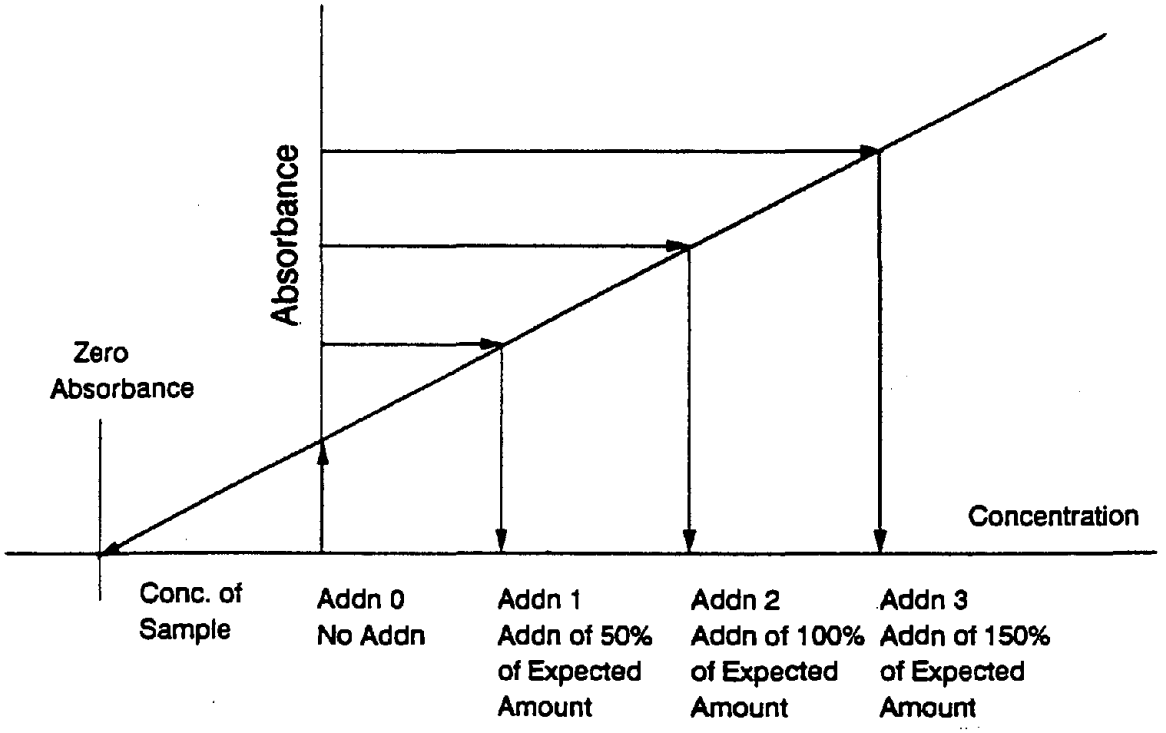
INSTRUMENT PARAMETERS (Ref. 1)

ELEMENT	WAVELENGTH (nm)	FUEL	OXIDANT	TYPE OF FLAME
Al	324.7	acetylene	nitrous oxide	fuel rich
Sb	217.6, 231.1	acetylene	air	fuel lean
Ba	553.6	acetylene	nitrous oxide	fuel rich
Be	234.9	acetylene	nitrous oxide	fuel rich
Cd	228.8	acetylene	air	fuel lean
Ca	422.7	acetylene	nitrous oxide	stoichiometric
Cr	357.9	acetylene	nitrous oxide	fuel rich
Co	240.7	acetylene	air	fuel lean
Cu	324.7	acetylene	air	fuel lean
Fe	248.3, 248.8, 271.8, 302.1, 252.7	acetylene	air	fuel lean
Pb	283.3, 217.0	acetylene	air	fuel lean
Li	670.8	acetylene	air	fuel lean
Mg	285.2	acetylene	air	fuel lean
Mn	279.5, 403.1	acetylene	air	fuel lean to stoichiometric
Mo	313.3	acetylene	nitrous oxide	fuel rich
Ni	232.0, 352.4	acetylene	air	fuel lean
Os	290.0	acetylene	nitrous oxide	fuel rich
K	766.5	acetylene	air	fuel lean
Ag	328.1	acetylene	air	fuel lean
Na	589.6	acetylene	air	fuel lean
Sr	460.7	acetylene	air	fuel lean
Tl	276.8	acetylene	air	fuel lean
Sn	286.3	acetylene	nitrous oxide	fuel rich
V	318.4	acetylene	nitrous oxide	fuel rich
Zn	213.9	acetylene	air	fuel lean

Note: If more than one wavelength is listed, the primary line is underlined.

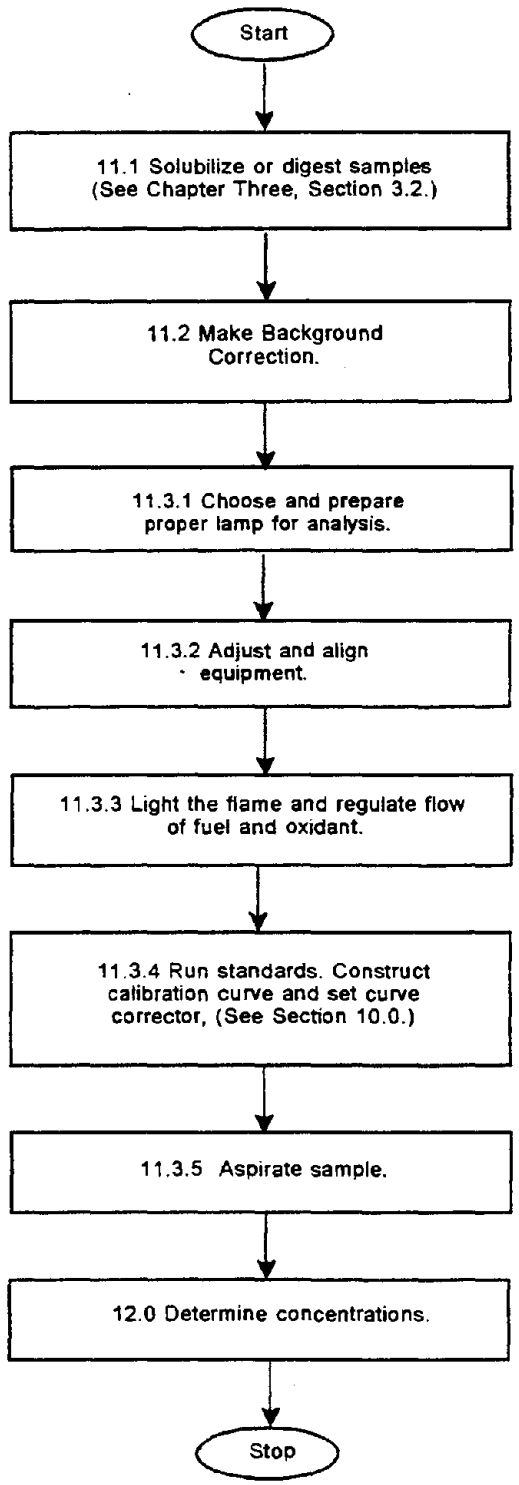
FIGURE 1

STANDARD ADDITION PLOT



METHOD 7000B

FLAME ATOMIC ABSORPTION SPECTROPHOTOMETRY



METHOD 7010

GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROPHOTOMETRY

1.0 SCOPE AND APPLICATION

1.1 Metals in solution may be readily determined by graphite furnace atomic absorption spectrophotometry (GFAA). The method is simple, quick, and applicable to a large number of metals in environmental samples including, but not limited to, ground water, domestic and industrial wastes, extracts, soils, sludges, sediments, and similar wastes. With the exception of the analyses for dissolved constituents, all samples require digestion prior to analysis. Analysis for dissolved elements does not require digestion if the sample has been filtered and then acidified.

NOTE: The analyst should be aware that organo-metallic species may not be detected if the sample is not digested.

This method is applicable to the following elements:

Element		CASRN[a]
Antimony	(Sb)	7440-36-0
Arsenic	(As)	7440-38-2
Barium	(Ba)	7440-39-3
Beryllium	(Be)	7440-41-7
Cadmium	(Cd)	7440-43-9
Chromium	(Cr)	7440-47-3
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Iron	(Fe)	7439-89-6
Lead	(Pb)	7439-92-1
Manganese	(Mn)	7439-96-5
Molybdenum	(Mo)	7439-98-7
Nickel	(Ni)	7440-02-0
Selenium	(Se)	7782-49-2
Silver	(Ag)	7440-22-4
Thallium	(Tl)	7440-28-0
Vanadium	(V)	7440-62-2
Zinc	(Zn)	7440-66-6

[a]Chemical Abstract Service Registry Number

1.2 Method detection limits, sensitivity, and optimum ranges of the metals will vary with the matrices and models of atomic absorption spectrophotometers. The data shown in Table 1 provide some indication of the detection limits obtainable by the furnace technique. The detection limits given in Table 1 are somewhat dependent on equipment (such as the type of spectrophotometer and furnace accessory, the energy source, the degree of electrical expansion of the output signal), and are greatly dependent on sample matrix. Method detection limits (MDLs) must be established, empirically, for each matrix type analyzed (refer to Chapter One for guidance) and are required for each preparatory/determinative method combination used.

1.3 Users of this method should state the data quality objectives prior to analysis and must document and have on file the required initial demonstration performance data described in the

following sections prior to using the method for analysis. When using furnace techniques, the analyst should be cautioned as to possible chemical reactions occurring at elevated temperatures which may result in either suppression or enhancement of the analysis element (see Sec. 4.0). To ensure valid data with furnace techniques, the analyst must examine each sample for interference effects (see Sec. 9.0) and, if detected, treat them accordingly, using either successive dilution, matrix modification, or the method of standard additions (see Sec. 9.7).

1.4 Other elements and matrices may be analyzed by this method as long as the method performance is demonstrated for these additional elements of interest, in the additional matrices of interest, at the concentration levels of interest in the same manner as the listed elements and matrices (see Sec. 9.0).

1.5 Use of this method is restricted to analysts who are knowledgeable in the chemical and physical interferences as described in this method.

2.0 SUMMARY OF THE METHOD

2.1 Although methods have been reported for the analysis of solids by atomic absorption spectrophotometry, the technique generally is limited to metals in solution or solubilized through some form of sample processing. Refer to Chapter Three for a description of appropriate digestion methods.

2.2 Preliminary treatment of wastes, both solid and aqueous, is always necessary because of the complexity and variability of sample matrix. Solids, slurries, and suspended material must be subjected to a solubilization process before analysis. This process may vary because of the metals to be determined and the nature of the sample being analyzed. Solubilization and digestion procedures are presented in Chapter Three.

2.3 When using the furnace technique in conjunction with an atomic absorption spectrophotometer, a representative aliquot of a sample is placed in the graphite tube in the furnace, evaporated to dryness, charred, and atomized. As a greater percentage of available analyte atoms is vaporized and dissociated for absorption in the tube rather than the flame, the use of smaller sample volumes or detection of lower concentrations of elements is possible. The principle is essentially the same as with direct aspiration atomic absorption, except that a furnace, rather than a flame, is used to atomize the sample. Radiation from a given excited element is passed through the vapor containing ground-state atoms of that element. The intensity of the transmitted radiation decreases in proportion to the amount of the ground-state element in the vapor. The metal atoms to be measured are placed in the beam of radiation by increasing the temperature of the furnace, thereby causing the injected specimen to be volatilized. A monochromator isolates the characteristic radiation from the hollow cathode lamp or electrodeless discharge lamp, and a photosensitive device measures the attenuated transmitted radiation.

3.0 DEFINITIONS

Refer to Chapter One and Chapter Three for a listing of applicable definitions.

4.0 INTERFERENCES

4.1 Although the problem of oxide formation is greatly reduced with furnace procedures (because atomization occurs in an inert atmosphere), the technique is still subject to chemical interferences. The composition of the sample matrix can have a major effect on the analysis. It is

those effects which must be determined and taken into consideration in the analysis of each different matrix encountered. See Sec. 9.6 for additional guidance.

4.2 Background correction is important when using flameless atomization, especially below 350 nm. Certain samples, when atomized, may absorb or scatter light from the lamp. This can be caused by the presence of gaseous molecular species, salt particles, or smoke in the sample beam. If no correction is made, sample absorbance will be greater than it should be, and the analytical result will be erroneously high. Zeeman background correction is effective in overcoming composition or structured background interferences. It is particularly useful when analyzing for As in the presence of Al and when analyzing for Se in the presence of Fe.

4.3 Memory effects occur when the analyte is not totally volatilized during atomization. This condition depends on several factors: volatility of the element and its chemical form, whether pyrolytic graphite is used, the rate of atomization, and furnace design. This situation is detected through blank burns. The tube should be cleaned by operating the furnace at full power for the required time period, as needed, at regular intervals during the series of determinations.

4.4 Gases generated in the furnace during atomization may have molecular absorption bands encompassing the analytical wavelength. When this occurs, use either background correction or choose an alternate wavelength. Background correction may also compensate for nonspecific broad-band absorption interference and light scattering.

4.5 Continuum background correction cannot correct for all types of background interference. When the background interference cannot be compensated for, chemically remove the analyte or use an alternate form of background correction, refer to Chapter Two. A single background correction device to be used with this method is not specified; however, it must provide an analytical condition that is not subject to the occurring interelement spectral interferences of palladium on copper, iron on selenium and aluminum on arsenic.

4.6 Interference from a smoke-producing sample matrix can sometimes be reduced by extending the charring time at a higher temperature or utilizing an ashing cycle in the presence of air. Care must be taken, however, to prevent loss of the analyte.

4.7 Samples containing large amounts of organic materials should be oxidized by conventional acid digestion before being placed in the furnace. In this way, broad-band absorption will be minimized.

4.8 Anion interference studies in the graphite furnace indicate that, under conditions other than isothermal, the nitrate anion is preferred. Therefore, nitric acid is preferable for any digestion or solubilization step. When another acid in addition to nitric acid is required, a minimum amount should be used. This applies particularly to hydrochloric and, to a lesser extent, to sulfuric and phosphoric acids.

4.9 Carbide formation resulting from the chemical environment of the furnace has been observed. Molybdenum may be cited as an example. When carbides form, the metal is released very slowly from the resulting metal carbide as atomization continues. Molybdenum may require 30 seconds or more atomization time before the signal returns to baseline levels. Carbide formation is greatly reduced and the sensitivity increased with the use of pyrolytically coated graphite. Elements that readily form carbides are noted with the symbol (p) in Table 1.

4.10 Spectral interference can occur when an absorbing wavelength of an element present in the sample, but not being determined, falls within the width of the absorption line of the element

of interest. The results of the determination will then be erroneously high, due to the contribution of the interfering element to the atomic absorption signal. Interference can also occur when resonant energy from another element in a multielement lamp, or from a metal impurity in the lamp cathode, falls within the bandpass of the slit setting when that other metal is present in the sample. This type of interference may sometimes be reduced by narrowing the slit width.

4.11 It is recommended that all graphite furnace analyses be carried out using an appropriate matrix modifier. The choice of matrix modifier is dependent on analytes, conditions, and instrumentation and should be chosen by the analyst as the situation dictates. Follow the instrument manufacturers instructions for the preferred matrix modifier. If necessary, refer to Chapter Two for additional guidance.

4.12 It is recommended that a stabilized temperature platform be used to maximize an isothermal environment within the furnace cell to help reduce interferences. Refer to Chapter Two for additional guidance.

4.13 Cross-contamination and contamination of the sample can be major sources of error because of the extreme sensitivities achieved with the furnace. The sample preparation work area should be kept scrupulously clean. All glassware should be cleaned as directed in Sec. 6.6. Pipet tips are a frequent source of contamination. The analyst should be aware of the potential for the yellow tips to contain cadmium. If suspected, they should be acid soaked with 1:5 nitric acid and rinsed thoroughly with tap and reagent water. The use of a better grade of pipet tip can greatly reduce this problem. Special attention should be given to assessing the contamination in method blanks during the analysis. Pyrolytic graphite, because of the production process and handling, can become contaminated. As many as five to ten high-temperature burns may be required to clean the tube before use. In addition, auto sampler tips may also be a potential source of contamination. Flushing the tip with a dilute solution of nitric acid between samples can help prevent cross-contamination.

4.14 Specific interference problems related to individual analytes are located in this section.

4.14.1 Antimony: High lead concentration may cause a measurable spectral interference on the 217.6 nm line. Choosing the secondary wavelength or using background correction may correct the problem.

4.14.2 Arsenic:

4.14.2.1 Elemental arsenic and many of its compounds are volatile; therefore, samples may be subject to losses of arsenic during sample preparation Likewise, caution must be employed during the selection of temperature and times for the dry and char (ash) cycles. A matrix modifier such as nickel nitrate or palladium nitrate should be added to all digestates prior to analysis to minimize volatilization losses during drying and ashing.

4.14.2.2 In addition to the normal interferences experienced during graphite furnace analysis, arsenic analysis can suffer from severe nonspecific absorption and light scattering caused by matrix components during atomization. Arsenic analysis is particularly susceptible to these problems because of its low analytical wavelength (193.7 nm). Simultaneous background correction must be employed to avoid erroneously high results. Aluminum is a severe positive interferant in the analysis of arsenic, especially using D_2 arc background correction.

Although Zeeman background correction is very useful in this situation, use of any appropriate background correction technique is acceptable.

4.14.3 Barium: Barium can form barium carbide in the furnace, resulting in less sensitivity and potential memory effects. Because of chemical interaction, nitrogen should not be used as a purge gas and halide acids should not be used.

4.14.4 Beryllium: Concentrations of aluminum greater than 500 ppm may suppress beryllium absorbance. The addition of 0.1% fluoride has been found effective in eliminating this interference. High concentrations of magnesium and silicon cause similar problems and require the use of the method of standard additions.

4.14.5 Cadmium: Cadmium analyses can suffer from severe non-specific absorption and light scattering caused by matrix components during atomization. Simultaneous background correction is required to avoid erroneously high results. Excess chloride may cause premature volatilization of cadmium; an ammonium phosphate matrix modifier may minimize this loss.

4.14.6 Chromium: Low concentrations of calcium and/or phosphate may cause interferences; at concentrations above 200 mg/L, calcium's effect is constant and eliminates the effect of phosphate. Therefore, add calcium nitrate (calcium nitrate solution: dissolve 11.8 g of calcium nitrate in 1 L reagent water) to ensure a constant effect. Nitrogen should not be used as the purge gas because of a possible CN band interference.

4.14.7 Cobalt: Since excess chloride may interfere, it is necessary to verify by standard additions that the interference is absent unless it can be shown that standard additions are not necessary.

4.14.8 Lead: If poor recoveries are obtained, a matrix modifier may be necessary. Add 10 uL of phosphoric acid to 1 mL of prepared sample.

4.14.9 Molybdenum: Molybdenum is prone to carbide formation; use a pyrolytically coated graphite tube.

4.14.10 Nickel: Severe memory effects for nickel may occur in graphite furnace tubes used for other GFAA analyses, due to the use of a nickel nitrate matrix modifier in those methods. Use of graphite furnace tubes and contact rings for nickel analysis that are separate from those used for arsenic and selenium analyses is strongly recommended.

4.14.11 Selenium:

4.14.11.1 Elemental selenium and many of its compounds are volatile; therefore, samples may be subject to losses of selenium during sample preparation. Likewise, caution must be employed during the selection of temperature and times for the dry and char (ash) cycles. A matrix modifier such as nickel nitrate or palladium nitrate should be added to all digestates prior to analysis to minimize volatilization losses during drying and ashing.

4.14.11.2 In addition to the normal interferences experienced during graphite furnace analysis, selenium analysis can suffer from severe nonspecific absorption and light scattering caused by matrix components during atomization. Selenium analysis is particularly susceptible to these problems because of its low analytical wavelength

(196.0 nm). Simultaneous background correction must be employed to avoid erroneously high results. High iron levels can give overcorrection with deuterium background. Although Zeeman background correction is very useful in this situation, use of any appropriate background correction technique is acceptable.

4.14.11.3 Selenium analysis suffers interference from chlorides (>800 mg/L) and sulfate (>200 mg/L). The addition of nickel nitrate such that the final concentration is 1% nickel will lessen this interference.

4.14.12 Silver: Silver chloride is insoluble, therefore HCl should be avoided unless the silver is already in solution as a chloride complex. In addition, it is recommended that the stock standard concentrations be kept below 2 ppm and the chloride content increased to prevent precipitation. If precipitation is occurring, a 5%:2% HCl:HNO_3 stock solution may prevent precipitation. Daily standard preparation may also be needed to prevent precipitation of silver. Analysts should be aware that this technique may not be the best choice for this analyte and that alternative techniques should be considered.

4.14.13 Thallium: HCl or excessive chloride will cause volatilization of thallium at low temperatures. Verification that losses are not occurring must be made for each matrix type (as detailed in 9.6.1).

4.14.14 Vanadium: Vanadium is refractory and prone to form carbides. Consequently, memory effects are common, and care should be taken to clean the furnace before and after analysis.

5.0 SAFETY

Refer to Chapter Three for a discussion on safety related references and issues.

6.0 EQUIPMENT AND SUPPLIES

6.1 Atomic absorption spectrophotometer - Single- or dual-channel, single- or double-beam instrument having a grating monochromator, photomultiplier detector, adjustable slits, a wavelength range of 190 to 800 nm, and provisions for interfacing with a graphical display. The instrument must be equipped with an adequate correction device capable of removing undesirable nonspecific absorbance over the spectral region of interest and provide an analytical condition not subject to the occurrence of interelement spectral overlap interferences.

6.2 Hollow cathode lamps - Single-element lamps are preferred but multielement lamps may be used. Electrodeless discharge lamps may also be used when available. Other types of lamps meeting the performance criteria of this method may be used.

6.3 Graphite furnace - Any furnace device capable of reaching the specified temperatures is satisfactory. For all instrument parameters (i.e., drying, ashing, atomizing, times and temperatures) follow the specific instrument manufacturers instructions for each element.

6.4 Data systems recorder - A recorder is recommended for furnace work so that there will be a permanent record and that any problems with the analysis such as drift, incomplete atomization, losses during charring, changes in sensitivity, peak shape, etc., can be easily recognized.

6.5 Pipets - Microliter, with disposable tips. Sizes can range from 5 to 100 µL as required. Pipet tips should be checked as a possible source of contamination when contamination is

suspected or when a new source or batch of pipet tips is received by the laboratory. The accuracy of variable pipets must be verified daily. Class A pipets can be used for the measurement of volumes equal to or larger than 1 mL.

6.6 Glassware - All glassware, polypropylene, or fluorocarbon (PFA or TFE) containers, including sample bottles, flasks and pipets, should be washed in the following sequence: 1:1 hydrochloric acid, tap water, 1:1 nitric acid, tap water, detergent, tap water, and reagent water. Chromic acid should not be used as a cleaning agent for glassware if chromium is to be included in the analytical scheme. If it can be documented through an active analytical quality control program using spiked samples and method blanks that certain steps in the cleaning procedure are not required for routine samples, those steps may be eliminated from the procedure. Leaching of polypropylene for longer periods at lower acid concentrations is necessary to prevent degradation of the polymer. Alternative cleaning procedures must also be documented. Cleaning for ultra-trace analysis should be reviewed in Chapter Three.

6.7 Volumetric flasks of suitable precision and accuracy.

7.0 REAGENTS AND STANDARDS

7.1 Reagents: Analytical reagent grade or trace metals grade chemicals should be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. All reagents should be analyzed to demonstrate that the reagents do not contain target analytes at or above the MDL .

7.2 Reagent water: All references to water in this method refer to reagent water unless otherwise specified. Refer to Chapter One for a definition of reagent water.

7.3 Nitric acid , HNO_3: Use a spectrograde acid certified for AA use. Prepare a 1:1 dilution with water by adding the concentrated acid to an equal volume of water. If the method blank does not contain target analytes at or above the MDL, then the acid may be used.

7.4 Hydrochloric acid (1:1), HCl: Use a spectrograde acid certified for AA use. Prepare a 1:1 dilution with water by adding the concentrated acid to an equal volume of water. If the method blank does not contain target analytes at or above the MDL, then the acid may be used.

7.5 Purge Gas: A mixture of H_2 (5%) and argon (95%). The argon gas supply must be high-purity grade, 99.99% or better. If performance can be documented, alternative gases may be used.

7.6 Stock standard metal solutions: Stock standard solutions are prepared from analytical reagent grade high purity metals, oxides, or nonhygroscopic salts using reagent water and redistilled nitric or hydrochloric acids. (See individual methods for specific instructions.) Sulfuric or phosphoric acids should be avoided as they produce an adverse effect on many elements. The stock solutions are prepared at concentrations of 1,000 mg of the metal per liter. Commercially available standard solutions may also be used. When using pure metals (especially wire) for standards preparation, cleaning procedures, as detailed in Chapter Three, should be used to ensure that the solutions are not compromised. Examples of appropriate standard preparations can be found in Sections 7.6.1 through 7.6.18.

7.6.1 Antimony: Carefully weigh 2.743 g of antimony potassium tartrate, $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$, and dissolve in reagent water. Dilute to 1 L with reagent water;

7.6.2 Arsenic: Dissolve 1.320 g of arsenic trioxide, As_2O_3, or equivalent in 100 mL of reagent water containing 4 g NaOH. Acidify the solution with 20 mL conc. HNO_3 and dilute to 1 L with reagent water.

7.6.3 Barium: Dissolve 1.779 g barium chloride, $BaCl_2 \cdot 2H_2O$, in reagent water and dilute to 1 L with reagent water.

7.6.4 Beryllium: Dissolve 11.659 g beryllium sulfate, $BeSO_4$, in reagent water containing 2 mL nitric acid (conc.) and dilute to 1 L with reagent water.

7.6.5 Cadmium: Dissolve 1.000 g cadmium metal in 20 mL of 1:1 HNO_3 and dilute to 1 L with reagent water.

7.6.6 Chromium: Dissolve 1.923 g of chromium trioxide, CrO_3, in reagent water, acidify with redistilled HNO_3, and dilute to 1 L with reagent water.

7.6.7 Cobalt: Dissolve 1.000 g of cobalt metal in 20 mL of 1:1 HNO_3 and dilute to 1 L with reagent water. Chloride or nitrate salts of cobalt(II) may be used. Although numerous hydrated forms exist, they are not recommended, unless the exact composition of the compound is known.

7.6.8 Copper: Dissolve 1.000 g of electrolytic copper in 5 mL of redistilled HNO_3 and dilute to 1 L with reagent water.

7.6.9 Iron: Dissolve 1.000 g iron wire in 10 mL redistilled HNO_3 and reagent water and dilute to 1 L with reagent water. Note that iron passivates in conc. HNO_3, and therefore some water should be present.

7.6.10 Lead: Dissolve 1.599 g of lead nitrate, $Pb(NO_3)_2$, in reagent water, acidify with 10 mL redistilled HNO_3, and dilute to 1 L with reagent water.

7.6.11 Manganese: Dissolve 1.000 g manganese metal in 10 mL redistilled HNO_3 and dilute to 1 L with reagent water.

7.6.12 Molybdenum: Dissolve 1.840 g of ammonium molybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and dilute to 1 L with reagent water.

7.6.13 Nickel: Dissolve 1.000 g nickel metal or 4.953 g nickel nitrate, $Ni(NO_3)_2 \cdot 6H_2O$ in 10 mL HNO_3 and dilute to 1 L with reagent water.

7.6.14 Selenium: Dissolve 0.345 g of selenious acid (actual assay 94.6% H_2SeO_3) or equivalent and dilute to 200 mL with reagent water.

NOTE: Due to the high toxicity of selenium, preparation of a smaller volume of reagent has been described. Larger volumes may be prepared if required.

7.6.15 Silver: Dissolve 1.575 g of anhydrous silver nitrate, $AgNO_3$, in reagent water. Add 10 mL of HNO_3 (conc.) and dilute to 1 L with reagent water. Because this standard is light sensitive, store in a amber glass bottle in a refrigerator.

7.6.16 Thallium: Dissolve 1.303 g thallium nitrate, $TlNO_3$, in reagent water, acidify with 10 mL conc. HNO_3, and dilute to 1 L with reagent water.

7.6.17 Vanadium: Dissolve 1.785 g of vanadium pentoxide, V_2O_5, in 10 mL of conc. HNO_3 and dilute to 1 L with reagent water.

7.6.18 Zinc: Dissolve 1.000 g zinc metal in 10 mL of conc. HNO_3 and dilute to 1 L with reagent water.

7.7 Common matrix modifiers: The use of a palladium modifier is strongly recommended for the determination of all analytes. This will correct for general chemical interferences as well as allow for higher char and atomization temperatures without allowing the premature liberation of analyte. Other matrix modifiers may also be used as recommended by the instrument manufacturer or when an interference is evident.

7.7.1 Palladium solution (Pd/Mg): Dissolve 300 mg of palladium powder in concentrated HNO_3 (1 mL of HNO_3, adding 0.1 mL of conc. HCl, if necessary). Dissolve 200 mg of $Mg(NO_3)_2$ in reagent water. Pour the two solutions together and dilute to 100 mL with reagent water.

7.7.2 Nickel nitrate solution (5%): Dissolve 25g of $Ni(NO_3)_2 \cdot 6H_2O$ in reagent water and dilute to 100 mL.

7.7.3 Nickel nitrate solution (1%): Dilute 20 mL of the 5% nickel nitrate solution to 100 mL with reagent water.

7.7.4 Ammonium phosphate solution (40%): Dissolve 40 g of ammonium phosphate, $(NH_4)_2HPO_4$, in reagent water and dilute to 100 mL.

7.7.5 Palladium chloride: Weigh 0.25 g of $PdCl_2$ to the nearest 0.0001 g and dissolve in 10 mL of 1:1 HNO_3. Dilute to 1 L with reagent water

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 See Chapter Three.

9.0 QUALITY CONTROL

9.1 All quality control data should be maintained and available for easy reference or inspection.

9.2 For each batch of samples processed, at least one method blank must be carried throughout the entire sample preparation and analytical process as described in Chapter One. A method blank is prepared by using a volume or weight of reagent water at the volume or weight specified in the preparation method and then carried through the appropriate steps of the analytical process. These steps may include but are not limited to digestion, dilution, filtering, and analysis. If the method blank does not contain target analytes at a level that interferes with the project-specific DQOs then the method blank would be considered acceptable. In the absence of project-specific DQOs, if the blank is less than the MDL or less than 10% of the lowest sample concentration for each analyte, whichever is greater, then the method blank would be considered acceptable. If the method blank cannot be considered acceptable, the method blank should be re-run once and if still unacceptable, then all contaminated samples after the last acceptable method blank must be

reprepped and reanalyzed along with the other appropriate batch QC samples. These blanks will be useful in determining if samples are being contaminated. Refer to Chapter One for the proper protocol when analyzing blanks.

9.3 For each batch of samples processed, at least one laboratory control sample must be carried throughout the entire sample preparation and analytical process as described in Chapter One. The laboratory control samples should be spiked with each analyte of interest at the project-specific action level or when lacking project-specific action levels, between the low and midlevel standards. Acceptance criteria should be set at a laboratory derived limit developed through the use of historical analyses. In the absence of historical data this limit should be set at ± 20% of the spiked value. After the determination of historical data, ±20% must still be the limit of maximum deviation to express acceptability. If the laboratory control sample cannot be considered acceptable, the laboratory control sample should be re-run once and if still unacceptable then all samples after the last acceptable laboratory control sample must be reprepped and reanalyzed. Refer to Chapter One for more information.

9.4 Matrix Spike/Matrix Spike Duplicates (MS/MSDs): At the laboratory's discretion, a separate spike sample and a separate duplicate sample may be analyzed in lieu of the MS/MSD. For each batch of samples processed, at least one MS/MSD sample must be carried throughout the entire sample preparation and analytical process as described in Chapter One. MS/MSDs are intralaboratory split samples spiked with identical concentrations of each analyte of interest. The spiking occurs prior to sample preparation and analysis. An MS/MSD is used to document the bias and precision of a method in a given sample matrix. Refer to the definitions of bias and precision, in Chapter One, for the proper data reduction protocols. MS/MSD samples should be spiked at the same level as the corresponding laboratory control sample that is at the project-specific action level or, when lacking project-specific action levels, between the low and midlevel standards. Acceptance criteria should be set at a laboratory derived limit developed through the use of historical analyses. In the absence of historical data this limit should be set at ± 20% of the spiked value for precision and ≤ 20 relative percent difference (RPD). After the determination of historical data, 20% must still be the limit of maximum deviation for both percent recovery and relative percent difference to express acceptability. Refer to Chapter One for guidance. If the bias and precision indicators are outside the laboratory control limits or if the percent recovery is less than 80% or greater than 120% or if the relative percent difference is greater than 20%, the interference test as discussed in Sec. 9.5.2 and 9.7 should be conducted.

9.5 Interference tests

9.5.1 Recovery test (post-digestion spike) - The recovery test must be done on every sample. To conduct this test withdraw an aliquot of the test sample and add a known amount of analyte to bring the concentration of the analyte to 2 to 5 times the original concentration. If spiking at 2-5 times would exceed the linear range of the instrument, a lesser spike may be used. If all of the samples in the batch have analyte concentrations below the detection limit, spike the selected sample at the project-specific action level or when lacking project-specific action levels, between the low and midlevel standards. Analyze the spiked sample and calculate the spike recovery. If the recovery is <85% or >115%, MSA should be used for the sample.

9.5.2 Dilution test - The dilution test is to be conducted when interferences are suspected (Sec. 9.5.1) and the sample concentration is high enough to allow for proper interpretation of the results. To conduct this test, determine the apparent concentration in the undiluted sample. Dilute the sample by a minimum of five fold (1+4) and reanalyze. Agreement within an RPD of 10 between the concentration for the undiluted sample and five

times the concentration for the diluted sample indicates the absence of interferences, and such samples may be analyzed without using the method of standard additions. If agreement between the dilutions is greater than 10%, the MSA should be used for all samples in the batch.

9.6 Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition (MSA) is recommended (see Section 9.7 below). Other options including, the use of different matrix modifiers, different furnace conditions, different preparatory methods or different analytical methods may also be attempted to properly characterize a sample. Section 9.5 provides tests to determine the potential for an interference and evaluates the need for using the MSA.

9.7 Method of standard additions - The standard addition technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique attempts to compensate for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interferences which cause a baseline shift. The method of standard additions may be appropriate for analysis of extracts, on analyses submitted as part of a delisting petition, whenever a new sample matrix is being analyzed and on every batch that fails the recovery test.

9.7.1 The simplest version of this technique is the single-addition method, in which two identical aliquots of the sample solution, each of volume V_x, are taken. To the first (labeled A) is added a known volume V_S of a standard analyte solution of concentration C_S. To the second aliquot (labeled B) is added the same volume V_S of reagent water. The analytical signals of A and B are measured and corrected for non-analyte signals. The unknown sample concentration C_x is calculated:

$$C_x = \frac{S_B V_S C_S}{(S_A - S_B) V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average, avoiding excess dilution of the sample. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure.

9.7.2 Improved results can be obtained by employing a series of standard additions. To equal volumes of the sample are added a series of standard solutions containing different known quantities of the analyte, and all solutions are diluted to the same final volume. For example, addition 1 should be prepared so that the resulting concentration is approximately 50 percent of the expected absorbance from the indigenous analyte in the sample. Additions 2 and 3 should be prepared so that the concentrations are approximately 100 and 150 percent of the expected endogenous sample absorbance. The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated to zero absorbance, the point of interception of the abscissa is the endogenous concentration of the analyte in the sample. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate. An example of a plot so obtained is shown in Figure 1. A linear regression program may be used to obtain the intercept concentration.

9.7.3 For the results of this MSA technique to be valid, the following limitations must be taken into consideration:

1. The apparent concentrations from the calibration curve must be linear (0.995 or greater) over the concentration range of concern. For the best results, the slope of the MSA plot should be nearly the same as the slope of the standard curve.

2. The effect of the interference should not vary as the ratio of analyte concentration to sample matrix changes, and the standard addition should respond in a similar manner as the analyte.

3. The determination must be free of spectral interference and corrected for nonspecific background interference.

9.8 All quality control measures described in Chapter One should be followed.

9.9 Independent source laboratory control sample or standard reference materials (SRMs) should be used to help assess the quality of the analytical scheme.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Calibration standards - All analyses require that a calibration curve be prepared to cover the appropriate concentration range. Usually, this means the preparation of a blank and standards which produce an absorbance of 0.0 to 0.7. Calibration standards can prepared by diluting the stock metal solutions in the same acids and acid concentrtions as the samples.

10.1.1 Calibration standards can be prepared fresh each time a batch of samples is analyzed. If the ICV solution is prepared daily and the ICV is analyzed within the acceptance criteria, calibration standards do not need to be prepared daily and may be prepared and stored for as long as the calibration standard viability can be verified through the use of the ICV. If the ICV is outside of the acceptance criteria, the calibration standards must be prepared fresh and the instrument recalibrated. Prepare a blank and at least three calibration standards in graduated amounts in the appropriate range of the linear part of the curve.

10.1.2 The calibration standards should be prepared using the same type of acid or combination of acids and at the same concentration as will result in the samples following processing.

10.1.3 Beginning with the blank and working toward the highest standard, inject the solutions and record the readings. Calibration curves are always required.

10.2 A calibration curve must be prepared each day with a minimum of a calibration blank and three standards. The curve must be linear and have a correlation coefficient of at least 0.995.

10.2.1 After initial calibration, the calibration curve must be verified by use of an initial calibration blank (ICB) and an initial calibration verification (ICV) standard. The ICV standard must be made from an independent (second source) material at or near mid-range. The acceptance criteria for the ICV standard must be ±10% of its true value and the ICB must not contain target analytes at or above the MDL for the curve to be considered valid. If the calibration curve cannot be verified within the specified limits, the cause must be determined

and the instrument recalibrated before samples are analyzed. The analysis data for the ICV must be kept on file with the sample analysis data.

10.2.2 The calibration curve must also be verified at the end of each analysis batch and/or after every 10 samples by use of a continuing calibration blank (CCB) and a continuing calibration verification (CCV) standard. The CCV standard should be made from the same material as the initial calibration standards at or near midrange. The acceptance criteria for the CCV standard must be ±10% of its true value and the CCB must not contain target analytes at or above the MDL for the curve to be considered valid. If the calibration cannot be verified within the specified limits, the sample analysis must be discontinued, the cause determined and the instrument recalibrated. All samples following the last acceptable CCV/CCB must be reanalyzed. The analysis data for the CCV/CCB must be kept on file with the sample analysis data.

10.3 It is recommended that each standard should be analyzed (injected) twice and an average value determined. Replicate standard values should be within ±10% RPD.

10.4 Standards are run in part to monitor the life and performance of the graphite tube. Lack of reproducibility or significant change in the signal for the standard indicates that the tube should be replaced. Tube life depends on sample matrix and atomization temperature. A conservative estimate would be that a tube will last at least 50 firings. A pyrolytic coating will extend that estimated life by a factor of three.

10.5 If conducting trace analysis, it is recommended that the lowest calibration standard be set at the laboratory's quantitation level. The laboratory can use a reporting limit that is below the quantitation level but all values reported below the low standard should be reported as estimated values.

11.0 PROCEDURE

11.1 Preliminary treatment of waste water, ground water, extracts, and industrial waste is always necessary because of the complexity and variability of sample matrices. Solids, slurries, and suspended material must be subjected to a solubilization process before analysis. This process may vary because of the metals to be determined and the nature of the sample being analyzed. Solubilization and digestion procedures are presented in Chapter Three. Samples which are to be analyzed only for dissolved constituents need not be digested if they have been filtered and acidified.

11.2 Furnace devices (flameless atomization) are a most useful means of extending detection limits. Because of differences between various makes and models of satisfactory instruments, no detailed operating instructions can be given for each instrument. Instead, the analyst should follow the instructions provided by the manufacturer of a particular instrument. A generalized set of instructions follows:

11.2.1 Inject an aliquot of sample into the furnace and atomize. If the concentration found is greater than the highest standard, the sample should be diluted in the same acid matrix and reanalyzed. The use of multiple injections can improve accuracy and help detect furnace pipetting errors.

11.2.2 To verify the absence of interference, follow the interference procedure given in Sec. 9.5.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 For determination of metal concentration by GFAA: Read the metal value from the calibration curve or directly from the read-out system of the instrument.

12.1.1 If dilution of sample was required:

$$\mu g/L \text{ metal in sample} = \frac{A\ (C+B)}{C}$$

where:

A = µg/L of metal in diluted aliquot from calibration curve.
B = Starting sample volume , mL.
C = Final volume of sample, mL.

12.1.2 For solid samples, report all concentrations in consistent units based on wet weight. Ensure that if the dry weight was used for the analysis, percent solids should be reported to the client. Hence:

$$\text{mg metal/kg sample} = \frac{A \times V}{W}$$

where:

A = mg/L of metal in processed sample from calibration curve.
V = Final volume of the processed sample, L.
W = Weight of sample, Kg.

12.1.3 Different injection volumes must not be used for samples and standards. Instead, the sample should be diluted and the same size injection volume be used for both samples and standards.

13.0 METHOD PERFORMANCE

13.1 See the individual methods from reference 1.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/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.

14.2 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, Department of Government Relations and Science Policy, 1155 16th Street, NW, Washington, DC 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits 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 Section 14.2.

16.0 REFERENCES

1. Methods for Chemical Analysis of Water and Wastes; U.S. Environmental Protection Agency. Office of Research and Development. Environmental Monitoring and Support Laboratory. ORD Publication Offices of Center for Environmental Research Information: Cincinnati, OH, 1983; EPA-600/4-79-020.

2. Rohrbough, W.G.; et al. Reagent Chemicals, American Chemical Society Specifications, 7th ed.; American Chemical Society: Washington, DC, 1986.

3. 1985 Annual Book of ASTM Standards, Vol. 11.01; "Standard Specification for Reagent Water"; ASTM: Philadelphia, PA, 1985; D1193-77.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain Tables 1 and 2, Figure 1, and a flow diagram of the method procedures.

TABLE 1

FURNACE ATOMIC ABSORPTION DETECTION LIMITS FOR ANALYTES IN REAGENT WATER

Metal	Furnace Procedure[a,b] Detection Limit (µg/L)
Antimony	3
Arsenic	1
Barium(p)	2
Beryllium	0.2
Cadmium	0.1
Chromium	1
Cobalt	1
Copper	1
Iron	1
Lead	1
Manganese	0.2
Molybdenum(p)	1
Nickel	1
Selenium	2
Silver	0.2
Thallium	1
Vanadium(p)	4
Zinc	0.05

NOTE: The symbol (p) indicates the use of pyrolytic graphite with the furnace procedure.

[a]For furnace sensitivity values, consult instrument operating manual.

[b]The listed furnace values are those expected when using a 20-µL injection and normal gas flow, except in the cases of arsenic and selenium, where gas interrupt is used.

Source: Reference 1.

TABLE 2

INSTRUMENT PARAMETERS

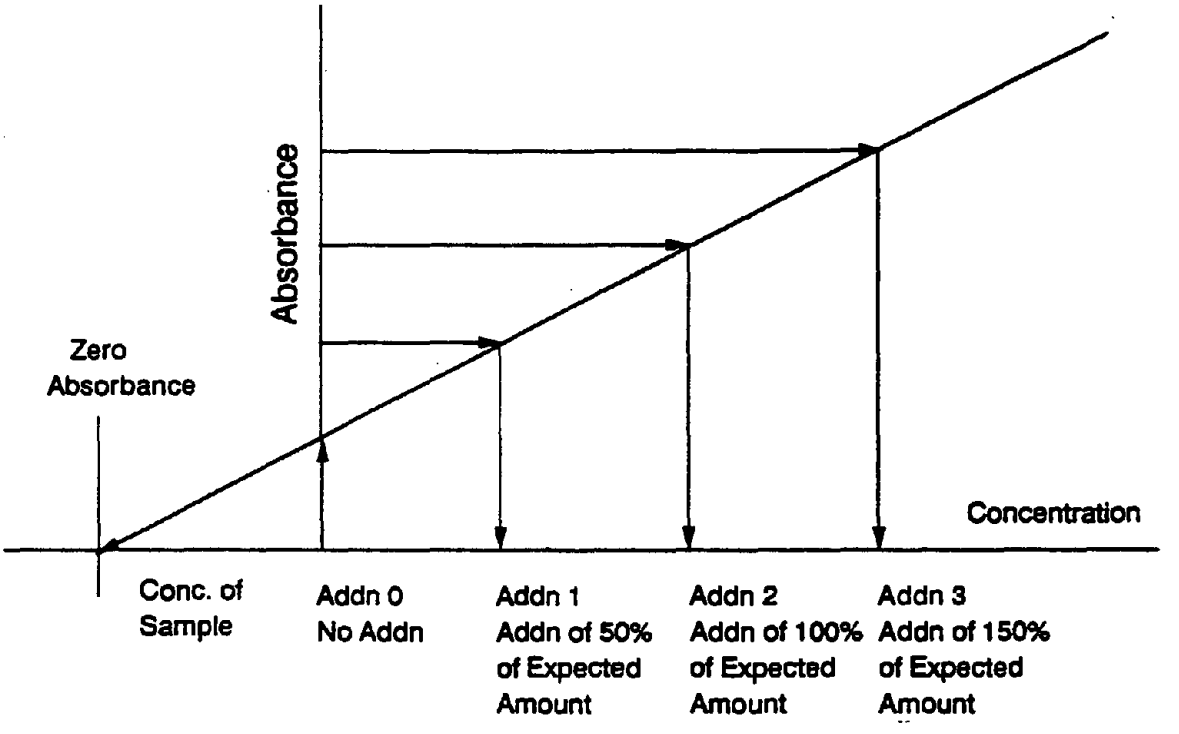
ELEMENT	WAVELENGTH (nm)	PURGE GAS[1]	COMMENTS
Sb	217.6, 231.1	argon or nitrogen	
As	193.7	argon	
Ba	553.6	argon	nitrogen should not be used
Be	234.9	argon	
Cd	228.8	argon	
Cr	357.9	argon	nitrogen should not be used
Co	240.7	argon	
Cu	324.7	argon or nitrogen	
Fe	248.3, 248.8, 271.8, 302.1, 252.7	argon or nitrogen	
Pb	283.3, 217.0	argon	
Mn	279.5, 403.1	argon or nitrogen	
Mo	313.3	argon	nitrogen should not be used
Ni	232.0, 352.4	argon or nitrogen	
Se	196.0	argon	
Ag	328.1	argon	
Tl	276.8	argon or nitrogen	
V	318.4	argon	nitrogen should not be used
Zn	213.9	argon or nitrogen	

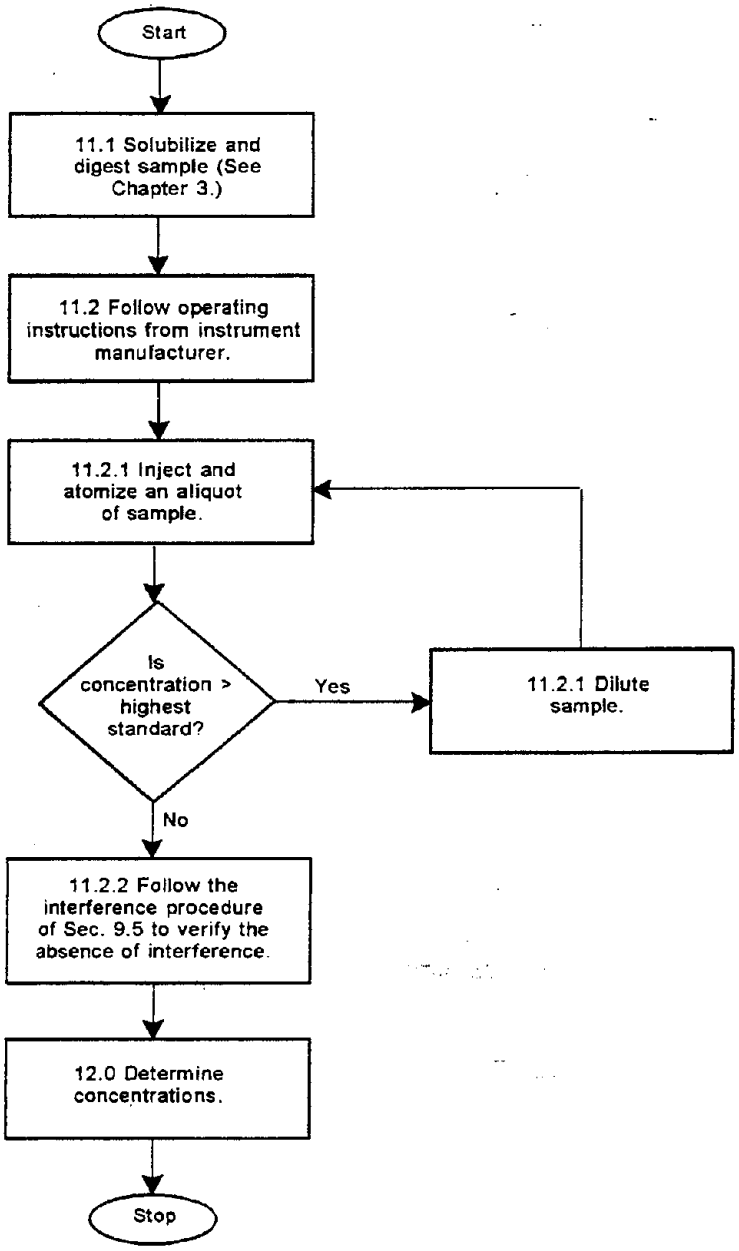
Note: If more than one wavelength is listed, the primary line is underlined.
[1]The argon/H_2 purge gas is also applicable.

Source: Reference 1

FIGURE 1

STANDARD ADDITION PLOT




Start
11.1 Solubilize and digest sample (See Chapter 3.)
11.2 Follow operating instructions from instrument manufacturer.
11.2.1 Inject and atomize an aliquot of sample.
Is concentration > highest standard?
Yes
11.2.1 Dilute sample.
No
11.2.2 Follow the interference procedure of Sec. 9.5 to verify the absence of interference.
12.0 Determine concentrations.
Stop

METHOD 7471B

MERCURY IN SOLID OR SEMISOLID WASTE (MANUAL COLD-VAPOR TECHNIQUE)

1.0 SCOPE AND APPLICATION

1.1 Method 7471 is approved for measuring total mercury (organic and inorganic) in soils, sediments, bottom deposits, and sludge-type materials. All samples must be subjected to an appropriate dissolution step prior to analysis. If this dissolution procedure is not sufficient to dissolve a specific matrix type or sample, then this method is not applicable for that matrix.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, the solid or semi-solid samples must be prepared according to the procedures discussed in this method.

2.2 This method is a cold-vapor atomic absorption method and is based on the absorption of radiation at the 253.7-nm wavelength by mercury vapor. The mercury is reduced to the elemental state and aerated from solution in a closed system. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer. Absorbance (peak height) is measured as a function of mercury concentration.

2.3 The typical instrument detection limit (IDL) for this method is 0.0002 mg/L.

3.0 DEFINITIONS

Refer to Chapter One and Chapter Three for a listing of applicable definitions.

4.0 INTERFERENCES

4.1 Potassium permanganate is added to eliminate possible interference from sulfide. Concentrations as high as 20 mg/Kg of sulfide, as sodium sulfide, do not interfere with the recovery of added inorganic mercury in reagent water.

4.2 Copper has also been reported to interfere; however, copper concentrations as high as 10 mg/Kg had no effect on recovery of mercury from spiked samples.

4.3 Samples high in chlorides require additional permanganate (as much as 25 mL) because, during the oxidation step, chlorides are converted to free chlorine, which also absorbs radiation of 254 nm. Care must therefore be taken to ensure that free chlorine is absent before the mercury is reduced and swept into the cell. This may be accomplished by using an excess of hydroxylamine sulfate reagent (25 mL). In addition, the dead air space in the BOD bottle must be purged before adding stannous sulfate.

4.4 Certain volatile organic materials that absorb at this wavelength may also cause interference. A preliminary run without reagents should determine if this type of interference is present.

5.0 SAFETY

Refer to Chapter Three for a discussion on safety related references and issues.

6.0 EQUIPMENT AND SUPPLIES

6.1 Atomic absorption spectrophotometer or equivalent: Any atomic absorption unit with an open sample presentation area in which to mount the absorption cell is suitable. Instrument settings recommended by the particular manufacturer should be followed. Instruments designed specifically for the measurement of mercury using the cold-vapor technique are commercially available and may be substituted for the atomic absorption spectrophotometer.

6.2 Mercury hollow cathode lamp or electrodeless discharge lamp.

6.3 Recording device: Any multirange variable-speed recorder compatible with the UV detection system or any other compatible data collection device.

6.4 Absorption cell: Standard spectrophotometer cells 10 cm long with quartz end windows may be used. Suitable cells may be constructed from Plexiglas tubing, 1 in. O.D. x 4.5 in. The ends are ground perpendicular to the longitudinal axis, and quartz windows (1 in. diameter x 1/16 in. thickness) are cemented in place. The cell is strapped to a burner for support and aligned in the light beam by use of two 2-in. x 2-in. cards. One inch diameter holes are cut in the middle of each card. The cards are then placed over each end of the cell. The cell is then positioned and adjusted vertically and horizontally to give the maximum transmittance.

6.5 Air pump: Any peristaltic pump capable of delivering 1 L/min air may be used. A Masterflex pump with electronic speed control has been found to be satisfactory.

6.6 Flowmeter: Capable of measuring an air flow of 1 L/min.

6.7 Aeration tubing: A straight glass frit with a coarse porosity. Tygon tubing is used for passage of the mercury vapor from the sample bottle to the absorption cell and return.

6.8 Drying tube: 6-in. x 3/4-in. diameter tube containing 20 g of magnesium perchlorate or a small reading lamp with 60-W bulb which may be used to prevent condensation of moisture inside the cell. The lamp should be positioned to shine on the absorption cell so that the air temperature in the cell is about 10°C above ambient.

6.9 The cold-vapor generator is assembled as shown in Figure 1 of reference 1 or according to the instrument manufacturers instructions. The apparatus shown in Figure 1 is a closed system. An open system, where the mercury vapor is passed through the absorption cell only once, may be used instead of the closed system. Because mercury vapor is toxic, precaution must be taken to avoid its inhalation. Therefore, a bypass has been included in the system either to vent the mercury vapor into an exhaust hood or to pass the vapor through some absorbing medium, such as:

1. Equal volumes of 0.1 M $KMnO_4$ and 10% H_2SO_4, or
2. Iodine 0.25% in a 3% KI solution.

A specially treated charcoal that will adsorb mercury vapor is also available from Barneby and Cheney, East 8th Avenue and North Cassidy Street, Columbus, Ohio 43219, Cat. #580-13 or #580-22.

6.10 Heating source - Adjustable and capable of maintaining a temperature of 95 ± 3°C. (e.g., hot plate, block digestor, microwave, etc.)

6.11 Graduated cylinder or equivalent volume measuring device.

7.0 REAGENTS AND STANDARDS

7.1 Reagent Water: Reagent water will be interference free. All references to water in this method refer to reagent water unless otherwise specified. Refer to Chapter One for a definition of reagent water.

7.2 Aqua regia: Prepare immediately before use by carefully adding three volumes of concentrated HCl to one volume of concentrated HNO_3.

7.3 Sulfuric acid, 0.5 N: Dilute 14.0 mL of concentrated sulfuric acid to 1 liter.

7.4 Stannous sulfate: Add 25 g stannous sulfate to 250 mL of 0.5 N sulfuric acid. This mixture is a suspension and should be stirred continuously during use. A 10% solution of stannous chloride can be substituted for stannous sulfate.

7.5 Sodium chloride-hydroxylamine sulfate solution: Dissolve 12 g of sodium chloride and 12 g of hydroxylamine sulfate in reagent water and dilute to 100 mL. Hydroxylamine hydrochloride may be used in place of hydroxylamine sulfate.

7.6 Potassium permanganate, mercury-free, 5% solution (w/v): Dissolve 5 g of potassium permanganate in 100 mL of reagent water.

7.7 Mercury stock solution: Dissolve 0.1354 g of mercuric chloride in 75 mL of reagent water. Add 10 mL of concentrated nitric acid and adjust the volume to 100.0 mL (1.0 mL = 1.0 mg Hg).

7.8 Mercury working standard: Make successive dilutions of the stock mercury solution to obtain a working standard containing 0.1 µg/mL. This working standard and the dilution of the stock mercury solutions should be prepared fresh daily. Acidity of the working standard should be maintained at 0.15% nitric acid. This acid should be added to the flask, as needed, before adding the aliquot.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

8.2 All sample containers must be prewashed with detergents, acids, and reagent water. Plastic and glass containers are both suitable.

8.3 Non-aqueous samples shall be refrigerated, when possible, and analyzed "as soon as possible."

9.0 QUALITY CONTROL

Refer to Method 7000.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Standard preparation: Transfer 0.0-, 0.5-, 1.0-, 2.0-, 5.0-, and 10-mL aliquots of the mercury working standard, containing 0-1.0 ug of mercury, to a series of 300-mL BOD bottles or equivalent. Add enough reagent water to each bottle to make a total volume of 10 mL. Add 5 mL

of aqua regia and heat 2 min at 95 ± 3°C. Allow the sample to cool; add 50 mL reagent water and 15 mL of $KMnO_4$ solution to each bottle and heat again at 95 ± 3°C for 30 min. Cool and add 6 mL of sodium chloride-hydroxylamine sulfate solution to reduce the excess permanganate. Add 50 mL of reagent water. Treating each bottle individually, add 5 mL of stannous sulfate solution, immediately attach the bottle to the aeration apparatus, and continue as described in Section 11.3.

10.2 Construct a calibration curve by plotting the absorbances of standards versus micrograms of mercury. Determine the peak height of the unknown from the chart or other recording device and read the mercury value from the standard curve. Duplicates, spiked samples, and check standards should be routinely analyzed.

11.0 PROCEDURE

11.1 Sample preparation: Weigh a 0.5 - 0.6g aliquot of a well homogenized sample and place in the bottom of a BOD bottle or other appropriate analysis vessel. Add 5 mL of reagent water and 5 mL of aqua regia. Heat two minutes at 95 ± 3°C. Cool; then add 50 mL reagent water and 15 mL potassium permanganate solution to each sample. Mix thoroughly, then heat for 30 min at 95 ± 3°C. Cool and add 6 mL of sodium chloride-hydroxylamine sulfate to reduce the excess permanganate.

CAUTION: Do this addition under a hood, as Cl_2 could be evolved. Add 55 mL of reagent water. Treating each bottle individually, add 5 mL of stannous sulfate and immediately attach the bottle to the aeration apparatus. Continue as described under Section 11.3.

11.2 An alternate digestion procedure employing an autoclave may also be used. In this method, 5 mL of concentrated H_2SO_4 and 2 mL of concentrated HNO_3 are added to the 0.5 - 0.6 g of sample. Add 5 mL of saturated $KMnO_4$ solution and cover the bottle with a piece of aluminum foil. The samples are autoclaved at 121 ± 3°C and 15 lb for 15 min. Cool, dilute to a volume of 100 mL with reagent water, and add 6 mL of sodium chloride-hydroxylamine sulfate solution to reduce the excess permanganate. Purge the dead air space and continue as described under Section 11.3. Refer to the caution statement in Section 11.1 for the proper protocol in reducing the excess permanganate solution and adding stannous sulfate.

11.3 Analysis: At this point, the sample is allowed to stand quietly without manual agitation. The circulating pump, which has previously been adjusted to a rate of 1 L/min, is allowed to run continuously. The absorbance, as exhibited either on the spectrophotometer or the recorder, will increase and reach maximum within 30 seconds. As soon as the absorbance reading levels off (approximately 1 minute), open the bypass valve and continue the aeration until the absorbance returns to its minimum value. Close the bypass valve, remove the fritted tubing from the BOD bottle, and continue the aeration.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Calculate metal concentrations: (1) by the method of standard additions, (2) from a calibration curve, or (3) directly from the instrument's concentration read-out. All dilution or concentration factors must be taken into account. Concentrations reported for multiphased or wet samples must be appropriately qualified (e.g., 5 µg/g dry weight).

13.0 METHOD PERFORMANCE

13.1 Precision and accuracy data are available in Method 245.5 of Methods for Chemical Analysis of Water and Wastes.

13.2 The data shown in Table 1 were obtained from records of state and contractor laboratories. The data are intended to show the precision of the combined sample preparation and analysis method.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/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.

14.2 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 Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits 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 Sec. 14.2.

16.0 REFERENCES

1. Methods for Chemical Analysis of Water and Wastes, EPA-600/4-82-055, December 1982, Method 245.5.

2. Gaskill, A., Compilation and Evaluation of RCRA Method Performance Data, Work Assignment No. 2, EPA Contract No. 68-01-7075, September 1986.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

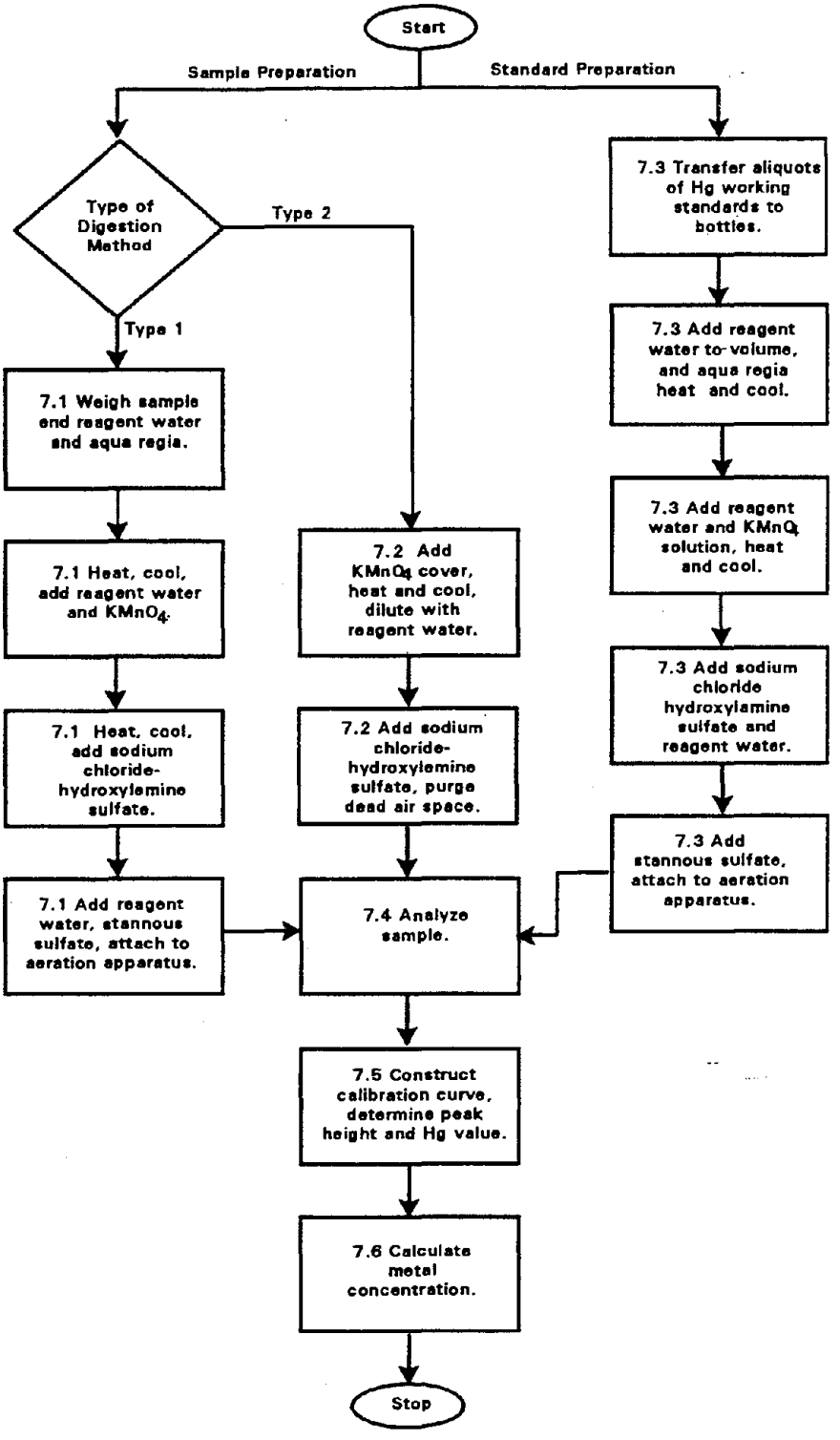
The pages to follow contain Table 1 and a flow diagram of the method procedure.

TABLE 1

METHOD PERFORMANCE DATA

Sample Matrix	Preparation Method	Laboratory Replicates
Emission control dust	Not known	12, 12 µg/g
Wastewater treatment sludge	Not known	0.4, 0.28 µg/g

MERCURY IN SOLID OR SEMISOLID WASTE (MANUAL COLD-VAPOR TECHNIQUE)



METHOD 7473

MERCURY IN SOLIDS AND SOLUTIONS BY THERMAL DECOMPOSITION, AMALGAMATION, AND ATOMIC ABSORPTION SPECTROPHOTOMETRY

1.0 SCOPE AND APPLICATION

1.1 Method 7473 is designated for the determination of mercury (CAS No. 7439-97-6) in solids, aqueous samples, and digested solutions in both the laboratory and field environments. Integration of thermal decomposition sample preparation and atomic absorption detection reduces the total analysis time of most samples to less than five minutes in either the laboratory or field setting. Total mercury (organic and inorganic) in soils, sediments, bottom deposits, and sludge-type materials as well as in aqueous wastes and ground waters can be determined without sample chemical pretreatment using this method, except as noted. Alternatively, this method can be used for the detection of total mercury from total decomposition sample preparation methods, such as Method 3052, or for detection of extracted or leached mercury compounds or species from methods such as the SW-846 3000 series methods (as detailed in Chapter Three).

NOTE: For unique circumstances when mercury could be bound in silicates or other matrices that may not thermally decompose, validation of direct analysis of the solid should be confirmed with total decomposition with an EPA approved method (such as Method 3052) and analysis with this method.

2.0 SUMMARY OF METHOD

2.1 Controlled heating in an oxygenated decomposition furnace is used to liberate mercury from solid and aqueous samples in the instrument. The sample is dried and then thermally and chemically decomposed within the decomposition furnace. The decomposition products are carried by flowing oxygen to the catalytic section of the furnace. Here oxidation is completed and halogens and nitrogen/sulfur oxides are trapped. The remaining decomposition products are then carried to an amalgamator that selectively traps mercury. After the system is flushed with oxygen to remove any remaining gases or decomposition products, the amalgamator is rapidly heated, releasing mercury vapor. Flowing oxygen carries the mercury vapor through absorbance cells positioned in the light path of a single wavelength atomic absorption spectrophotometer. Absorbance (peak height or peak area) is measured at 253.7 nm as a function of mercury concentration.

2.2 The typical working range for this method is 0.05 - 600 ng. The mercury vapor is first carried through a long pathlength absorbance cell and then a short pathlength absorbance cell. (The lengths of the first cell and the second cell are in a ratio of 10:1 or another appropriate ratio.) The same quantity of mercury is measured twice, using two different sensitivities (see Figure 1), resulting in a dynamic range that spans at least four orders of magnitude.

2.3 The instrument detection limit (IDL) for this method is 0.01 ng total mercury.

3.0 DEFINITIONS

3.1 Thermal Decomposition: Partial or complete degradation of sample components using convection and conduction heating mechanisms resulting in the release of volatile components such as water, carbon dioxide, organic substances, elements in the form of oxides or complex compounds, and elemental gases.

3.2 Amalgamation: The process by which mercury forms a metal alloy with gold.

3.3 Amalgamator: A system composed of gold particles at a high surface area to volume ratio for the purpose of amalgamating mercury vapor.

3.4 Primary Calibration: A complete calibration of the instrument's working range. This calibration is performed initially and when any significant instrumental parameters are changed. For example, in this method a primary calibration should be performed after the decomposition tube, amalgamator, or oxygen tank is replaced.

3.5 Daily Calibration: A calibration performed with minimal standards to ensure that the primary calibration is valid. For example, when two standards within the range of interest are analyzed and agree within 10% of their true value the primary calibration is assumed to be valid.

3.6 Memory Effects: Mercury vapor may remain in the decomposition tube, amalgamator, or absorbance cells and be released in a subsequent analysis resulting in a positive bias. For example, this may result when a low concentration sample is analyzed after a sample of high mercury content.

3.7 Sample Boat: The non-amalgamating thermally stable vessel used for containment and transport of the solid or liquid sample for thermal decomposition.

4.0 INTERFERENCES

4.1 In areas where mercury contamination is an existing problem, the background signal may be significantly increased.

4.2 Memory effects between analyses may be encountered when analyzing a sample of high mercury concentration ($\geq$ 400 ng) prior to analyzing one of low concentration ($\leq$ 25 ng). Typically, to minimize memory effects, analyze the samples in batches of low and high concentrations, always analyzing those of low concentration first. If this batching process cannot be accomplished, a blank analysis with an extended decomposition time may be required following the analysis of a highly concentrated sample to limit memory effects.

4.3 Co-absorbing gases, such as free chlorine and certain organics (as indicated in Methods 7470 and 7471), should not interfere due to the release of decomposition products by the decomposition furnace, removal of some decomposition products by the decomposition catalyst, and the selective entrapment of mercury vapor on the amalgamator.

5.0 SAFETY

5.1 Refer to Chapter Three for a discussion on safety related references and issues.

5.2 Many mercury compounds are highly toxic if swallowed, inhaled, or absorbed through the skin. Extreme care must be exercised in the handling of concentrated mercury reagents. Concentrated mercury reagents should only be handled by analysts knowledgeable of their risks and of safe handling procedures.

6.0 EQUIPMENT AND SUPPLIES

6.1 The working scheme of the mercury analysis system is illustrated in Figure 2. The sample introduction device consists of a motorized support with a metal or metal alloy sample boat

that is appropriate for solids and liquids. An example of an appropriate boat would be made of nickel with a liquid capacity of 0.5 - 1.0 mL. Once the sample is either manually or automatically dispensed into the sample boat, the boat is mechanically introduced automatically into a quartz decomposition tube. The decomposition tube is heated by two independently programmable ovens, the decomposition and catalyst furnaces, each furnace is capable of maintaining a temperature of at least 750°C. The sample is dried and thermally decomposed in an oxygen environment, releasing mercury vapor. The mercury vapor is transported by oxygen over the amalgamator that traps the mercury. Once the sample is completely decomposed the trapped mercury is desorbed rapidly by heating the amalgamator with the mercury release furnace. The mercury vapor passes through two absorbance cuvette, in series, that are separated by a collection flask outside the optical axis. The flow path through the spectrometer and cuvettes is maintained at approximately 120°C, by a heating unit, to prevent condensation and minimize carry-over effects. A mercury vapor lamp is used as the light source. The detector is connected to a computer for data acquisition and analysis.

6.2 The DMA 80 automatic mercury analyzer (Milestone, Inc.) is the instrument used for the scheme outlined above. It has been tested for use with this method. Other instruments based on these principles may also be appropriate.

6.3 This method is not limited to mercury vapor generation by thermal decomposition. Alternatively, other mercury vapor introduction systems, such as mercury cold vapor generation, may be appropriate. Alternative sample introduction apparatus may be applied after validation with data similar to those in Tables 1 and 2.

6.4 This method is not limited to analyzing total mercury content. This detection scheme can be used for analysis of individual species of mercury that have been separated by an appropriate method or instrument system.

7.0 REAGENTS AND STANDARDS

7.1 Reagent water: Reagent water will be interference free. All references to water in this method refer to reagent water unless otherwise specified.

7.2 High purity oxygen gas: High purity oxygen should be interference and mercury free. If the oxygen is possibly contaminated with mercury vapor, a gold mesh filter should be inserted between the gas cylinder and the mercury analysis instrument to prevent any mercury from entering the instrument.

7.3 Mercury stock solution: Dissolve 0.1354 g of mercuric chloride in 75 mL of reagent water. Add 10 mL of concentrated nitric acid and adjust the volume to 100.0 mL (1.0 mL = 1.0 mg Hg). Stock solutions may also be purchased. Verify the quality of the standard by checking it against a second source standard (Sec. 9.2).

7.4 Mercury working standards: Make successive dilutions of the stock mercury solution to obtain standards containing 100 ppm and 10 ppm. For calibration of the high range, standards of 0, 1, 2, 3, 4, 5, and 6 ppm are recommended. These are prepared by dilution of the 100 ppm standard. For calibration of the low range, standards of 0.00, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 ppm are recommended. These are prepared by dilution of the 10 ppm standard. A blank calibration solution is also used for a zero calibration. Acidity of the working standards should be maintained at least 0.15% nitric acid, as also recommended in Methods 7470 and 7471.

NOTE: The concentrations listed above are only recommended concentrations. The concentration of the working standards may need adjustment according to specific instrumental working ranges and/or manufactures' recommendations.

NOTE: The stability of the mercury standards is limited to 24 - 48 hours. Fresh mercury standards must be prepared daily.

7.5 Standard reference material: In place of aqueous mercury standards, solid reference material with a certified value for mercury may by used for calibration.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 All samples should be collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

8.2 All sample containers must be prewashed with detergents, acids, and reagent water. Glass, plastic, and PTFE containers are suitable in most cases. Polymers are not suitable for samples containing metallic mercury.

8.3 Metallic mercury, some inorganic mercury compounds, and many organic mercury compounds are volatile and unstable. It is advantageous to analyze the samples as soon as possible to determine the total mercury in the sample but in no cases exceed the 28-day limit as defined in Chapter Three of this manual. Non-aqueous samples shall be analyzed as soon as possible. If solid samples are not analyzed immediately, refrigeration is necessary.

9.0 QUALITY CONTROL

9.1 All quality control data should be maintained and available for easy reference or inspection.

9.2 If more than 10 samples per day are analyzed, the working standard curve must be verified by measuring satisfactorily a mid-range standard or reference standard after every 10 samples. This sample value must be within 20% of the true value, or the previous 10 samples must be reanalyzed.

9.3 Matrix Spike/Matrix Spike Duplicates (MS/MSDs): At the laboratory's discretion, a separate spike sample and a separate duplicate sample may be analyzed in lieu of the MS/MSD. For each batch of samples processed, at least one MS/MSD sample must be carried throughout the entire sample preparation and analytical process as described in Chapter One. MS/MSDs are intralaboratory split samples spiked with identical concentrations of each analyte of interest. The spiking occurs prior to sample preparation and analysis. An MS/MSD is used to document the bias and precision of a method in a given sample matrix. Refer to the definitions of bias and precision, in Chapter One, for the proper data reduction protocols. MS/MSD samples should be spiked at the same level as the corresponding laboratory control sample that is at the project-specific action level or, when lacking project-specific action levels, between the low and midlevel standards. Acceptance criteria should be set at a laboratory derived limit developed through the use of historical analyses. In the absence of historical data this limit should be set at ± 20% of the spiked value for precision and ≤ 20 relative percent difference (RPD). After the determination of historical data, 20% must still be the limit of maximum deviation for both percent recovery and relative percent difference to express acceptability. Refer to Chapter One of this manual for guidance.

9.4 For each batch of samples processed, at least one method blank must be carried throughout the entire sample preparation and analytical process as described in Chapter One. A method blank is prepared by using a volume or weight of reagent water at the volume or weight specified in the preparation method and then carried through the appropriate steps of the analytical process. These steps may include but are not limited to digestion, dilution, filtering, and analysis. If the method blank does not contain target analytes at a level that interferes with the project-specific DQOs then the method blank would be considered acceptable. In the absence of project-specific DQOs, if the blank is less than the MDL or less than 10% of the lowest sample concentration for each analyte, whichever is greater, then the method blank would be considered acceptable. If the method blank cannot be considered acceptable, the method blank should be re-run once and if still unacceptable then all samples after the last acceptable method blank must be reprepped and reanalyzed along with the other appropriate batch QC samples. These blanks will be useful in determining if samples are being contaminated. Refer to Chapter One for the proper protocol when analyzing blanks.

9.5 For each batch of samples processed, at least one laboratory control samples must be carried throughout the entire sample preparation and analytical process as described in Chapter One. The laboratory control samples should be spiked with each analyte of interest at the project-specific action level or when lacking project-specific action levels, between the low and midlevel standards. Acceptance criteria should be set at a laboratory derived limit developed through the use of historical analyses. In the absence of historical data this limit should be set at ± 20% of the spiked value. After the determination of historical data, ±20% must still be the limit of maximum deviation to express acceptability. If the laboratory control sample cannot be considered acceptable, the laboratory control sample should be re-run once and if still unacceptable then all samples after the last acceptable laboratory control sample must be reprepped and reanalyzed. Refer to Chapter One for more information.

9.6 The method of standard additions can be used to verify linearity or if matrix interference is suspected. Refer to Method 7000 for standard addition procedures.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Primary calibration: 100 µL of a working standard is dosed onto the sample boat. Analytical parameters for drying, decomposition, and wait times as recommended by the manufacturer are chosen for the analysis (Section 11.1). Each standard solution is analyzed twice. For the DMA 80, parameters of 70 seconds drying, 100 seconds decomposition, and 40 seconds wait times (abbreviated 70/100/40) would be chosen for each standard analysis. Typical calibration curves obtained in laboratory conditions are illustrated in Figures 3a and b and a calibration curve obtained in field conditions is illustrated in Figure 4. Conduct curve using standards described in Section 7.4.

10.2 Daily calibration: At least a high and low concentration standard for each working range is analyzed using the analytical parameters as recommended by the manufacturer. The working calibration standards must be measured within 10% of their true value for the curve to be considered valid.

10.3 An alternative calibration using standard reference materials (SRMs) may be used. In this method, an amount of the reference material is weighed (accurate to ± 0.001 g or better) onto a tared sample boat. The analytical parameters chosen are based on the weight, moisture content, and organic content of the soil and should be as similar to the matrix of interest as possible (refer to Sec. 11.1). This procedure is repeated with several different weights of the

standard reference material containing mercury concentrations in the desired working range (see Figure 5).

> NOTE: Do not dry the standard reference material as indicated on the certificate of analysis unless the SRM was prepared and analyzed that way for mercury certification. Drying may result in loss of mercury that is thermally unstable. Drying a separate sample at the time of analysis and correcting for moisture content is appropriate.

11.0 PROCEDURE

11.1 General analytical parameters: the analytical parameters depend on the sample size and matrix and are instrument specific. The following table shows the guidelines given for the DMA 80. Consult the operating manual for manufactures' recommendations.

Analytical parameters as recommended by Milestone, Inc. for the DMA 80.

Sample Type	Maximum Capacity	Drying Time (s) [1]	Decomposition Time (s) [1]	Wait Time (s)
Aqueous	500 µL or 1000 µL[2]	= [0.7 s * vol. (µL)]	100	40
Solid (dry)	500 mg	10	= [0.4 s * wt. (mg) + 100 s]	40
Solid (moist)	500 mg	= [0.7 s * wt (mg) * % water content]	= [0.4 s * wt. (mg) + 100 s]	40
Solid (high organic content)	500 mg	= [0.7 s * wt (mg) * % water content]	100	40

[1] The variability of some matrices requires calculating the drying and decomposition times.
[2] Maximum sample size is dependent on the volume of the sample boat. Typical sample boat sizes are either 0.5 or 1.0 mL.

11.2 Sample analysis: For solids, a homogenized amount of the sample is weighed (to ± 0.001 g or better) onto a tared sample boat. The sample boat is inserted into the instrument with appropriate clean techniques. The analytical parameters chosen are based on the weight, moisture content, and organic content of the soil (refer to Sec. 11.1). For example, for 200 mg of sediment with a water content of 45%, the parameters for the DMA 80 would be: 63/180/40. For aqueous samples and previous prepared samples (using appropriate SW-846 3000 series methods), a known volume of the sample is dosed onto the sample boat (3, 4). The analytical parameters chosen are based on the volume of the sample dosed (refer to Sec. 11.1). For example, for 200 µL of prepared sample, the parameters for the DMA 80 would be: 140/100/40.

11.3 Field analysis: With a stable power supply, this method can be transported to the field for direct sample analysis without acid digestions.

11.4 Construct a calibration curve by plotting the absorbances of the standards versus nanograms of mercury. Determine the peak height or peak area of the sample from the chart and calculate the mercury value from the standard curve. Duplicates, spiked samples, and check standards should be routinely analyzed as detailed in Section 9.0 of this method. Samples exceeding the calibration range should be diluted and reanalyzed. Refer to Section 10.0 for additional guidance on calibrating the instrument.

12.0 DATA ANALYSIS AND CALCULATIONS

Calculate metal concentrations: (1) by the method of standard addition, (2) from a calibration curve, or (3) directly from the instrument's concentration read-out. All dilution or concentration factors must be taken into account. Concentrations reported for multiphased or wet samples must be appropriately qualified (e.g., 5 µg/g dry weight).

13.0 METHOD PERFORMANCE

13.1 This method has been validated with both solid samples and digests of solid samples. National Institute of Standards and Technology (NIST) Solid Standard Reference Materials (SRMs) were selected for there homogeneity and availability. The selected SRMs encompass various chemical forms of mercury, including biological forms, geological forms, and contaminated environmental forms. The SRMs were analyzed directly as the solid and as the digested sample as prepared by Method 3052. These results are summarized in Table 1.

Field capabilities of this instrumental method were tested. Direct analysis of various SRMs were performed in a field setting. A summary of the results is given in Table 2. Field analysis with this instrumental method resulted in the data in Table 2. Using this method randomly collected field soil samples were tested. A sample was collected and homogenized in approximately ten minutes and was analyzed in triplicate in an additional 15 minutes. Field data of randomly collected soil samples indicate that typical % RSD of less than 10% can be achieved, however this is dependent on many factors including concentration of mercury and homogeneity of the sample.

13.2 The following documents may provide additional guidance and insight on this method and technique:

13.2.1 Salvato, N. and Pirola, C.; Analysis of Mercury Traces by Means of Solid Sample Atomic Absorption Spectrometry. *Mikrochimica Acta.* Vol. 123, 63 - 71, 1996.

13.2.2 Walter, P.J., and Kingston, H.M.; "The Fate of Mercury in Sample Preparation", The Pittsburgh Conference, Atlanta, GA, March 1997, paper #1223.

13.2.3 Kingston, H.M., Walter, P.J., Chalk, S., Lorentzen E., and Link, D.; "Chapter 3: Environmental Microwave Sample Preparation: Fundamentals, Methods, and Applications" in *Microwave Enhanced Chemistry*; Kingston, H.M. and Haswell, S., Eds.; American Chemical Society, Washington DC, 1997.

13.2.4 Milestone, Inc., DMA 80 Operating Manual, 160B Shelton Rd., Monroe, CN 06468.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/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.

14.2 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.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits 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.

16.0 REFERENCES

1. Boylan, H.M., Walter, P.J., and Kingston, H.M.; "Direct Mercury Analysis: Field and Laboratory Validation for EPA Method 7473".

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain Tables 1 and 2, Figures 1 through 5, and a method procedure flow diagram.

TABLE 1

LABORATORY ANALYSIS RESULTS (MEAN ± 95% CONFIDENCE INTERVAL) OF DIRECT AND DIGESTED (METHOD 3052) ANALYSES OF VARIOUS NIST SRMs USING THE DMA 80 (MILESTONE, INC.)
(Ref. 1)

Standard Reference Material	Direct Analysis (ng/g)	Digested Sample Analysis (ng/g)	Certified Value (ng/g)
Apple Leaves NIST SRM 1515	48.3 ± 2.4	NA	44 ± 4
Citrus Leaves NIST SRM 1572	100 ± 12	97 ± 9	80 ± 20
Estuarine Sediment NIST SRM 1646	75.2 ± 4.9	65.7 ± 8.7	63 ± 12
Oyster Tissue NIST SRM 1566a	67.1 ± 3.2	NA	64.2 ± 6.7
Coal Fly Ash NIST SRM 1633b	139 ± 6	132 ± 12	141 ± 19
Buffalo River SedimentNIST SRM 2704	1,450 ± 24	1,450 ± 26	1,440 ± 70
Montana Highly Contaminated Soil NIST SRM 2710	33,100 ± 310	33,400 ± 230	32,600 ± 1,800

NA: Not analyzed
$n \geq 3$

TABLE 2

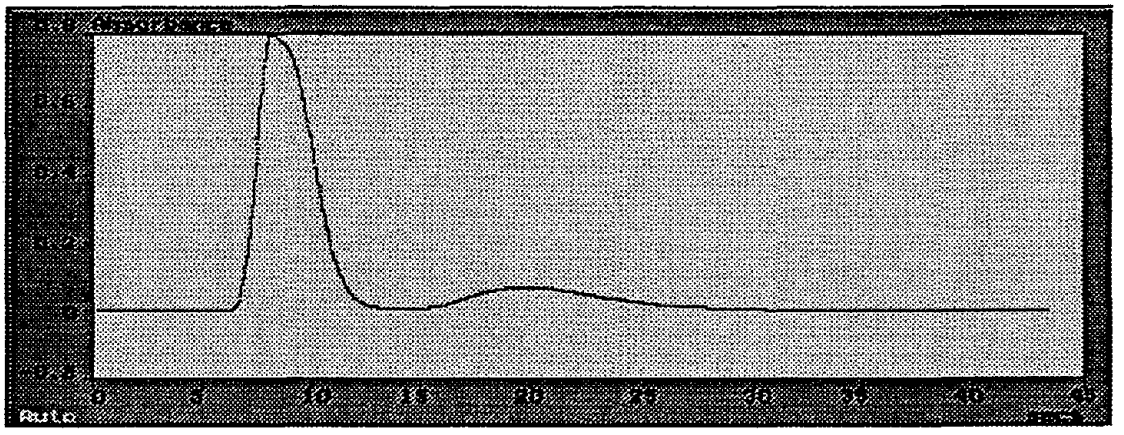
FIELD ANALYSIS RESULTS (MEAN ± 95% CONFIDENCE INTERVAL) OF DIRECT ANALYSES OF VARIOUS NIST SRMs USING THE DMA 80 (MILESTONE, INC.)
(Ref. 1)

Standard Reference Material	Direct Analysis (ng/g)	Certified Value (ng/g)
EstuarineSediment NIST SRM 1646	74.7 ± 2.4	63 ± 12
Oyster Tissue NIST SRM 1566a	68.0 ± 2.0	64.2 ± 6.7
Coal Fly Ash NIST SRM 1633b	139.2 ± 2.2	141 ± 19

FIGURE 1

SPECTRAL OUTPUT OF DMA 80

The two individual peaks correspond to the two absorbance cells of different sensitivities. The maximum intensity of the long pathlength cuvette (low range cell) occurs at ~8 seconds and the maximum intensity of the short pathlength cuvette (high range cell) occurs at ~20 seconds.



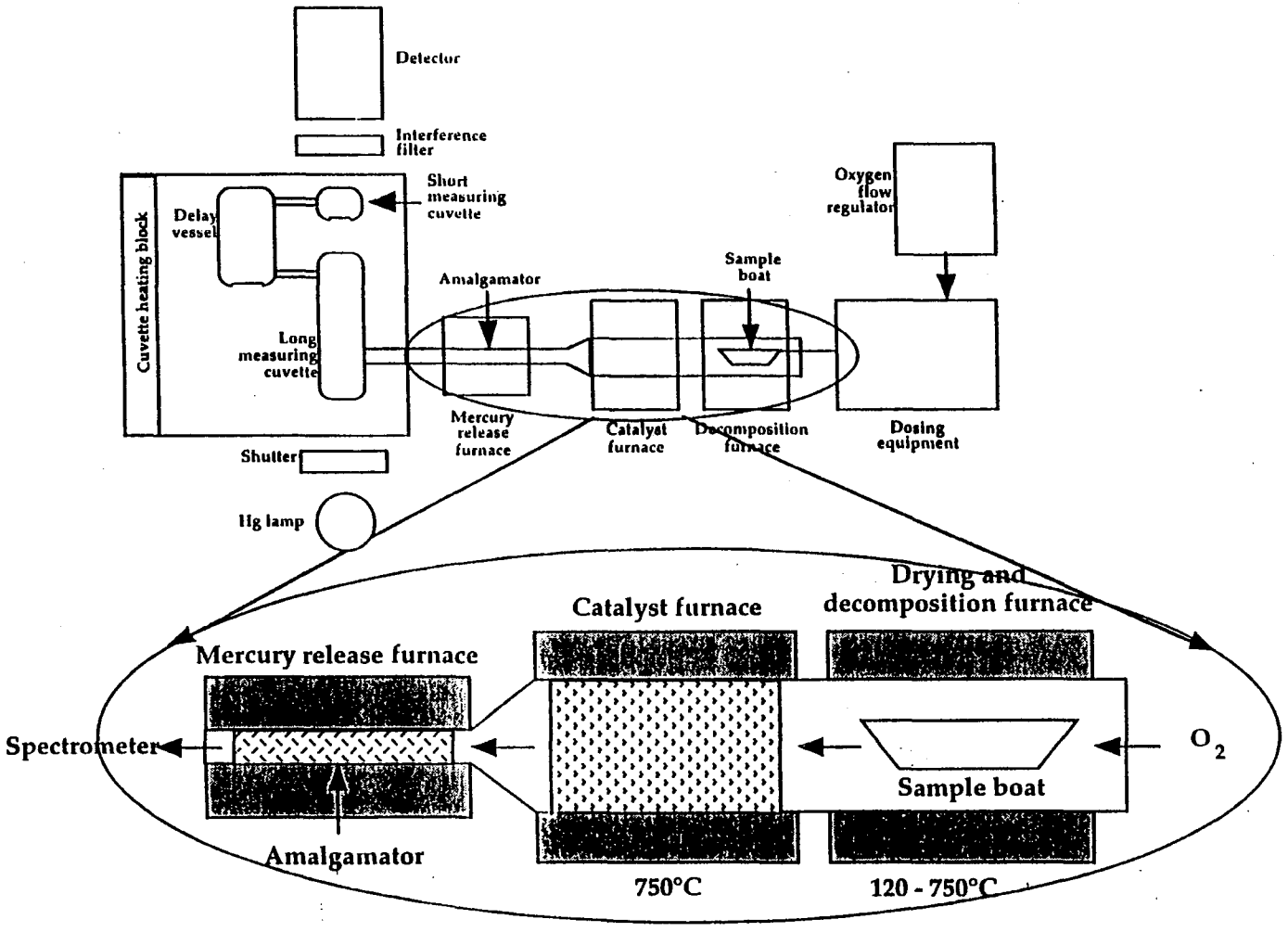


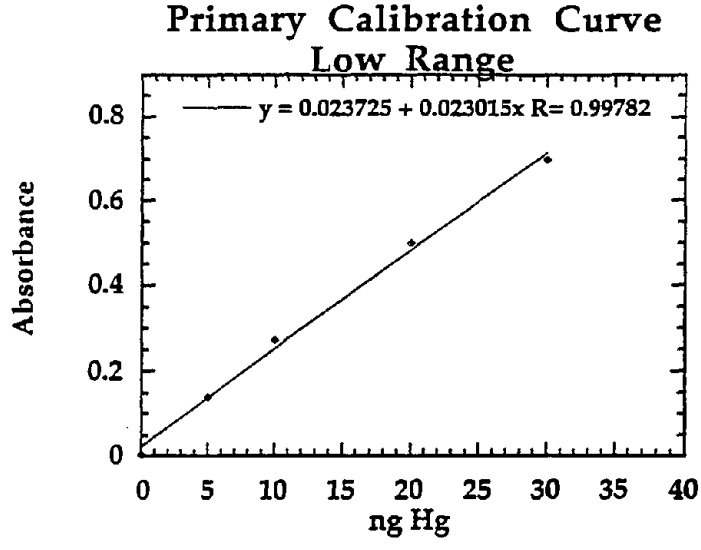
FIGURE 2

DIAGRAM OF THE MERCURY ANALYSIS SYSTEM

FIGURES 3a AND 3b

PRIMARY CALIBRATION CURVES USING THE DMA 80

The low range curve (3a) corresponds to the long pathlength cell. The high range curve (3b) corresponds to the short pathlength cell.



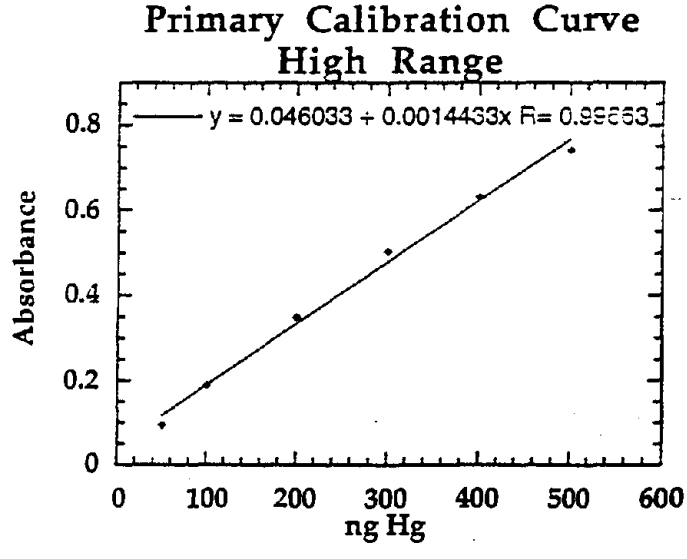


FIGURE 4

PRIMARY CALIBRATION CURVE USING THE DMA 80 IN FIELD ANALYSIS CONDITIONS

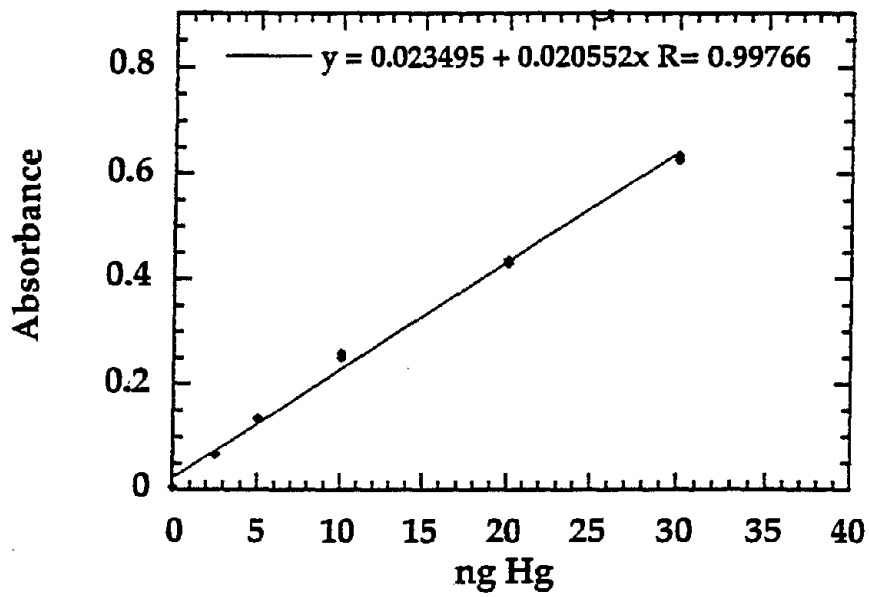
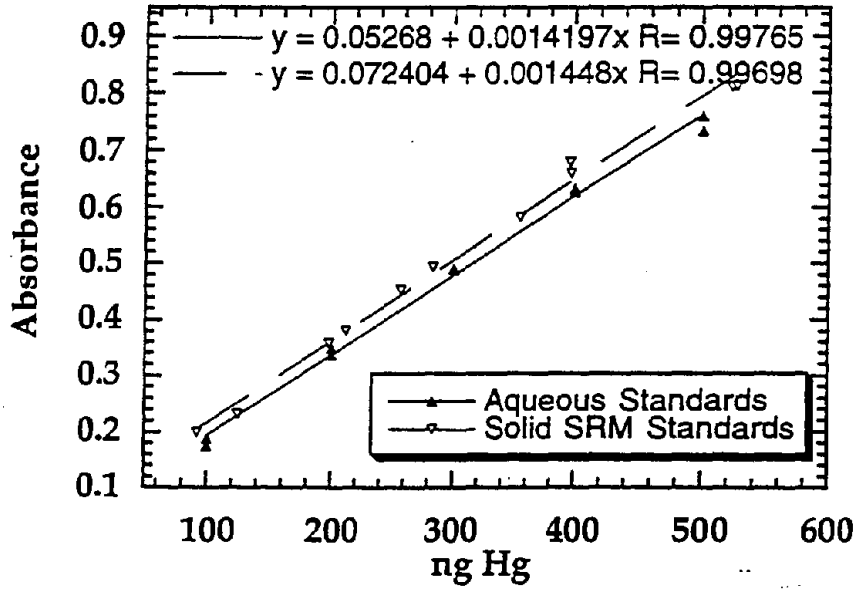
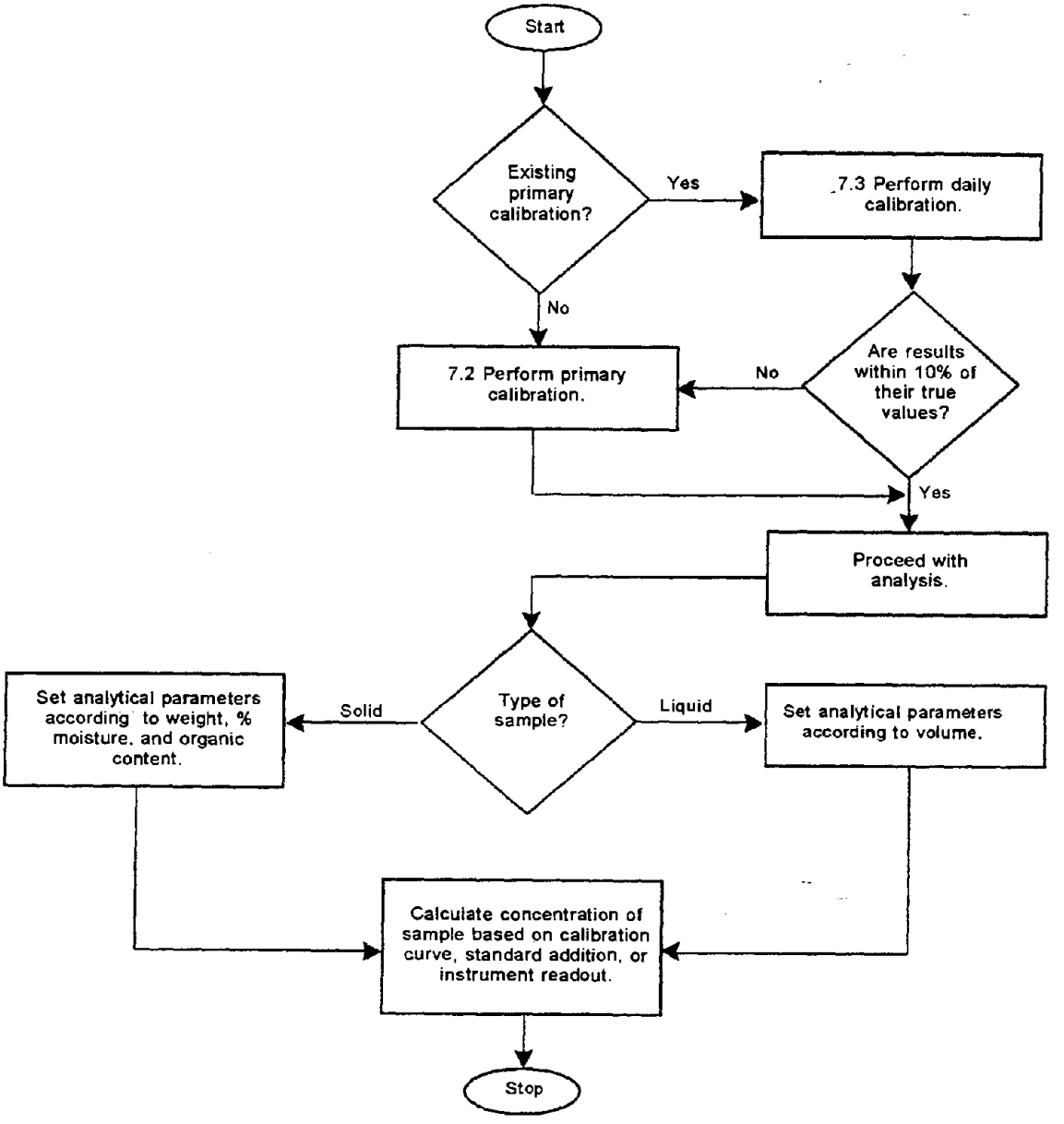


FIGURE 5

PRIMARY CALIBRATION CURVES USING THE DMA 80 -
COMPARISON OF THE CALIBRATION USING AQUEOUS STANDARD SOLUTIONS AND
SOLID NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY STANDARD
REFERENCE MATERIAL 2704 (BUFFALO RIVER SEDIMENT)



MERCURY IN SOLIDS AND SOLUTIONS BY THERMAL DECOMPOSITION, AMALGAMATION, AND ATOMIC ABSORPTION SPECTROPHOTOMETRY



METHOD 7474

MERCURY IN SEDIMENT AND TISSUE SAMPLES BY ATOMIC FLUORESCENCE SPECTROMETRY

1.0 SCOPE AND APPLICATION

1.1 This procedure measures total mercury in sediment and tissue samples.

1.2 The range of this method is from approximately 1 part per billion to the part per million range. Analysis of the entire range cannot be accomplished at once, but rather different portions of this range can be analyzed depending upon the instrument gain settings.

1.3 This method should only be used by analysts experienced with the analysis of trace elements at very low concentrations when analyzing samples in the ppb range.

2.0 SUMMARY OF THE METHOD

2.1 A representative portion of sample is digested in a microwave unit (a variation of Method 3052) using nitric and hydrochloric acids in a closed fluorocarbon container. The sample is digested under pressure to aid in the dissolution of organic compounds containing mercury.

2.2 An aliquot of the digested sample is diluted and subjected to cold digestion with an acid/bromate/bromide mixture.

2.3 Stannous chloride is added to the digested sample as a reducing agent to produce Hg^0. The reduced mercury is separated from the sample/reagent mixture as a vapor that is carried to the fluorescence detector by a stream of high purity argon.

3.0 DEFINITIONS

Refer to Chapter One and Chapter Three for a listing of applicable definitions.

4.0 INTERFERENCES

4.1 Method 3052 will overcome the problems associated with incomplete digestion. Incomplete digestion may lead to the incomplete solubilization of sparingly soluble Hg compounds.

4.2 High purity argon (99.999%) must be used as the carrier gas. Nitrogen will reduce the sensitivity by a factor eight-fold, while the use of air will reduce the sensitivity thirty-fold.

4.3 The presence of water vapor in the fluorescence detector may produce scattering effects, positive interferences and degradation of the analytical signal. The use of a dryer tube is required to remove any water vapor from the flow before reaching the detector.

4.4 Contamination is always a potential problem in trace element determinations. See Chapter Three for clean laboratory procedures.

5.0 SAFETY

5.1 Refer to Chapter Three for a discussion on safety related references and issues.

5.2 Many mercury compounds are highly toxic if swallowed, inhaled, or absorbed through the skin. Extreme care must be exercised in the handling of concentrated mercury reagents. Concentrated mercury reagents should only be handled by analysts knowledgeable of their risks and of safe handling procedures.

6.0 EQUIPMENT AND SUPPLIES

6.1 Atomic fluorescence system:

6.1.1 Autosampler (optional) - A multi-position computer controlled autosampler may be used. However, it is essential that the autosampler have a wash cycle or "wash pot" to rinse the probe between sampling positions. The autosampler wash water should closely approximate the wash water (Section 7.13) in acid strength.

6.1.2 Peristaltic pump - A three channel peristaltic pump that can deliver reagents and sample at flow rates up to 10 mL/min by varying the pump speed, the pump tubing, or both is required. Silicone pump tubing is required for ppt determinations as PVC pump tubing has been found to adsorb mercury.

6.1.3 Solenoid switching valve - A software controlled valve is required to switch between wash and sample at the proper intervals in the analysis cycle.

6.1.4 Mass flow controllers - Mass flow controllers are required for the carrier and sheath gas flows when analyzing near the detection limit where maximum stability of conditions is critical.

6.1.5 Gas liquid separator - A gas-liquid separator is required to sparge reduced mercury from the liquid stream, direct the mercury vapor and argon carrier gas to the fluorescence detector, and direct the liquid reagents to waste.

6.1.6 Dryer tube - A dryer tube is to be placed in line between the gas-liquid separator and the detector to remove water vapor from the carrier gas stream. Any dryer tube which does not degrade the analysis or sensitivity is acceptable. (PermaPure MD-250-12 or equivalent.)

6.1.7 Fluorescence detector - A fluorescence detector with a high intensity mercury light source and a photomultiplier tube at a right angle to the source is required. Use of 254 nm filter coupled with the chemistry of the stannous chloride reduction in the vapor generator/gas-liquid separator makes the detector highly specific for mercury.

6.1.8 Computer controller - A computer controller and software is required to operate and coordinate the various components of the system and acquire the data as it is produced.

6.1.9 Argon gas supply - High purity argon (99.999%) is required. A gas purifier cartridge is also recommended.

6.1.10 Microwave apparatus. Refer to Method 3051 for a description of an appropriate microwave digestion apparatus.

6.2 Data systems recorder - A recorder is recommended so that there will be a permanent record and that any problems with the analysis can be easily recognized.

6.3 Pipets - Microliter, with disposable tips. Pipet tips should be checked as a possible source of mercury contamination prior to their use. Class A pipets can be used for the measurement of volumes equal to or larger than 1 mL.

6.4 Glassware - All glassware, vessels, pipets, etc., must be very clean. Glass, plastic, and fluorocarbon polymer (PFA or TFM) containers may be used but polymers are not suitable for samples containing metallic mercury. The following is an example of a cleaning procedure successfully used in a trace level laboratory. Soak glassware overnight in a cleaning solution (such as Micro®). Rinse four times with Type I water (ASTM Type I water) and soak overnight in an acid/bromate/bromide mixture. The acid/bromate/bromide mixture is made by adding the bromate/bromide solution from Section 7.9 to dilute acid (approximately 5% v/v) until a yellow color forms (the exact composition is not critical). The container should be covered or closed, as an open container will pick up mercury from the atmosphere and permit bromine vapor to escape to the air. After soaking overnight, add enough 5% hydroxylamine from Section 7.10 to eliminate the yellow color. Rinse six times with Type I water. Cap the glassware tightly if it is not to be used immediately. Store in a reduced mercury atmosphere.

When running samples on a daily basis, vessels require the rigorous cleaning procedure described above every 5 to 7 uses. In between and after each use, the vessels should be soaked in cleaning solution for 2 hours to loosen deposits. They are then cleaned thoroughly with cotton swabs (tested for mercury contamination) and soaked again in cleaning solution overnight. Rinse six times with reagent water. Repeat the soaking and rinsing steps if necessary.

6.5 Balance - A top-loader balance with an accuracy of $\pm$ 0.01 g is required.

6.6 Muffle furnace - A muffle furnace capable of reaching and maintaining a temperature of 150°C is required for purifying the potassium bromate and potassium bromide reagents.

7.0 REAGENTS AND STANDARDS

7.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. All reagents should be analyzed to provide proof that all constituents are below the MDLs.

7.2 Reagent water: All references to water in this method refer to reagent water unless otherwise specified. Refer to Chapter One for a definition of reagent water.

7.3 Nitric acid, HNO_3: Use a trace metal grade with negligible mercury content. If the reagent blank is less than the MDL, the acid may be used.

7.4 Hydrochloric acid, HCl: Use a trace metal grade with negligible mercury content. If the reagent blank is less than the MDL, the acid may be used.

7.6 Mercury stock standard solution: A mercury stock solution should be purchased from a reputable source with a concentration of 1.0 mg Hg/mL.

7.7 Potassium bromate (CAS 7758-01-2): Volatilize trace mercury impurities by heating in a muffle furnace at 150°C for at least 8 hours. This procedure is recommended every time the compound is used but if it is stored in a desiccator, ensure that is not contaminated prior to use.

7.8 Potassium bromide (CAS 7758-02-3): Volatilize trace mercury impurities by heating in a muffle furnace at 150°C for at least 8 hours.

7.9 Bromate/Bromide solution: Dissolve 1.39 g potassium bromate and 5.95 g potassium bromide in 500 mL reagent water. Prepare weekly.

7.10 Hydroxylamine hydrochloride (CAS 5470-11-1): Use a source that is specified as suitable for mercury analysis.

7.11 Hydroxylamine hydrochloride solution (5% w/v): Dissolve 2.5 g hydroxylamine in 50 mL of reagent water. Prepare weekly.

7.12 Stannous chloride solution (CAS 10025-69-1), 2% in 10% HCl: Add approximately 500 mL of reagent water to a 1L volumetric flask followed by the addition of 100 mL conc. HCl. Add 20.0 g stannous chloride and stir to dissolve. Dilute to 1 L with reagent water. The solution should be sparged with argon for 30 minutes prior to analysis to remove any traces of mercury. Prepare daily.

7.13 Wash water (reagent blank), 5% HCl: Add approximately 1000 mL of reagent water to a 2 L flask. Add 100 mL conc. HCl and 80 mL of the bromate/bromide solution (Section 7.9). Bring to volume with reagent water. Prepare daily.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 See Chapter Three, Inorganic Analytes.

9.0 QUALITY CONTROL

9.1 All quality control data should be maintained and available for easy reference or inspection.

9.2 For each batch of samples processed, method blanks must be carried throughout the entire sample preparation and analytical process according to the frequency described in Chapter One. A method blank is prepared by using a volume or weight of reagent water at the volume or weight specified in the preparation method and then carried through the appropriate steps of the analytical process. These steps may include but are not limited to digestion, dilution, filtering, and analysis. These blanks will be useful in determining if samples are being contaminated. Refer to Chapter One for the proper protocol when analyzing blanks.

9.3 Matrix Spike/Matrix Spike Duplicates (MS/MSDs): MS/MSDs are intralaboratory split samples spiked with identical concentrations of target analytes. The spiking occurs prior to sample preparation and analysis. An MS/MSD is used to document the bias and precision of a method in a given sample matrix. MS/MSDs are to be analyzed at the frequency of one per analytical batch as described in Chapter One. Refer to the definitions of bias and precision, in Chapter One, for the proper data reduction protocols. MS/MSD samples should be spiked at the project-specific action level or when lacking project-specific action levels, between the low and midlevel standards. Acceptance criteria should be set at a laboratory derived limit developed through the use of historical analyses. In the absence of historical data this limit should be set at ± 20% of the spiked value for precision and ≤ 20 relative percent difference (RPD). After the determination of historical data, 20% must still be the limit of maximum deviation for both percent recovery and relative percent difference to express acceptability.

9.4 For each batch of samples processed, laboratory control samples must be carried throughout the entire sample preparation and analytical process according to the frequency of one per analytical batch as described in Chapter One. The laboratory control samples should be spiked at the project-specific action level or when lacking project-specific action levels, between the low and midlevel standards. Acceptance criteria should be set at a laboratory derived limit developed through the use of historical analyses. In the absence of historical data this limit should be set at ± 20% of the spiked value. Refer to Chapter One for more information.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Calibration standards - All analyses require that a calibration curve be prepared to cover the appropriate concentration range. Calibration standards are prepared by diluting the stock metal solutions at the time of analysis and digesting them using the same procedure used for actual samples. If running more than one batch of samples during the same week, the microwave-digested standards can be kept in clean dedicated glassware from which dilutions can be made daily which are then prepared with acid and bromate/bromine just as the samples are prepared after microwaving.

10.1.1 Calibration standards must be prepared fresh (or from the weekly microwave digest described in 10.1) each time a batch of samples is analyzed. Prepare a reagent blank and at least three calibration standards in graduated amounts in the appropriate range of the linear part of the curve.

10.1.2 The calibration standards should be prepared using the same type of bromine, acid or combination of acids and at the same concentration as will result in the samples following processing.

10.2 A calibration curve must be prepared each day with a minimum of a reagent blank and three standards. After calibration, the calibration curve must be verified by use of at least a calibration blank and a check standard (made from a reference material or other independent standard material) at or near the mid-range. The calibration curve must also be verified at the end of each analysis batch and/or after every ten samples. The calibration check standard must be measured within 20% of its true value for the curve to be considered valid.

10.3 The working standard curve must be verified by measuring satisfactorily a mid-range standard or check standard and a reagent blank at the end of each analysis batch and/or after every 10 samples. This sample value must be within 10% of the true value, or the previous ten samples must be reanalyzed. The reagent blank must be less than the MDL. If the aforementioned criteria are not met, reanalyze the samples analyzed since the last passing calibration check and calibration blank.

11.0 PROCEDURE

11.1 Prepare samples in a microwave unit using only nitric and hydrochloric acids. Follow instrument manufacturers instructions.

11.1.2 Transfer approximately 1.0 gram of wet sample to a digestion vessel. Add 2.0 mL of concentrated nitric and 6.0 mL of concentrated hydrochloric and cap.

11.1.3 Microwave the samples with a program appropriate for complete digestion. Typically, the temperature should be ramped slowly to 190°C without overpressurization and held at 190°C for 10 minutes or until the digestion is complete.

11.1.4 Cool the samples. During the cooling period, vent and swirl the samples occasionally to release dissolved gases. After the samples have cooled and the dissolved gases have been dissipated, transfer 1.0 mL of the digested sample into a graduated 50 mL centrifuge tube that contains reagent water, 2.0 mL bromate/bromide solution (Sec. 7.9) and 2.5 mL HCl. Bring to volume with reagent water and cold digest for 15 minutes. Document the accuracy of the centrifuge tubes through mass/volume records or use volumetric glassware.

11.2 Set up a software controlled timing sequence for the analysis. Follow your instrument manufacturer's instructions for all settings. Timing sequences that should be addressed are:

11.2.1 Delay: The delay step allows times for the sample line to fill with sample.

11.2.2 Rise: The rise step allows sample to enter the gas/liquid separator and react with the stannous chloride. Mercury in the digested sample is reduced to Hg^0 and the argon carrier stream carries the Hg^0 as mercury vapor to the detector.

11.2.3 Analysis: The analysis time allows for the peak height to rise to its maximum while the software measures the peak height or area.

11.2.4 Memory: The memory time allows the signal to return to the baseline level.

11.2.5 Set the gas flows at a level providing adequate sensitivity for the desired analytical range. Flow rates for the following gases should be established: carrier gas, sheath gas, and dryer tube gas.

11.2.6 When using a variable speed peristaltic pump, choose appropriate sized tubing to obtain an approximate ratio of 2:1 between sample/wash (Sec. 7.13) flow rates and the reductant (Sec. 7.12) flow rate.

11.2.7 Set the gain on the detector to the sensitivity range required for the analysis.

11.2.8 If an autosampler is used, set up a wash solution for the autosampler probes. The autosampler wash solution should closely approximate the wash water (Sec. 7.13) in acid concentration or contain acid at a sufficient strength (typically 5%) to preclude any sample carryover.

11.2.9 Allow 30 minutes for the system to equilibrate before initiating sample analysis.

11.3 Sample Analysis

11.3.1 Add 0.10 mL of the hydroxylamine solution (Sec. 7.11) to remove excess bromine and decolorize the sample.

11.3.2 Allow precipitate or sediment in diluted samples to settle to avoid fouling the valves with solid material during analysis.

11.3.3 Any sample that gives a response greater than the highest standard must be diluted and rerun. Add appropriate amounts of reagents to ensure the reagent concentration of the diluted sample match that of the other samples and the wash (Sec. 7.13).

11.3.4 Any samples that fall outside the laboratory calculated and derived QC range must be re-digested and reanalyzed.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Maintain an instrument log book that contains all information necessary to reproduce the analytical conditions associated with a sample run.

12.2 Sample calculation:

$$\text{Hg in } \mu g/Kg = \frac{\text{result ng Hg}}{\text{L}} \times \frac{\text{0.05 L final vol.}}{\text{0.001 L dig sample}} \times \frac{\text{0.008 L dig soln.}}{\text{g sample (x \%solids)}}$$

13.0 METHOD PERFORMANCE

13.1 Accuracy: Results for accuracy from the US EPA Region IV laboratory are given in Table 1. Data for liquid reference materials and liquid calibration checks used with this procedure are included along with the sediment and tissue materials.

13.2 Precision: Results for precision from the Region IV laboratory are provided in Table 2.

13.3 The laboratory for which accuracy and precision data are presented here also participated in three intercomparison studies.

13.3.1 In the first study, two sediments and two tissues were analyzed for the National Oceanic and Atmospheric Administration in the Seventh Round Intercomparison for Trace Metals in Marine Sediments and Biological Tissues.

13.3.1.1 The accepted value for the sample identified as Sediment T is 0.107 mg/Kg with an acceptable range of 0.087 to 0.127. The value reported from this laboratory was 0.100 with a standard deviation of 0.003 and percent relative standard deviation of 3.0. The number of labs reporting results for this sample was 32.

13.3.1.2 The accepted value for the sample identified as BCSS-1 is 0.163 mg/Kg with an acceptable range of 0.096 to 0.230. The value reported from this laboratory was 0.199 with a standard deviation of 0.013 and percent relative standard deviation of.3. The number of laboratories reporting results for this sample was 28.

13.3.1.3 The accepted value for the tissue sample identified as Tissue S is 0.0618 mg/Kg with an acceptable range of 0.0409 to 0.0827. The value reported from this laboratory was 0.0574 with a standard deviation of 0.0047 and percent relative standard deviation of 8.3. The number of laboratories reporting results for this sample was 33.

13.3.1.4 The certified value for the tissue sample identified as NIST 1566a is 0.0642 mg/Kg with an acceptable range of 0.0575 to 0.0709. The value reported from this laboratory was 0.0631 with a standard deviation of 0.0042 and

percent relative standard deviation of 6.8. The number of laboratories reporting results for this sample was 27.

13.3.2 Two sediments and two tissues were analyzed in the Eighth Round Intercomparision for Trace Metals in Marine Sediments and Biological Tissues for the National Oceanic and Atmospheric Administration.

13.3.2.1 The accepted value for the sediment sample identified as Sediment U is 0.55 mg/Kg with an acceptable range of 0.42 to 0.68. The value reported from this laboratory was 0.63 with a standard deviation of 2.7. The number of laboratories reporting results for this sample was 28.

13.3.2.2 The accepted value for the sediment sample identified as BCSS-1 is 0.180 mg/Kg with an acceptable range of 0.109 to 0.251. The value reported from this laboratory was 0.23 with a standard deviation of 0.01 and percent relative standard deviation of 5.7. The number of laboratories reporting results for this sample was 26.

13.3.2.3 The accepted value for the tissue sample identified as Tissue V is 0.0654 mg/Kg with an acceptable range of 0.0462 to 0.0846. The value reported from this laboratory was 0.058 with a standard deviation of 0.006 and percent relative standard deviation of 8.0. The number of laboratories reporting results for this sample was 32.

13.3.2.4 The certified value for the tissue sample identified as NIST 1566a is 0.0654 mg/Kg with an acceptable range of 0.0587 to 0.0721. The value reported from this laboratory was 0.066 with a standard deviation of 0.003 and percent relative standard deviation of 5.2. The number of laboratories reporting results for this sample was 28.

13.3.3 Ten sediment samples from the Florida Everglades were analyzed and the results from two laboratories are presented in Table 3.

13.4 Comparison data for this method (CVAF) versus cold vapor atomic absorption generated within the Region IV laboratory are presented in Tables 4 and 5. Attention should be drawn to the fact that the lowest results in Table 5 are near the limits of detection for the CVAA method, but well within the CVAF range, while the higher results are obtained by diluting samples for the CVAF method, but are well within the range for the CVAA method. Therefore, the CVAA method may be more appropriate for the samples with higher levels of mercury and the CVAF method is more appropriate for lower level samples.

13.5 The following documents may provide additional guidance and insight on this method and technique:

13.5.1 W. Van Delft and G. Vos, *Analytica Chimica Acta*, 209 (1988) 147-156.

13.5.2 "Yorkshire Water Methods of Analysis", 5th Edition, 1988. (ISBN 090507236)

13.5.3 "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

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

13.5.5 "Proposed OSHA Safety and Health Standards, Laboratories", Occupational Safety and Health Administration, FR July 24, 1986.

13.5.6 1985 Annual Book of ASTM Standards, Vol. 11.01; "Standard Specification for Reagent Water"; ASTM: Philadelphia, PA, 1985; D1193-77.

13.5.7 Standard Methods, 18th Edition, 1992.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/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.

14.2 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, Department of Government Relations and Science Policy, 1155 16th Street, NW, Washington, D.C. 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits 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 Section 14.2.

16.0 REFERENCES

1. "Method for Total Mercury in Drinking Water, Surface, Ground, Industrial and Domestic Waste Waters and Saline Waters", P.S. Analytical Ltd., Sevenoaks, Kent, U.K.

2. "Method for the Determination of Ultra Trace Level Total Mercury in Sediment and Tissue Samples by Atomic Fluorescence Spectrometry", EPA Region IV.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The following pages contain Tables 1 through 5 and a method procedure flow diagram.

TABLE 1

RECOVERY DATA FOR SEDIMENT AND TISSUE REFERENCE MATERIALS, SPIKES, METHODS CHECKS (MC), AND CALIBRATION CHECKS

Matrix	True value[1]	Avg. % Rec.	# of samples	Std dev
NIST 8406 SED	60 µg/Kg	103.3	70	9.3
NRCC BEST1 SED	92.0 µg/Kg	100.9	21	7.4
Sediment spike	22.7 to 68.9 µg/Kg	89.2	38	13.7
NIST 1575 Plant tissue	150 µg/Kg	95.6	13	7.5
NIST 1566 Oyster tissue	84.2 µg/Kg	96.7	72	12.3
NRCC DORM-1 Fish tissue	798 µg/Kg	108	9	8.1
NBS 1641B Inorg water (MC)	38 to 60.8 ng/L	95.6	15	6.4
NBS 1641C Inorg water (MC)	36.8 to 73.5 ng/L	105.6	6	4.3
EPA WS024 Inorg+org water	43.2 to 108 ng/L	88.2	16	7.0
EPA WS029 Inorg+org water	10.1 to 50.6 ng/L	97.3	45	7.3
EPA WS030 Inorg+org water	43.2 ng/L	100.6	24	8.6
EPA WS031 inorg+org water	9.08 to 45.4 ng/L	100.8	49	11.1
Calib checks water	20.0 to 100 ng/L	101.5	139	4.2

[1]True values analyzed at various dilutions.
Source: Reference 2

TABLE 2

PRECISION DATA FOR SEDIMENT AND TISSUE SAMPLES[1]

Replicates	Avg %RSD	# Of samples	Std dev
Sediment original	10.2	280	9.2
Sediment duplicate	8.8	43	8.8
Sediment original vs duplicate	7.5	43	7.0
Tissue original	11.4	61	10.5
Tissue duplicate	12.0	13	10.6
Tissue original vs duplicate	5.7	13	4.7

[1]All samples analyzed twice.
Source: Reference 2.

TABLE 3

EVERGLADES SEDIMENT SAMPLE COMPARISON DATA (µg/Kg)

Sample	Reference Laboratory			Region IV Laboratory	
	Rep 1	Rep 2	Fluorescence Rep 1	Fluorescence Rep 2	Method 245.5
1	11	13	8.6	10.3	
2	6		5.6	5.4	< 25
3	76		76	73	68
4	58		63	64	59
5	410		458	444	424
6	296		501	463	453
7	42		48	46	
8	40		47	58	
9	36		37	37	
10	34	40	42	39	

Source: Reference 2

TABLE 4

COMPARISON OF CVAF METHOD VS. CVAA METHOD 245.5 RESULTS
ON EVERGLADES SEDIMENTS, PEAT AND MARL (µg/Kg)

SAMPLE	AVG	%RSD	CVAA	CVAA %RSD
1	169	2.35	148	11.8
2	197	0.35	248	20.3
3	89	8.04	46	56.6
4	64	6.21	48	26.0
5	28	3.09	23	16.4
6	56	3.76	47	15.4
7	97	2.88	75	22.6
8	172	0.11	161	5.7
9	79	7.89	71	10.0
10	75	4.52	68	8.3
11	79	0.70	76	2.8
12	99	1.54	81	18.1
13	67	1.97	58	12.8
14	146	19.25	130	10.6
15	63	0.52	59	6.3
16	157	0.10	140	10.7
17	144	2.37	144	0.2
18	450	2.55	424	5.5
19	482	6.99	453	5.6
20	247	2.89	226	7.7
21	161	1.97	195	17.8
22	108	4.76	118	7.6
23	278	15.96	356	22.0
24	278	13.71	175	40.0
25	273	2.68	209	23.5
26	456	7.79	285	41.1
27	136	6.83	128	5.42
28	263	3.95	215	17.9
29	89	0.75	80	9.3
30	108	4.79	119	8.4

Source: Reference 2

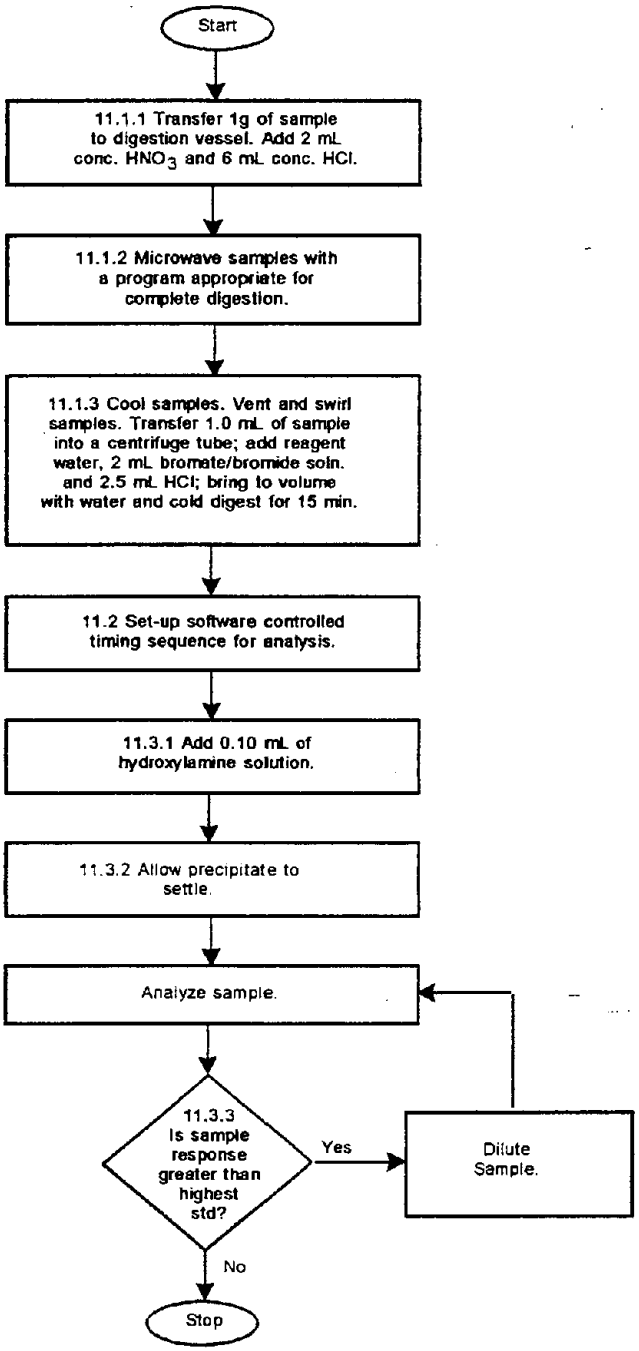
TABLE 5

COMPARISON OF CVAF METHOD VS. CVAA METHOD 245.5 RESULTS
ON TISSUE SAMPLES (µg/Kg)

Sample	Avg	% RSD	CVAA	%RSD CVAA
Alligator Liver 1	802	18.7	950	15.0
Alligator Liver 2	133	19.9	170	21.1
Alligator Liver 3	216	5.2	230	5.5
Alligator Liver 4	62.7	4.6	72	11.7
Gambusia 1	38.2	6.4	66	47.4
Gambusia 2	165	26.7	120	28.2
Mixed Fish Comp.	70.1	10.6	74	3.4
Bass Filet 1	460	22.2	370	19.4
Bass Filet 2	987	5.5	910	7.2
Bass Filet 3	559	15.9	560	0.2
Bass Filet 4	274	52.8	202	26.9
Bass Filet 5	172	2.6	155	9.5
Catfish Filet 1	62.2	12.3	80	22.3
Catfish Filet 2	133	0.5	150	10.4
Catfish Filet 3	119	7.1	130	7.9
Bluegill Filet 1	36.4	5.1	37	1.6
Bluegill Filet 2	53.7	0.0	36	35.1
Clam Tissue 1	28.5	87.1	25	11.8
Clam Tissue 2	21.1	11.6	30	31.0
Clam Tissue 3	17.6	12.2	30	46.3

Source: Reference 2

MERCURY IN SEDIMENT AND TISSUE SAMPLES BY ATOMIC FLUORESCENCE SPECTROMETRY



METHOD 9000

DETERMINATION OF WATER IN WASTE MATERIALS
BY KARL FISCHER TITRATION

1.0 SCOPE AND APPLICATION

1.1 The Karl Fischer titration technique is capable of quantifying the water content of materials from 1 ppm to nearly 100%. Coulometric titration is used for direct analysis of samples with water contents between 1 ppm and 5%, while volumetric titration is more suitable for direct analysis of higher levels (100 ppm to 100%). With proper sample dilution, the range of the coulometric technique can also be extended to 100% water. Both coulometric and volumetric procedures are presented.

1.2 Multiphasic samples should be separated into physical phases (liquid, solid, etc.) prior to analysis to assure representative aliquots are analyzed.

1.3 Establishing the water content in a sample may be useful for the reasons to follow.

1.3.1 It is useful in determining the total composition of a sample. In combination with other analytical results, the mass balance of a sample can be determined.

1.3.2 It is useful in identifying which samples can be analyzed by Infrared Spectroscopy using sodium chloride cells or which require zinc selenide cells.

1.3.3 It is useful in determining the amount of alcohol in an aqueous solution.

1.3.4 It is useful when distinguishing an aqueous from a nonaqueous solution.

1.3.5 It is useful when setting the proper mixture of feed materials in the incineration of waste.

2.0 SUMMARY OF METHOD

2.1 In the volumetric procedure, the sample or an extract of it, is added to a Karl Fischer solvent consisting of sulfur dioxide and an amine dissolved in anhydrous methanol. This solution is titrated with an anhydrous solvent containing iodine. The iodine titrant is first standardized by titrating a known amount of water.

2.2 In the coulometric procedure, the sample or an extract of it, is injected into an electrolytic cell containing the Karl Fischer solvent, where the iodine required for reaction with water is produced by anodic oxidation of iodide. With this technique, no standardization of reagents is required.

2.3 In both procedures, the endpoint is determined amperometrically with a platinum electrode that senses a sharp change in cell resistance when the iodine has reacted with all of the water in the sample.

2.4 In the coulometric procedure, the coulombs of electricity required to generate the necessary amount of iodine are converted to micrograms of water by the instrument microprocessor, while in the volumetric procedure, the volume of iodine titrant required to reach the endpoint is

converted to micrograms of water. Most instruments will also calculate concentration (ppm or percent) if the sample weight is keyed in.

3.0 DEFINITIONS

Refer to Chapter One and Chapter Three for a listing of applicable definitions.

4.0 INTERFERENCES

4.1 Interfering side-reactions can occur between the various species in the Karl Fischer reagent and the sample components, resulting in an overestimation of the water content in the sample.

4.1.1 Hydroxide ions will titrate as water when injected directly into the titration cell. This is a significant problem with samples having a pH > 14. When this is suspected to be a problem, a water vaporization module (furnace) should be used. The sample is heated in this module and the water vapor carried to the titration cell, while the hydroxide remains in the module.

4.1.2 Ketones can interfere with some Karl Fischer reagents by reacting with alcoholic solvents like methanol to form ketals and acetals which can decompose to form water. This problem can be avoided by substitution of a non-reactive alcohol or increasing the pH.

4.1.3 The reduction of iodine by oxidizable species such as thiols, ammonia and thiosulfate results in the consumption of iodine and an overestimation of the water content.

4.2 Undesired interfering side-reactions can also result in the underestimation of the water content in the sample. These include:

4.2.1 Sulfur dioxide, base, carbonyl functional groups on aldehydes and ketones and other substances that form bisulfite complexes.

4.2.2 Oxidation of iodide and bisulfite complexes.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis.

5.2 Care should be taken in avoiding the inhalation of the reagent vapors or skin contact with the reagents. If any of the reagents comes in contact with the skin, wash thoroughly with copious amounts of water. To avoid inhalation of vapors, fill and empty the cell or electrode assembly in a working laboratory hood. Once the cell is assembled, solvent vapors are contained so long as the system remains sealed.

5.3 Protective laboratory clothing, eyewear and gloves should be worn at all times.

6.0 EQUIPMENT AND SUPPLIES

6.1 Coulometric water titrator - An automatic Karl Fischer titration system with amperometric, potentiometric or potential difference end point detection. It consists of an electrolytic titration cell, dual platinum electrode, magnetic stirrer and control unit.

6.2 Volumetric water titrator - An automatic Karl Fischer titration system consisting of a titration cell, dual platinum electrode, magnetic stirrer, dispensing buret and control unit.

6.3 Syringes - 5 µL, 10 µL and 100 µL.

6.4 Analytical balance - Minimum capacity of 160 g and capable of weighing to 0.0001 grams.

6.5 Screw cap vials, 20 mL.

6.6 Furnace module for determining water in the presence of high levels of hydroxide or in samples not otherwise amenable to direct titration or extraction. This is interfaced with the titration cell. An appropriate sample introduction apparatus will also be required.

7.0 REAGENTS AND STANDARDS

7.1 Coulometric cell solutions.

7.1.1 Anode reagent - Main ingredients consisting of methanol, organic base, sulfur dioxide and a suitable iodine compound.

7.1.2 Cathode reagent - Main ingredients consisting of methanol, organic base, sulfur dioxide and possibly carbon tetrachloride.

7.2 Volumetric reagents.

7.2.1 Volumetric titrant - A mixture of an organic amine, sulfur dioxide and iodine dissolved in a non-hygroscopic solvent. Reagents with titers of 1, 2 and 5 mg H_2O/mL can be commercially obtained.

7.2.2 Karl Fischer Solvent - Typically consisting of an organic amine and sulfur dioxide dissolved in anhydrous methanol.

7.3 The reagents described in 7.1 and 7.2 are commercially available.

7.4 Methanol or other appropriate solvent for extracting samples, anhydrous, > 99.8%.

7.5 Water - Reagent water (as defined in Chapter One).

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected and stored in containers which will protect them from changes in volume or water content. Storage in glass with Teflon-lined caps is required if analytes requiring such storage are to be determined.

8.2 Samples should be refrigerated at 4°C ± 2°C and then brought to room temperature prior to analysis if analytes requiring such storage are to be determined.

9.0 QUALITY CONTROL

9.1 For each batch of twenty samples processed, at least one duplicate sample must be carried throughout the entire sample preparation and analytical process as described in Chapter One. The relative standard deviation of the duplicate analyses should be <10%.

9.2 For each batch of twenty samples processed, at least one spiked sample must be carried throughout the entire sample preparation and analytical process as described in Chapter One. The spike recovery should be 90 to 110%. In the absence of other information, a spike of 50% water is recommended. Spikes to some matrices, e.g., paints, may not be meaningful due to their high water levels and problems with spiking emulsions. In these cases, a spike of their extract may be the best option.

9.3 Certified reference materials should be analyzed where available.

9.4 To assess the accuracy of coulometric titrators, three 5 mg injections of reagent water are to be performed daily with average recoveries of 90 to 110% and relative standard deviations of < 5% to be achieved. If the recoveries fall outside of this range, the instrument problem must be corrected before continuing with sample analysis.

9.5 Background levels of water in reagents are minimized by using anhydrous reagents and by pre-titration of reagents prior to sample analysis.

9.6 To prevent the carryover of moisture into the syringe, the syringe should be rinsed once with methanol between samples and twice with the sample prior to loading the volume to be analyzed. Alternatively, use several syringes that have been oven dried, rotating the drying/use cycle so that the syringe in use reaches room temperature prior to use.

9.7 Only small aliquots of samples should be handled near the titrator to prevent contamination of the bulk sample by Karl Fischer reagent solvents.

9.8 When methanol or other solvent extractions are performed, three solvent blanks should be analyzed with these extracts and the extract results corrected for the mean of these blanks.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Coulometric procedure - Since coulometric titrators generate iodine on demand by the titration cell, standardization of titrant is not required.

10.2 Volumetric procedure - The titer of the titrant must be checked on a daily basis. Using a 5 uL syringe, 3.0 uL of water is injected into the titration cell containing solvent that has been pre-titrated to remove residual moisture. The titer is calculated as follows:

3.0 mg H_2O/mL of titrant consumed = mg/mL H_2O equivalent of titrant.

11.0 PROCEDURES

11.1 Sample introduction - The approach which should be used will depend on the viscosity and solubility of the sample and is left to the discretion of the analyst.

11.2 Direct injection - A sample (5 µL for coulometric, 100 µL for volumetric) is weighed by difference in a cleaned and dried syringe which has the needle inserted in a small piece of silicone rubber to reduce sample evaporation. The sample is injected into the titration cell septum and the syringe reweighed to determine the actual weight injected. The sample is titrated to the endpoint and the results recorded.

11.3 Extraction - A 500 mg sample is extracted with 10 mL of an appropriate solvent by shaking in a 20 mL vial for 2-3 minutes. The mixture is allowed to settle. Centrifugation may be required. A 100 µL aliquot of the supernatant is titrated. The volume of the aliquot to be titrated can be varied to achieve results within the linear range of the titrator. Methanol is most commonly used, but is not appropriate for all materials. Toluene, DMF, pyridine and diglyme are suitable for paints.

11.4 Water Vaporization - A 10 to 100 mg sample is weighed into a sampling tube and introduced into the furnace or injected into hot mineral oil and the water vapor carried by a gas stream into the titration cell. This approach is most commonly used with samples which cannot be directly titrated or extracted or for samples containing high levels of hydroxide ion as described in 4.1.1. Consult the instrument instruction manual for proper use.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 The water content in ppm or percent in the sample is either calculated by the instrument, if sample weight was keyed in, or the instrument readout in g H_2O found is divided by the g of sample injected with appropriate corrections for any dilutions or extractions performed.

12.2 Data analysis worksheets should be prepared for all samples analyzed. The information to be included is the sample identification, sample weight, water content measured, water content in the original sample and results of quality control tests performed as described in Section 9.

13.0 METHOD PERFORMANCE

13.1 Coulometric Procedure: Crude oil analysis: In ASTM Method D4928-89, crude oils containing 0.02 to 5% water were tested in an interlaboratory study. Within laboratory precision was 5 to 10% and between laboratory 7 to 20% relative standard deviation between 0.1 and 5% water.

13.2 Volumetric Procedure

13.2.1 Used oil analysis: A series of used oil standards was prepared by spiking dried used oil with water over the range 0 to 20%. Additional standards were made by spiking a hydrocarbon based cutting fluid at 25% and 50%. The results in w/w percent are shown in Table 1. Over the range 1 to 50% water, a linear regression of the results by the method vs. the spiked water content followed the relationship: $y = 1.0137x + 0.0917$ with $R^2 = 0.9997$.

Certified reference materials covering the range 2 to 90% water were analyzed using the direct injection procedure and volumetric titration. The results are shown in Table 2 and

agreed with those obtained using Method 9001 and the certified value. The relative standard deviations ranged from 1 to 10% for 6 to 10 determinations.

13.2.2 Paint analysis: A certified reference material, ERM-19, Water and Volatiles in Latex Paint, was analyzed 10 times by the extraction procedure and volumetric titration. A 500 mg sample was extracted with diglyme, centrifuged and 125 µL of the supernatant titrated. The results agreed with results determined by Method 9001:

	ERM-19, %w/w
Method 9000	42.34 ± 1.25
Method 9001	44.91 ± 0.31

In ASTM Method D4017-90, paints containing 25 to 75% water were tested in an interlaboratory study. Within laboratory precision was 1.7% and between laboratory 5.3% relative standard deviation.

13.2.3 Other wastes: In ASTM Method D5530, hazardous waste fuels containing 13 to 32% water yielded within laboratory precision of 1.3% and between laboratory of 4.3% relative standard deviation.

13.2.4 Soil analysis: A marine sediment was dried and spiked with water over the range 0 to 40% (w/w). The results are shown in Table 3 and followed the relationship $y = 0.9972x + 0.1103$ with $R^2 = 0.9991$.

13.2.5 Alcohol analysis: Mixtures of ethanol and water covering the range 0 to 100% water and three distilled spirits were analyzed by this method and Method 9001. The results are given in Table 4. Because total dissolved solids like sugars and other carbohydrates often present in beers, wines and distilled spirits will be counted as "alcohol" when water content is used to estimate alcohol content, their contribution must be considered and if necessary, determined and subtracted from the non-water content to determine the alcohol content.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. 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 waste cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 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 Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

15.1 Spent reagents and samples should be stored and disposed appropriately.

15.2 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly with hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult the *Management Manual for Laboratory Personnel*, available from the American Chemical Society at the address listed in Section 14.2.

16.0 REFERENCES

1. ASTM Method D3401-92, Standard Test Method for Water in Halogenated Organic Solvents and Their Admixtures.

2. ASTM Method D4017-90, Standard Test Method for Water in Paints and Paint Materials by Karl Fischer Method.

3. ASTM Method D5530-94, Standard Test Method for Total Moisture of Hazardous Waste Fuel by Karl Fischer Titrimetry.

4. ASTM Method D4928-89 Standard Test Methods for Water in Crude Oils by Coulometric Karl Fischer Titration.

5. ASTM Method D4377-93a Standard Test Method for Water in Crude Oils by Potentiometric Karl Fischer Titration.

6. Validation Data for Draft Methods 9000 and 9001 for the Determination of Water Content in Liquid and Solid Matrices, Dexsil Corp., Hamden, CT.

7. MacLeod, Steven K. "Moisture Determination Using Karl Fischer Titrations." Analytical Chemistry. Volume 63. Pages 557-566. May 15, 1991.

8. U.S. EPA NEIC Method. "Water Content of Waste Material Samples by Coulometric Karl Fischer Titration." Pages 1-12, August 1991.

17.0 TABLES, DIAGRAMS, FLOW CHARTS AND VALIDATION DATA

The following pages contain Tables 1 through 4 and a method procedure flow diagram.

TABLE 1

DETERMINATION OF WATER IN USED OIL
(w/w %)

Expected	Method 9001	Method 9000
0	0.161	0.061
0.1	0.149	0.145
0.2	0.226	0.255
0.5	0.459	0.561
1.0	0.948	1.07
2.0	2.36	2.46
5.0	5.03	5.05
10.0	9.82	9.97
20.0	20.2	20.0
25.0	26.37	26.05
50.0	50.05	50.60

Source: Reference 6

TABLE 2

ANALYSIS OF USED OIL CERTIFIED REFERENCE MATERIALS[a]

CRM	Certified Value, wt %	Method 9001, wt %	Method 9000, wt %
ERM-34	1.95	1.92±0.02	1.86±0.09
ERM-35	5.86	5.91±0.61	6.13±0.55
ERM-36	10.3	10.30±0.85	10.3±0.81
ERM-41	87.4	88.4±6.7	86.4±6.6

[a]ERM-34 to 41 Water Content in Used Oil Mixtures from Environmental Reference Materials, Inc.

Source: Reference 6

TABLE 3

DETERMINATION OF WATER IN MARINE SEDIMENT
(w/w%)

Expected	Method 9001	Method 9000
0	1.14	0.579
10	10.06	9.74
20	18.99	19.67
30	28.52	29.95
40	38.47	40.34

Source: Reference 6

TABLE 4

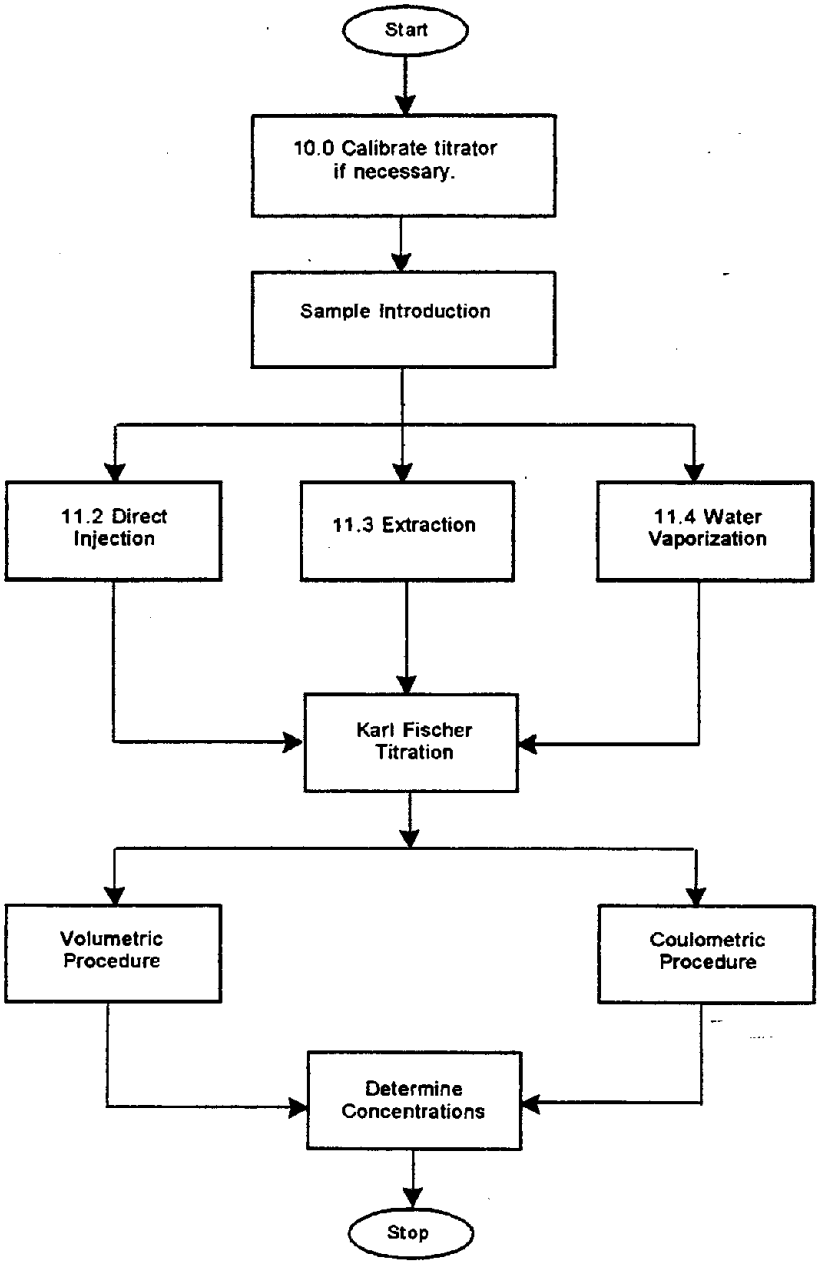
DETERMINATION OF ALCOHOL IN WATER/ALCOHOL MIXTURES

Expected % Alcohol, v/v	Method 9001 (% v/v)	Method 9000 (% v/v)
0	0	0
10	10.0	10.3
25	25.6	25.0
40	40.9	38.7
50	48.5	49.1
80	80.6	79.8
100	99.9	100.0
Vodka, 40	41.9	42.0
Whiskey, 40	40.0	41.9
Gin, 47	47.2	48.7

Source: Reference 6.

METHOD 9000

DETERMINATION OF WATER IN WASTE MATERIALS BY KARL FISCHER TITRATION



METHOD 9001

DETERMINATION OF WATER IN WASTE MATERIALS BY QUANTITATIVE CALCIUM HYDRIDE REACTION

1.0 SCOPE AND APPLICATION

1.1 This quantitative calcium hydride reaction method is capable of determining water in the concentration range from 0.1% to 100% in liquid and solid materials including oils, paints, soils and water/alcohol mixtures. It is intended to be used as either a field or laboratory method.

1.2 Multiphasic samples should be separated into physical phases (liquid, solid, etc.) prior to analysis to assure representative aliquots are analyzed.

1.3 Establishing the amount of water in a sample may be useful for the reasons to follow.

1.3.1 It is useful in determining the total composition of a sample. In combination with other analytical results, the mass balance of a sample can be determined.

1.3.2 It is useful in the distinction of which samples can be analyzed by infrared spectroscopy using sodium chloride cells or which require zinc selenide cells.

1.3.3 It is useful in determining the amount of alcohol in an aqueous alcohol solution.

1.3.4 It is useful when distinguishing aqueous from nonaqueous solutions.

1.3.5 It is useful when setting the proper mixture of feed materials in the incineration of waste.

2.0 SUMMARY OF METHOD

2.1 A sample of the material to be tested is treated with a specially formulated calcium hydride reagent which reacts with water in the sample to liberate hydrogen gas as shown below:

$$CaH_2 + 2H_2O \rightarrow Ca(OH)_2 + 2H_2 \uparrow$$

2.2 The reaction is carried out in a sealed pressure vessel and the resulting pressure is then measured using a specially designed meter. The results are displayed directly in weight or volume percent, depending on the sampling method used.

2.3 The reaction is quantitative, measuring all water present in the sample over the range 0.1 to 100%.

3.0 DEFINITIONS

Refer to Chapter One and Chapter Three for a listing of applicable definitions.

4.0 INTERFERENCES

4.1 This method has no known positive interferences. Tests conducted on 20% (w/w) solutions of the compounds listed below using twice the normal sample size produced no response. The following compounds are representative of substances either known to react with calcium hydride and/or which are likely to be present in materials to be tested using this method:

Ethanol
Methanol
Acetone
Methyl ethyl ketone
Tetrahydrofuran
Diethylene glycol dimethyl ether
Ethylene glycol
Diethylene glycol
Dipropylene glycol
Stearic acid
2-Ethyl hexanoic acid
Lead oxide (II and III)
Aluminum oxide (Brockman I)

4.2 Nitric acid reacts with calcium hydroxide to form calcium nitrate tetra-hydrate crystals, which trap water in the acid before it can react with calcium hydride. This yields results as much as 80% lower than the actual water content. This interference is only significant when determining the water content of concentrated nitric acid mixtures.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis.

5.2 Protective laboratory clothing, eyewear and gloves should be worn at all times.

5.3 The amount of hydrogen gas generated is minimal and is not a hazard to the user.

6.0 EQUIPMENT AND SUPPLIES

6.1 Quantitative calcium hydride reaction test kit - Hydroscout test system (Dexsil Corporation, One Hamden Park Drive, Hamden, CT), or equivalent. Each commercially available test kit will supply or specify the apparatus and materials necessary for successful completion of the test.

7.0 REAGENTS AND STANDARDS

7.1 Each commercially available test kit will supply or specify the reagents necessary for successful completion of the test. Reagents should be labeled with appropriate expiration dates.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Samples should be collected and stored in containers which will protect them from changes in volume or water content. Storage in glass with PTFE-lined caps is required if analytes requiring such storage are to be determined.

8.2 Samples should be refrigerated at 4°C ± 2°C and brought to room temperature prior to analysis if analytes requiring such storage are to be determined.

9.0 QUALITY CONTROL

9.1 Follow the manufacturer's instructions for quality control procedures.

9.2 For each batch of twenty samples processed, at least one duplicate sample must be carried throughout the entire sample preparation and analytical process as described in Chapter One. The relative standard deviation of the duplicate determinations should be <10%.

9.3 For each batch of twenty samples processed, at least one spiked sample must be carried throughout the entire sample preparation and analytical process as described in Chapter One. The spike recovery should be 90 to 110%. In the absence of other information, a spike of 50% water is recommended. Spikes to some matrices (e.g., oils and paints) may not be meaningful due to their high water levels and problems with spiking emulsions. In these cases, a spike of their extract may be the best option.

9.4 A test sample provided with the kit should be analyzed to verify proper performance of the test and meter operation.

9.5 A blank correction for water is not required. Reagents are ampulized instead of bulk packaged and thus are less likely to absorb water from the air.

9.6 Certified reference materials should be analyzed where available.

10.0 CALIBRATION AND STANDARDIZATION

10.1 The meter provided with the kit is factory calibrated to read directly in percent water. Every time the meter is turned on, a new zero calibration point is determined.

11.0 PROCEDURE

11.1 Follow the directions provided by your kit manufacturer.

11.2 Oil samples are analyzed by directly reacting a measured 0.4 to 0.8 mL (for v/v measurements) or 1 g (for w/w measurements) sample with the calcium hydride reagent. Samples up to 5 mL can be used to determine water in the 0.1 to 1.0% range. The resulting pressure due

to hydrogen gas is converted by the meter to percent water. The meter has separate programs for reporting results in v/v or w/w percent water.

11.3 Paint and soil samples are analyzed after extracting 1 g samples with a dilution solvent. A 0.8 mL aliquot of the extract is reacted with the calcium hydride reagent. The meter results are reported in w/w percent water.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 The meter provided with the kit is factory calibrated and the equations converting pressure to percent water are stored in designated programs. The matrix and sample size determine the appropriate program to use.

12.2 Data analysis worksheets should be prepared for all samples analyzed. The information to be included is the sample identification, sample weight or volume, water content (as read from the instrument readout), water content in the original sample (accounting for any dilutions or extractions) and results of quality control tests performed as described in Section 9.0.

13.0 METHOD PERFORMANCE

13.1 Used oil analysis: A series of used oil standards were prepared by spiking dried used oil with water over the range 0 to 20%. Additional standards were made by spiking a hydrocarbon based cutting fluid at 25% and 50%. The results in w/w percent are shown in Table 1. Over the range 1 to 50% water, a linear regression of the results by the method vs. the spiked water content followed the relationship: $y = 1.007x + 0.1024$ with $R^2 = 0.9993$.

Certified reference materials covering the range 2 to 90% water were analyzed using this method and Method 9000. The results are shown in Table 2. The relative standard deviations ranged from 1 to 10% for 6 to 10 determinations and the results agreed with the certified value and those determined by Method 9000.

13.2 Paint analysis: A certified reference material, ERM-19, Water and Volatiles in Latex Paint, was analyzed 10 times. The results in w/w% were 44.91 ± 0.31%. The RSD of the measurements was 0.7%. The results by this method agreed with those obtained using Method 9000 (43.38 ± 1.29%).

13.3 Soil analysis: A marine sediment was dried and spiked with water over the range 0 to 40% (w/w). The results are shown in Table 3 and followed the relationship $y = 0.9311x + 0.8149$ with $R^2 = 0.9994$.

13.4 Alcohol analysis: Mixtures of ethanol and water covering the range 0 to 100% water and three distilled spirits were analyzed by this method and Method 9000. The results are given in Table 4. Because total dissolved solids like sugars and other carbohydrates often present in beers, wines and distilled spirits will be counted as "alcohol" when water content is used to estimate alcohol content, their contribution must be considered and if necessary, determined and subtracted from the non-water content to determine the alcohol content.

13.5 Other wastes: Concentrated sulfuric and nitric acids and 10 N sodium hydroxide were analyzed. The water content of the sulfuric acid was determined to be 4.33% vs. the bottle assay value of 4.2%. The water content of 10 N NaOH was found to be greater than 20%, the upper limit of the method for undiluted samples. This is expected for 10 N NaOH, which has a nominal water

content in excess of 50%. The water content of concentrated nitric acid was determined to be around 6% vs. the assay value of 30%.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/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 waste cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 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 Relations and Science Policy, 1155 16th St., N.W. Washington, D.C., 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

15.1 On completion of a test, the reaction tube will contain water, the original sample matrix and a solution of calcium hydroxide. Samples requiring dilution with an organic solvent will also require disposal of the solvent. Reacted samples and spent solvents should be stored and disposed appropriately.

15.2 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly with hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult the *Management Manual for Laboratory Personnel*, available from the American Chemical Society at the address listed in Section 14.2.

16.0 REFERENCES

1. Operating Manual, Hydroscout System, Dexsil Corporation.

2. Lynn, Theodore B., Validation Data for Draft Methods 9000 and 9001 for the Determination of Water Content in Liquid and Solid Matrices, Dexsil Corp., Hamden, CT.

17.0 TABLES, DIAGRAMS, FLOW CHARTS AND VALIDATION DATA

The pages to follow contain Tables 1 through 4 and a method procedure flow diagram.

TABLE 1

DETERMINATION OF WATER IN USED OIL
(w/w %)

Expected	Method 9001	Method 9000
0	0.161	0.061
0.1	0.149	0.145
0.2	0.226	0.255
0.5	0.459	0.561
1.0	0.948	1.07
2.0	2.36	2.46
5.0	5.03	5.05
10.0	9.82	9.97
20.0	20.2	20.0
25.0	26.37	26.05
50.0	50.05	50.60

Source: Reference 2

TABLE 2

ANALYSIS OF USED OIL CERTIFIED REFERENCE MATERIALS[a]

CRM	Certified Value, wt %	Method 9001, wt %	Method 9000, wt %
ERM-34	1.95	1.92±0.02	1.86±0.09
ERM-35	5.86	5.91±0.61	6.13±0.55
ERM-36	10.3	10.30±0.85	10.3±0.81
ERM-41	87.4	88.4±6.7	86.4±6.6

[a]ERM-34 to 41 Water Content in Used Oil Mixtures from Environmental Reference Materials, Inc.

Source: Reference 2

TABLE 3

DETERMINATION OF WATER IN MARINE SEDIMENT
(w/w %)

Expected	Method 9001	Method 9000
0	1.14	0.579
10	10.06	9.74
20	18.99	19.67
30	28.52	29.95
40	38.47	40.34

Source: Reference 2.

TABLE 4

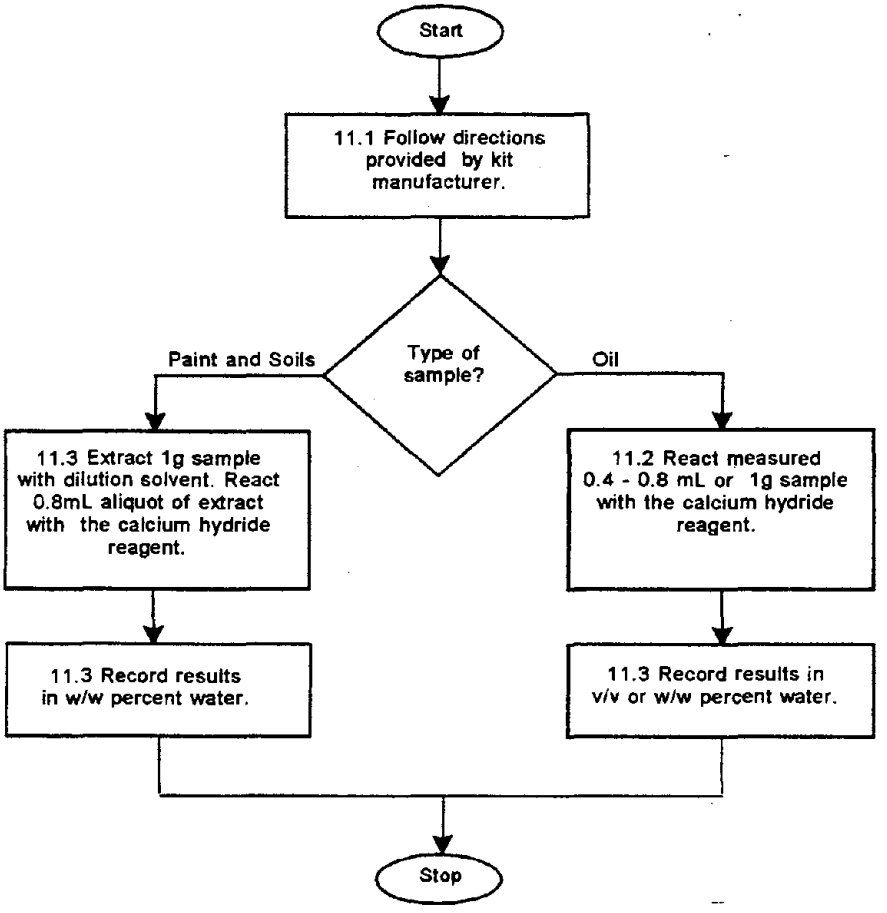
DETERMINATION OF ALCOHOL IN WATER/ALCOHOL MIXTURES

Expected % Alcohol, v/v	Method 9001 (% v/v)	Method 9000 (% v/v)
0	0	0
10	10.0	10.3
25	25.6	25.0
40	40.9	38.7
50	48.5	49.1
80	80.6	79.8
100	99.9	100.0
Vodka, 40	41.9	42.0
Whiskey, 40	40.0	41.9
Gin, 47	47.2	48.7

Source: Reference 2

METHOD 9001

DETERMINATION OF WATER IN WASTE MATERIALS BY QUANTITATIVE CALCIUM HYDRIDE REACTION



CHAPTER FOUR
ORGANIC ANALYTES

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in this chapter is provided by EPA as guidance to be used by the analyst and the regulated community in making judgements necessary to meet the data quality objectives or needs for the intended use of the data.

4.1 SAMPLING CONSIDERATIONS

4.1.1 Introduction

Following the initial and critical step of designing a sampling plan (Chapter Nine) is the implementation of that plan such that a representative sample of the solid waste is collected. Once the sample has been collected it must be stored and preserved to maintain the chemical and physical properties that it possessed at the time of collection. The sample type, type of containers and their preparation, possible forms of contamination, and preservation methods are all items which must be thoroughly examined in order to maintain the integrity of the samples. This section highlights considerations which must be addressed in order to maintain a sample's integrity and representativeness. This section is, however, applicable only to trace analyses.

Quality Control (QC) requirements need not be met for all compounds presented in the Table of Analytes for the method in use, rather, they must be met for all compounds reported. A report of non-detect is considered a quantitative report, and must meet all applicable QC requirements for that compound and the method used.

4.1.2 Sample Handling and Preservation

This section deals separately with volatile and semivolatile organics. Refer to Chapter Two and Table 4-1 of this section for sample containers, sample preservation, and sample holding time information.

Volatile Organics

Standard 40 mL glass screw-cap VOA vials with Teflon lined silicone septa may be used for liquid matrices. Special 40 mL VOA vials for purge-and-trap of solid samples are described in Method 5035. VOA vials for headspace analysis of solid samples are described in Method 5021. Standard 125 mL widemouth glass containers may be used for Methods 5031 and 5032. However, the sampling procedures described in Method 5035 may minimize sample preparation analyte loss better than the procedures described in Methods 5031 and 5032. The vials and septa should be washed with soap and water and rinsed with distilled deionized water. After thoroughly cleaning the vials and septa, they should be placed in an oven and dried at 100°C for approximately one hour.

NOTE: Do not heat the septa for extended periods of time (i.e., more than one hour, because the silicone begins to slowly degrade at 105°C).

When collecting the samples, liquids and solids should be introduced into the vials gently to reduce agitation which might drive off volatile compounds.

In general, liquid samples should be poured into the vial without introducing any air bubbles within the vial as it is being filled. Should bubbling occur as a result of violent pouring, the sample must be poured out and the vial refilled. The vials should be completely filled at the time of sampling, so that when the septum cap is fitted and sealed, and the vial inverted, no headspace is visible. The sample should be hermetically sealed in the vial at the time of sampling, and must not be opened prior to analysis to preserve their integrity.

- Due to differing solubility and diffusion properties of gases in LIQUID matrices at different temperatures, it is possible for the sample to generate some headspace during storage. This headspace will appear in the form of micro bubbles, and should not invalidate a sample for volatiles analysis.

- The presence of a macro bubble in a sample vial generally indicates either improper sampling technique or a source of gas evolution within the sample. The latter case is usually accompanied by a buildup of pressure within the vial, (e.g. carbonate-containing samples preserved with acid). Studies conducted by the USEPA (EMSL-Ci, unpublished data) indicate that "pea-sized" bubbles (i.e., bubbles not exceeding 1/4 inch or 6 mm in diameter) did not adversely affect volatiles data. These bubbles were generally encountered in wastewater samples, which are more susceptible to variations in gas solubility than are groundwater samples.

Immediately prior to analysis of liquid samples, the aliquot to be analyzed should be taken from the vial using the instructions from the appropriate sample introduction technique:

- For smaller analysis volumes, a gas-tight syringe may be inserted directly through the septum of the vial to withdraw the sample.

- For larger analysis volumes, (e.g. purge-and-trap analyses) the sample may be carefully poured into the syringe barrel. Opening a volatile sample to pour a sample into a syringe destroys the validity of the sample for future analysis. Therefore, if there is only one VOA vial, it is strongly recommended that the analyst fill a second syringe at this time to protect against possible loss of sample integrity. This second sample is maintained only until such time as the analyst has determined that the first sample has been analyzed properly.

If these guidelines are not followed, the validity of the data generated from the samples may be suspect.

VOA vials for samples with solid or semi-solid matrices (e.g., sludges) should be filled according to the guidance given in the appropriate 5000 series sample introduction method (see Table 4-1) to be used. When 125-mL widemouth glass containers are used, the containers should be filled as completely as possible. The 125-mL vials should be tapped slightly as they are filled to try and eliminate as much free air space as possible. A minimum of two vials should also be filled per sample location.

At least two VOA vials should be filled and labeled immediately at the point at which the sample is collected. They should NOT be filled near a running motor or any type of exhaust system because discharged fumes and vapors may contaminate the samples. The two vials from each sampling location should then be sealed in separate plastic bags to prevent cross-contamination between samples, particularly if the sampled waste is suspected of containing high levels of volatile organics. (Activated carbon may also be included in the bags to prevent cross-contamination from highly contaminated samples). VOA samples may also be contaminated by diffusion of volatile

organics through the septum during shipment and storage. To monitor possible contamination, a trip blank prepared from organic-free reagent water (as defined in Chapter One) should be carried throughout the sampling, storage, and shipping process.

Semivolatile Organics (including Pesticides, PCBs and Herbicides.)

Containers used to collect samples for the determination of semivolatile organic compounds should be soap and water washed followed by methanol (or isopropanol) rinsing (see Sec. 4.1.4 for specific instructions on glassware cleaning). The sample containers should be of glass or Teflon, and have screw-caps with Teflon lined septa. In situations where Teflon is not available, solvent-rinsed aluminum foil may be used as a liner. However, acidic or basic samples may react with the aluminum foil, causing eventual contamination of the sample. Plastic containers or lids may NOT be used for the storage of samples due to the possibility of sample contamination from the phthalate esters and other hydrocarbons within the plastic. Sample containers should be filled with care so as to prevent any portion of the collected sample coming in contact with the sampler's gloves, thus causing contamination. Samples should not be collected or stored in the presence of exhaust fumes. If the sample comes in contact with the sampler (e.g. if an automatic sampler is used), run organic-free reagent water through the sampler and use as a field blank.

4.1.3 Safety

Safety should always be the primary consideration in the collection of samples. A thorough understanding of the waste production process, as well as all of the potential hazards making up the waste, should be investigated whenever possible. The site should be visually evaluated just prior to sampling to determine additional safety measures. Minimum protection of gloves and safety glasses should be worn to prevent sample contact with the skin and eyes. A respirator should be worn even when working outdoors if organic vapors are present. More hazardous sampling missions may require the use of supplied air and special clothing.

4.1.4 Cleaning of Glassware

In the analysis of samples containing components in the parts per billion range, the preparation of scrupulously clean glassware is necessary. Failure to do so can lead to a myriad of problems in the interpretation of the final chromatograms due to the presence of extraneous peaks resulting from contamination. Particular care must be taken with glassware such as Soxhlet extractors, Kudema-Danish evaporative concentrators, sampling-train components, or any other glassware coming in contact with an extract that will be evaporated to a smaller volume. The process of concentrating the compounds of interest in this operation may similarly concentrate the contaminating substance(s), which may seriously distort the results.

The basic cleaning steps are:

1. Removal of surface residuals immediately after use;
2. Hot soak to loosen and float most particulate material;
3. Hot water rinse to flush away floated particulates;
4. Soak with an oxidizing agent to destroy traces of organic compounds;
5. Hot water rinse to flush away materials loosened by the deep penetrant soak;

6. Distilled water rinse to remove metallic deposits from the tap water;

7. Alcohol, e.g., isopropanol or methanol, rinse to flush off any final traces of organic materials and remove the water; and

8. Flushing the item immediately before use with some of the same solvent that will be used in the analysis.

Each of these eight fundamental steps are discussed here in the order in which they appeared on the preceding page.

1. As soon possible after glassware (i.e., beakers, pipets, flasks, or bottles) has come in contact with sample or standards, the glassware should be flushed with alcohol before it is placed in the hot detergent soak. If this is not done, the soak bath may serve to contaminate all other glassware placed therein.

2. The hot soak consists of a bath of a suitable detergent in water of 50°C or higher. The detergent, powder or liquid, should be entirely synthetic and not a fatty acid base. There are very few areas of the country where the water hardness is sufficiently low to avoid the formation of some hard-water scum resulting from the reaction between calcium and magnesium salts with a fatty acid soap. This hard-water scum or curd would have an affinity particularly for many chlorinated compounds and, being almost wholly water-insoluble, would deposit on all glassware in the bath in a thin film.

   There are many suitable detergents on the wholesale and retail market. Most of the common liquid dishwashing detergents sold at retail are satisfactory but are more expensive than other comparable products sold industrially. Alconox, in powder or tablet form, is manufactured by Alconox, Inc., New York, and is marketed by a number of laboratory supply firms. Sparkleen, another powdered product, is distributed by Fisher Scientific Company.

3. No comments required.

4. The most common and highly effective oxidizing agent for removal of traces of organic compounds is the traditional chromic acid solution made up of concentrated sulfuric acid and potassium or sodium dichromate. For maximum efficiency, the soak solution should be hot (40-50°C). Safety precautions must be rigidly observed in the handling of this solution. Prescribed safety gear should include safety goggles, rubber gloves, and apron. The bench area where this operation is conducted should be covered with fluorocarbon sheeting because spattering will disintegrate any unprotected surfaces.

   The potential hazards of using chromic-sulfuric acid mixture are great and have been well publicized. There are now commercially available substitutes that possess the advantage of safety in handling. These are biodegradable concentrates with a claimed cleaning strength equal to the chromic acid solution. They are alkaline, equivalent to ca. 0.1 N NaOH upon dilution, and are claimed to remove dried blood, silicone greases, distillation residues, insoluble organic residues, etc. They are further claimed to remove radioactive traces and will not attack glass or exert a corrosive effect on skin or clothing. One such product is "Chem Solv 2157," manufactured by Mallinckrodt and available through laboratory supply firms. Another comparable product is "Detex," a product of Borer-Chemie, Solothum, Switzerland.

5, 6, and 7. No comments required.

8. There is always a possibility that between the time of washing and the next use, the glassware could pick up some contamination from either the air or direct contact. To ensure against this, it is good practice to flush the item immediately before use with some of the same solvent that will be used in the analysis.

The drying and storage of the cleaned glassware is of critical importance to prevent the beneficial effects of the scrupulous cleaning from being nullified. Pegboard drying is not recommended. It is recommended that laboratory glassware and equipment be dried at 100°C. Under no circumstances should such small items be left in the open without protective covering. The dust cloud raised by the daily sweeping of the laboratory floor can most effectively recontaminate the clean glassware.

As an alternate to solvent rinsing, the glassware can be heated to a minimum of 300°C to vaporize any organics. Do not use this high temperature treatment on volumetric glassware, glassware with ground glass joints, or sintered glassware.

4.1.5 High Concentration Samples

Cross contamination of trace concentration samples may occur when prepared in the same laboratory with high concentration samples. Ideally, if both type samples are being handled, a laboratory and glassware dedicated solely to the preparation of high concentration samples would be available for this purpose. If this is not feasible, as a minimum when preparing high concentration samples, disposable glassware should be used or, at least, glassware dedicated entirely to the high concentration samples. Avoid cleaning glassware used for both trace and high concentration samples in the same area.

TABLE 4-1.
SAMPLE CONTAINERS, PRESERVATION, TECHNIQUES, AND HOLDING TIMES

VOLATILE ORGANICS			
Sample Matrix	Container	Preservative	Holding Time
Concentrated Waste Samples	Method 5035: 40-mL vials with stirring bar. Method 5021: See method. Methods 5031 & 5032: 125-mL widemouth glass container. Use Teflon-lined lids for all procedures.	Cool to 4°C.	14 days
Aqueous Samples With No Residual Chlorine Present	Methods 5030, 5031, & 5032: 2 X 40-mL vials with Teflon-lined septum caps	Cool to 4°C and adjust pH to less than 2 with H_2SO_4, HCl, or solid $NaHSO_4$.	14 days
Aqueous Samples WITH Residual Chlorine Present	Methods 5030, 5031, & 5032: 2 X 40-mL vials with Teflon-lined septum caps	Collect sample in a 125-mL container which has been pre-preserved with 4 drops of 10% sodium thiosulfate solution. Gently swirl to mix sample and transfer to a 40-mL VOA vial. Cool to 4°C and adjust pH to less than 2 with H_2SO_4, HCl, or solid $NaHSO_4$.	14 days
Acrolein and Acrylonitrile in Aqueous Sample	Methods 5030, 5031, & 5032: 2 X 40-mL vials with Teflon-lined septum caps	Adjust to pH 4-5. Cool to 4°C.	14 days
Solid Samples (e.g. soils, sediments, sludges, ash)	Method 5035: 40-mL vials with septum and stirring bar. Method 5021: See method. Methods 5031 & 5032: 125-mL widemouth glass container with Teflon-lined lids.	See the individual methods.	14 days

TABLE 4-1 (Continued)

SEMIVOLATILE ORGANICS/ORGANOCHLORINE PESTICIDES/PCBs AND HERBICIDES			
Sample Matrix	Container	Preservative	Holding Time
Concentrated Waste Samples	125-mL widemouth glass with Teflon-lined lid	None	Samples extracted within 14 days and extracts analyzed within 40 days following extraction.
Aqueous Samples With No Residual Chlorine Present	1-gal., 2 x 0.5-gal., or 4 x 1-L amber glass container with Teflon-lined lid	Cool to 4°C	Samples extracted within 7 days and extracts analyzed within 40 days following extraction.
Aqueous Samples WITH Residual Chlorine Present	1-gal., 2 x 0.5-gal., or 4 x 1-L, amber glass container with Teflon-lined lid.	Add 3-mL 10% sodium thiosulfate solution per gallon (or 0.008%). Addition of sodium thiosulfate solution to sample container may be performed in the laboratory prior to field use. Cool to 4°C.	Samples extracted within 7 days and extracts analyzed within 40 days following extraction.
Solid Samples (e.g. soils, sediments, sludges, ash)	250-mL widemouth glass container with Teflon-lined lid	Cool to 4°C	Samples extracted within 14 days and extracts analyzed within 40 days following extraction.

4.2 SAMPLE PREPARATION METHODS

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgements necessary to meet the data quality objectives or needs for the intended use of the data.

4.2.1 EXTRACTIONS AND PREPARATIONS

The following methods are included in this section:

Method 3500B:	Organic Extraction and Sample Preparation
Method 3510C:	Separatory Funnel Liquid-Liquid Extraction
Method 3520C:	Continuous Liquid-Liquid Extraction
Method 3535A:	Solid-Phase Extraction (SPE)
Method 3540C:	Soxhlet Extraction
Method 3541:	Automated Soxhlet Extraction
Method 3542:	Extraction of Semivolatile Analytes Collected Using Method 0010 (Modified Method 5 Sampling Train)
Method 3545A:	Pressurized Fluid Extraction (PFE)
Method 3550B:	Ultrasonic Extraction
Method 3560:	Supercritical Fluid Extraction of Total Recoverable Petroleum Hydrocarbons
Method 3561:	Supercritical Fluid Extraction of Polynuclear Aromatic Hydrocarbons
Method 3562:	Supercritical Fluid Extraction of Polychlorinated Biphenyls (PCBs) and Organochlorine Pesticides
Method 3580A:	Waste Dilution
Method 3585:	Waste Dilution for Volatile Organics
Method 5000:	Sample Preparation for Volatile Organic Compounds
Method 5021:	Volatile Organic Compounds in Soils and Other Solid Matrices Using Equilibrium Headspace Analysis
Method 5030B:	Purge-and-Trap for Aqueous Samples
Method 5031:	Volatile, Nonpurgeable, Water-Soluble Compounds by Azeotropic Distillation
Method 5032:	Volatile Organic Compounds by Vacuum Distillation
Method 5035:	Closed-System Purge-and-Trap and Extraction for Volatile Organics in Soil and Waste Samples
Method 5041A:	Analysis for Desorption of Sorbent Cartridges from Volatile Organic Sampling Train (VOST)

METHOD 3535A

SOLID-PHASE EXTRACTION (SPE)

1.0 SCOPE AND APPLICATION

1.1 This method describes a procedure for isolating target organic analytes from aqueous samples using solid-phase extraction (SPE) media. The method describes conditions for extracting a variety of organic compounds from aqueous matrices that include: groundwater, wastewater, and TCLP leachates. The method describes the use of disk extraction media for eight groups of analytes and the use of cartridge extraction media for one group of analytes. Other solid-phase extraction media may be employed as described in see Sec. 4.0. The extraction procedures are specific to the analytes of interest and vary by group of analytes and type of extraction media. The groups of analytes that have been evaluated thus far are listed below, along with the types of media that have been evaluated, and the determinative methods in which the corresponding performance data can be found.

Analyte Group	Extraction Media Type	Determinative Method
Phthalate esters	Disks	8061
Organochlorine pesticides	Disks	8081
Polychlorinated biphenyls (PCBs)	Disks	8082
Organophosphorus pesticides	Disks	8141
Nitroaromatics and nitramines	Disks and Cartridges	8330
TCLP leachates containing organochlorine pesticides	Disks	8081
TCLP leachates containing semivolatiles	Disks	8270
TCLP leachates containing phenoxyacid herbicides	Disks	8321

1.2 The technique may also be applicable to other semivolatile or extractable compounds. It may also be used for the extraction of additional target analytes or may employ other solid-phase media, provided that the analyst demonstrates adequate performance (e.g., recovery of 70 - 130%, or project-specific recovery criteria) using spiked sample matrices and an appropriate determinative method of the type included in Chapter Four (Sec. 4.3). The use of organic-free reagent water alone is not considered sufficient for conducting such performance studies, and must be supported by data from actual sample matrices.

1.3 This method also provides procedures for concentrating extracts and for solvent exchange.

1.4 Solid-phase extraction is called liquid-solid extraction in some methods associated with the Safe Drinking Water Act.

1.5 This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Sample preparation procedures vary by analyte group. Extraction of some groups requires that the pH of the sample be adjusted to a specified value prior to extraction (see Sec. 7.2). Other groups do not require a pH adjustment.

2.2 Following any necessary pH adjustment, a measured volume of sample is extracted by passing it through the solid-phase extraction medium (disks or cartridges), which is held in an extraction device designed for vacuum filtration of the sample.

2.3 Target analytes are eluted from the solid-phase media using an appropriate solvent (see Secs. 7.8 and 7.9) which is collected in a receiving vessel. The resulting solvent extract is dried using sodium sulfate and concentrated, as needed.

2.4 As necessary for the specific analysis, the concentrated extract may be exchanged into a solvent compatible extract with subsequent cleanup procedures (Chapter Four, Sec. 4.2) or determinative procedures (Chapter Four, Sec. 4.3) for the measurement of the target analytes.

3.0 INTERFERENCES

3.1 Refer to Method 3500.

3.2 The decomposition of some analytes has been demonstrated under basic extraction conditions. Organochlorine pesticides may dechlorinate and phthalate esters may hydrolyze. The rates of these reactions increase with increasing pH and reaction times.

3.3 Bonded-phase silica (e.g., C_{18}) will hydrolyze on prolonged exposure to aqueous samples with pH less than 2 or greater than 9. Hydrolysis will increase at the extremes of this pH range and with longer contact times. Hydrolysis may reduce extraction efficiency or cause baseline irregularities. Styrene divinylbenzene (SDB) extraction disks should be considered when hydrolysis is a problem.

3.4 Phthalates are a ubiquitous laboratory contaminant. All glass extraction apparatus should be used for this method because phthalates are used as release agents when molding rigid plastic (e.g., PVC) and as plasticizers for flexible tubing. A method blank, as described in Chapter One, should be analyzed, demonstrating that there is no phthalate contamination of the sodium sulfate or other reagents listed in this method.

3.5 Sample particulates may clog the solid-phase media and result in extremely slow sample extractions. Use of an appropriate filter aid will result in shorter extractions without loss of method performance if clogging is a problem. Even when a filter aid is employed, this method may not be appropriate for aqueous samples with high levels of suspended solids (>1%), as the extraction efficiency may not be sufficient, given the small volumes of solvents employed and the short contact time.

4.0 APPARATUS AND MATERIALS

The apparatus and materials described here are based on data provided to EPA for the extraction of eight groups of analytes using disk-type materials and for the extraction of one group of analytes using cartridge-type materials. Other solid-phase extraction media configurations may

be employed, provided that the laboratory demonstrates adequate performance for the analytes of interest. The use of other SPE configurations will require modifications to the procedures described in Sec. 7.0. Consult the manufacturer's instructions regarding such modifications.

4.1 Solid-phase disk extraction system - Empore™ manifold that holds three 90-mm filter standard apparatus or six 47-mm standard filter apparatus, or equivalent. Other manual, automatic, or robotic sample preparation systems designed for solid-phase media may be utilized for this method if adequate performance is achieved and all quality control requirements are satisfied.

4.1.1 Manifold station - (Fisher Scientific 14-378-1B [3-place], 14-378-1A [6-place], or equivalent).

4.1.2 Standard filter apparatus - (Fisher Scientific 14-378-2A [47-mm], 14-378-2B [90-mm], or equivalent), consisting of a sample reservoir, clamp, fritted disk and filtration head with drip tip.

4.1.3 Collection tube - 60-mL. The collection tube should be of appropriate ID and length so that the drip tip of the standard filter apparatus can be positioned well into the neck of the tube to prevent splattering.

4.1.4 Filter flask - 2-L with a ground-glass receiver joint (optional). May be used to carry out individual disk extractions with the standard filter apparatus and collection vial in an all-glass system.

4.2 Solid-phase cartridge extraction system - Visiprep solid-phase extraction manifold (Supelco) or equivalent system suitable for use with the extraction cartridges (see Sec. 4.4). Consult the manufacturer's recommendations for the associated glassware and hardware necessary to perform sample extractions.

4.3 Solid-phase extraction disks - Empore™, 47-mm, 90-mm, or equivalent. Disks are available in 47-mm and 90-mm diameters, composed of a variety of solid-phase materials. Other solid phases may be employed, provided that adequate performance is demonstrated for the analytes of interest. Guidance for selecting the specific disk is provided in Table 1.

4.3.1 C_{18} disks - Empore™ disks, 47-mm diameter (3M product number 98-0503-0015-5), 90-mm diameter (3M product number 98-0503-0019-7), or equivalent.

4.3.2 C_{18} fast flow disks - Empore™ disks, 47-mm diameter (3M product number 98-0503-0138-5), 90-mm diameter (3M product number 98-0503-0136-9), or equivalent. These disks may be a better choice for samples that are difficult to filter even with the use of a filter aid.

4.3.3 Styrene divinylbenzene (SDB-XC) disks - Empore™ disks, 47-mm diameter (3M product number 98-0503-0067-6), 90-mm diameter (3M product number 98-0503-0068-4), or equivalent.

4.3.4 Styrene divinylbenzene reversed-phase sulfonated (SDB-RPS) disks - Empore™ disks, 47-mm diameter (3M product number 98-0503-0110-4), 90-mm diameter (3M product number 98-0503-0111-2), or equivalent.

4.4 Solid-phase extraction cartridges - Porapak® R SPE device, Waters Corporation, or equivalent. Other solid phases may be employed, provided that adequate performance is demonstrated for the analytes of interest.

4.5 Filtration aid (optional)

4.5.1 Filter Aid 400 - (Fisher Scientific 14-378-3, or equivalent).

4.5.2 In-situ glass micro-fiber prefilter - (Whatman GMF 150, 1-µm pore size, or equivalent).

4.6 Drying column - 22-mm ID glass chromatographic column with a PTFE stopcock (Kontes K-420530-0242, or equivalent).

NOTE: Fritted glass discs used to retain sodium sulfate in some columns are difficult to decontaminate after contact with highly contaminated or viscous extracts. Columns suitable for this method use a small pad of glass wool to retain the drying agent.

4.7 Kuderna-Danish (K-D) apparatus

4.7.1 Concentrator tube - 10-mL, graduated. A ground-glass stopper is used to prevent evaporation of extracts during short-term storage.

4.7.2 Evaporation flask - 500-mL, or other size appropriate for the volumes of solvents to be concentrated. Attach to concentrator tube using springs or clamps.

4.7.3 Three-ball macro-Snyder column.

4.7.4 Two-ball micro-Snyder column (optional).

4.7.5 Springs - ½-inch.

4.8 Solvent Vapor Recovery System - Kontes 545000-1006 or K-547300-0000, Ace Glass 6614-30, or equivalent.

NOTE: The glassware in Sec. 4.6 is recommended for the purpose of solvent recovery during the concentration procedures (Secs. 7.10 and 7.11) requiring the use of Kuderna-Danish evaporative concentrators. Incorporation of this apparatus may be required by State or local municipality regulations that govern air emissions of volatile organics. EPA recommends the incorporation of this type of reclamation system as a method to implement an emissions reduction program. Solvent recovery is a means to conform with waste minimization and pollution prevention initiatives.

4.9 Boiling chips - Solvent extracted, approximately 10/40 mesh (silicon carbide, or equivalent).

4.10 Water bath - Heated, with concentric ring cover, capable of temperature control to within ± 5°C. The bath should be used in a hood.

4.11 Nitrogen evaporation apparatus (optional) - N-Evap, 12- or 24-position (Organomation Model 112, or equivalent).

4.12 Vials, glass - Sizes as appropriate, e.g., 2-mL or 10-mL, with PTFE-lined screw caps or crimp tops for storage of extracts.

4.13 pH indicator paper - Wide pH range.

4.14 Vacuum system - Capable of maintaining a vacuum of approximately 66 cm (26 inches) of mercury.

4.15 Graduated cylinders - Sizes as appropriate.

4.16 Pipets - disposable.

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without decreasing the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

5.2 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Sodium sulfate (granular, anhydrous), Na_2SO_4 - Purify by heating at 400°C for 4 hours in a shallow tray, or by precleaning the sodium sulfate with methylene chloride.

5.4 Solutions for adjusting the pH of samples before extraction.

5.4.1 Sulfuric acid solution (1:1 v/v), H_2SO_4 - Slowly add 50 mL of concentrated H_2SO_4 (sp. gr. 1.84) to 50 mL of organic-free reagent water.

5.4.2 Sodium hydroxide solution (10N), NaOH - Dissolve 40 g NaOH in organic-free reagent water and dilute to 100 mL.

5.5 Extraction, washing, and exchange solvents - At a minimum, all solvents must be pesticide quality or equivalent.

5.5.1 Methylene chloride, CH_2Cl_2.

5.5.2 Hexane, C_6H_{14}.

5.5.3 Ethyl acetate, $CH_3C(OH)OCH_2CH_3$.

5.5.4 Acetonitrile, CH_3CN.

5.5.5 Methanol, CH_3OH.

5.5.6 Acetone, $(CH_3)_2CO$.

5.5.7 Methyl-*tert*-butyl ether (MTBE), $C_5H_{12}O$.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

See the introductory material to Chapter Four, Organic Analytes, Sec. 4.1, Method 3500, Sec. 7.1 of this method, and the specific determinative methods to be employed.

7.0 PROCEDURE

The procedures for solid-phase extraction are very similar for most organic analytes. Therefore, this section describes procedures for sample preparation, pH adjustment, preparation of the extraction apparatus, and extract concentration that apply to all target analytes. The procedures for disk washing, disk conditioning, sample extraction, and sample elution vary among the groups of analytes.

7.1 Sample preparation

Most of the specific procedures described in this method were developed for a nominal sample size of 1 L, as this sample size is usually employed for other extraction methods such as separatory funnel or continuous liquid-liquid extraction. This method also may be employed with smaller samples when overall analytical sensitivity is not a concern or when high levels of the target analytes are anticipated. However, such samples are best collected in a container of appropriate size. The extraction of aqueous samples presents several challenges that must be considered during sample preparation. First, the analytes of interest are often associated with the particulate matter in the sample and sample preparation procedures must ensure that any particulates in the original sample are included in the sample aliquot that is extracted. Secondly, the majority of the organic analytes are hydrophobic and may preferentially adhere to the surfaces of the sample container. For this reason, most extraction methods have traditionally specified that once the sample has been transferred to the extraction apparatus, the sample container be rinsed with solvent which is added to the apparatus. As a result, it is generally <u>not</u> appropriate to extract only part of the sample from a sample container, e.g., 250 mL from a 1-L sample bottle.

The appropriate sample volume may vary with the intended use of the results and, in general, is the volume necessary to provide the analytical sensitivity necessary to meet the objectives of the project (see Chapter Two). Under ideal conditions, the sample should be collected by completely filling the container. The sample should generally be collected without additional volume and with little or no headspace. Thus, a 1-L sample is collected in a 1-L container, a 250-mL sample is collected in a 250-mL container, etc.

Any surrogates and matrix spiking compounds (if applicable) are added to the sample in the original container. The container is then recapped and shaken to mix the spiked analytes into the sample. The extraction of some groups of analytes also requires that the pH of the sample be adjusted to a specified value (see Table 1). When pH adjustment is necessary, it should be performed <u>after</u> the surrogates and matrix spiking compounds (if applicable) have been added and mixed with the sample. Otherwise, the recoveries of these compounds will have little relevance to those of the target analytes in the sample.

If this approach is not possible, then a sample aliquot may be transferred to a graduated cylinder and spiked. However, in such instances, the analyst must take great care to mix the sample well, by shaking, to ensure a homogeneous distribution of the particulate matter and must record the fact that the container was not rinsed.

NOTE: This method may not be appropriate for aqueous samples with greater than 1% solids, as such samples can be difficult to filter and the extraction efficiency may be reduced as a result of the small volumes of solvents employed and the short contact time. If the particulate load significantly slows or prevents filtration, it may be more appropriate to employ an alternative extraction procedure.

7.1.1 Mark the level of the sample on the outside of the sample container for later determination of the sample volume used. Shake the container for several minutes, with the cap tightly sealed, to ensure that any particulate matter is evenly distributed throughout the sample.

7.1.2 Prepare a method blank from a 1-L volume of organic-free reagent water, or a volume similar to that of the samples (e.g., a 250-mL blank should be used when the sample size is 250 mL, etc.). The blank may be prepared in a graduated cylinder, beaker, or other suitable container. The frequency of method blank preparation is described in Chapter One.

7.1.3 Add any surrogate standards listed in the determinative method to the samples in their original containers and to the blank. For disk extractions, also add 5.0 mL of methanol to each sample in the original container. All samples, blanks, and QC samples should receive the same amount of methanol. (This step is not necessary for the cartridge extraction of nitroaromatics and nitramines.) Shake the samples to mix the surrogates and allow the sample to stand for at least several minutes. This will permit the surrogates to dissolve in the sample and will also allow the particulate matter to settle after spiking, which will speed the filtration process somewhat.

7.1.4 Prepare matrix spikes by adding listed matrix spike standards to representative sample replicates in their original containers . The frequency with which matrix spikes are prepared and analyzed is described in Chapter One or as part of the determinative method. Mix the matrix spike samples as described in Sec.7.1.3 and allow to stand.

7.1.5 If cleanup procedures are to be employed that result in the loss of extract, adjust the amount of surrogate and spiking cocktail(s) accordingly. In the case of Method 3640, Gel Permeation Cleanup, double the amount of standards to compensate for the loss of one half of the extract concentrate when loading the GPC column.

7.2 pH adjustment

Check the pH of the sample with wide-range pH paper and, if necessary, adjust the pH to the range listed below. If pH adjustment is required, this step should be performed in the original sample container to ensure that analytes are not lost in precipitates or flocculated material. Any adjustment of the sample pH should take place after the surrogates and matrix spiking compounds are added, so that they are affected by the pH in the same manner as the target analytes.

NOTE: The efficiency of solid-phase extraction of acid herbicide compounds is greatly affected by pH. If acid herbicides are to be extracted from TCLP leachates or other samples, adjust the pH to 1.0 before extraction.

Analyte Group	Extraction pH
Phthalate esters	5 - 7
Organochlorine pesticides	5 - 9
Polychlorinated biphenyls (PCBs)	5 - 9
Organophosphorus pesticides	as received
Nitroaromatics and nitramines	as received
TCLP leachates containing organochlorine pesticides	as produced by TCLP
TCLP leachates containing semivolatiles	as produced by TCLP
TCLP leachates containing phenoxyacid herbicides	1.0

7.3 Setting up the extraction apparatus

7.3.1 Assemble a manifold for multiple disk extractions (Figure 1) using 47-mm or 90-mm extraction disks. Use a filter flask with the standard filter apparatus for single extractions. The solid-phase disks that are generally appropriate for each group of analytes are listed below, and in Table 1.

Analyte Group	Disk Medium
Phthalate esters	C_{18}
Organochlorine pesticides	C_{18}
Polychlorinated biphenyls (PCBs)	C_{18}
Organophosphorus pesticides	SDB-RPS
Nitroaromatics and nitramines	SDB-RPS
TCLP leachates containing organochlorine pesticides	SDB-XC
TCLP leachates containing semivolatiles	SDB-XC
TCLP leachates containing phenoxyacid herbicides	SDB-XC

For nitroaromatics and nitramines, samples also may be extracted using an SPE cartridge. Assemble the cartridge apparatus according to the manufacturer's instructions, using Porapak R, or equivalent, SPE cartridges, and proceed to Sec. 7.6.

7.3.2 If samples contain significant quantities of particulates, the use of a filter aid or prefilter is advisable for disk extractions. Empore™ Filter Aid 400, Whatman GMF 150, or equivalent prefilters are recommended.

7.3.2.1 Pour about 40 g of Filter Aid 400 onto the surface of the disk after assembling the standard filter apparatus.

7.3.2.2 Alternatively, place the Whatman GMF 150 on top of the extraction disk prior to clamping the glass reservoir into the standard filter apparatus.

7.3.2.3 Do not add the filter aid if using the cartridge extraction procedure for nitroaromatics and nitramines.

7.4 Washing the extraction apparatus

Prior to use, the extraction disks must undergo two separate washing steps, usually with different solvents. The steps involved in washing the extraction apparatus before use depend on the analytes of interest and the sample matrix.

7.4.1 First washing step

The following table illustrates the solvents recommended for the first washing step.

Analyte Group	1st solvent wash volume
Phthalate esters	20 mL methylene chloride
Organochlorine pesticides	20 mL methylene chloride
Polychlorinated biphenyls (PCBs)	20 mL methylene chloride
Organophosphorus pesticides	5 mL acetone
Nitroaromatics and nitramines	5 mL acetonitrile
TCLP leachates containing organochlorine pesticides	5 mL acetone
TCLP leachates containing semivolatiles	5 mL acetone
TCLP leachates containing phenoxyacid herbicides	5 mL acetonitrile

Wash the extraction apparatus and disk with the volume of the solvent listed above by rinsing the solvent down the sides of the glass reservoir. Pull a small amount of solvent through the disk with a vacuum. Turn off the vacuum and allow the disk to soak for about one minute. Pull the remaining solvent through the disk and allow the disk to dry.

7.4.1.1 When using a filtration aid, adjust the volume of all wash solvents so the entire filtration bed is submerged.

7.4.1.2 In subsequent conditioning steps, volumes should be adjusted so that a level of solvent is always maintained above the entire filter bed.

7.4.2 Second washing step

The following table illustrates the solvents recommended for the second washing step.

Analyte Group	2nd solvent wash volume
Phthalate esters	10 mL acetone
Organochlorine pesticides	10 mL acetone
Polychlorinated biphenyls (PCBs)	not required
Organophosphorus pesticides	5 mL methanol
Nitroaromatics and nitramines	15 mL acetonitrile
TCLP leachates containing organochlorine pesticides	5 mL ethyl acetate
TCLP leachates containing semivolatiles	5 mL ethyl acetate
TCLP leachates containing phenoxyacid herbicides	not required

7.5 Disk conditioning

The extraction disks are composed of hydrophobic materials which will not allow water to pass unless they are pre-wetted with a water-miscible solvent before being used for sample extraction. This step is referred to as conditioning, and the solvent used is dependent on the analytes of interest. The following table illustrates the solvents recommended for specific groups of analytes.

NOTE: Beginning with the conditioning step, it is CRITICAL that the disk NOT go dry until after the extraction steps are completed. Should a disk accidentally go dry during the conditioning steps, the conditioning steps for that disk must be repeated prior to adding the sample.

Analyte Group	Conditioning steps
Phthalate esters	20 mL methanol, soak 1 min, 20 mL reagent water
Organochlorine pesticides	20 mL methanol, soak 1 min, 20 mL reagent water
Polychlorinated biphenyls (PCBs)	20 mL methanol, soak 1 min, 20 mL reagent water
Organophosphorus pesticides	5 mL methanol, soak 1 min, 20 mL reagent water
Nitroaromatics and nitramines	15 mL acetonitrile, soak 3 min 30 mL reagent water
TCLP leachates containing organochlorine pesticides	5 mL methanol soak 1 min, 15 mL reagent water
TCLP leachates containing semivolatiles	5 mL methanol soak 1 min, 15 mL reagent water
TCLP leachates containing phenoxyacid herbicides	5 mL methanol soak 1 min, 15 mL reagent water

7.5.1 Add the conditioning solvent to the extraction apparatus. Apply a vacuum until a few drops of solvent pass through the disk, ensuring that the disk is soaked with the solvent. Turn off the vacuum and allow the disk to soak in the solvent for the time specified above.

7.5.2 When using a filtration aid, adjust the volume of conditioning solvents so that the entire filtration bed remains submerged until the extraction is completed.

7.5.3 Once the soaking time is over, apply the vacuum again, drawing all but a thin layer of solvent through the disk. Stop the vacuum just before the disk goes dry.

7.5.4 Add the volume of organic-free reagent water listed above and apply vacuum to draw the water through the disk. Stop the vacuum just before the disk goes dry, leaving 2-3 mm of water above the surface of the disk.

7.5.5 Proceed to Sec. 7.7 for the sample extraction instructions.

7.6 Cartridge procedure for nitroaromatics and nitramines

Aqueous samples to be analyzed for nitroaromatics and nitramines may also be extracted using the SPE cartridge technique described below. The same sample preparation considerations discussed in Sec. 7.1 also apply to this procedure.

7.6.1 After assembling the SPE cartridge in the extraction apparatus (see Sec. 7.3.1), wash the cartridge with 10 mL of acetonitrile, using gravity flow. Do not allow the cartridge to go dry.

7.6.2 When only a thin layer of solvent remains above the sorbent bed in the cartridge, add 30 mL of reagent water to the cartridge and allow it to flow through the sorbent bed under gravity flow. Stop the flow just before the cartridge goes dry.

7.6.3 Attach a connector to the top of the cartridge. The other end of the connector should be fitted with flexible PTFE tubing long enough to reach into the sample bottle or other container (e.g., a beaker) holding the sample.

7.6.4 Turn on the vacuum, and draw the sample through the cartridge at a rate of about 10 mL/min, until all of the sample has passed through the cartridge. As particulate matter plugs the cartridge and slows the flow, increase the vacuum to maintain a reasonable flow rate.

7.6.5 Once all of the sample has been pulled through the cartridge, shut off the vacuum and add 5 mL of reagent water to the cartridge. Allow the reagent water to pass through the cartridge under gravity flow, if practical, or apply a vacuum to complete the process. Shut off the flow once the water has been drawn through the cartridge.

7.6.6 Method blanks and matrix spike aliquots (Sec. 7.1) are handled in the same manner as the samples.

7.6.7 Proceed with sample elution, as described in Sec. 7.9.

7.7 Sample extraction using SPE disks

7.7.1 Pour the sample into the reservoir and, under full vacuum, filter it as quickly as the vacuum will allow (at least 10 minutes). Transfer as much of the measured volume of water as possible.

NOTE: With heavily particle-laden samples, allow the sediment in the sample to settle and decant as much liquid as is practical into the reservoir. After most of the aqueous portion of the sample has passed through the disk, swirl the portion of the sample containing sediment and add it to the reservoir. Use additional portions of organic-free reagent water to transfer any remaining particulates to the reservoir. Particulates must be transferred to the reservoir before all of the aqueous sample has passed through the disk.

7.7.2 After the sample has passed through the solid-phase media, dry the disk by maintaining vacuum for about 3 minutes. Method blanks and matrix spike aliquots (Sec. 7.1) are handled in the same manner as the samples.

7.8 Elution of the analytes from the disk

The choice of elution solvent is critical to the success of solid-phase extraction. The recommended elution solvent for each group of analytes is listed below.

Analyte Group	Sample elution steps
Phthalate esters	5 mL acetone, soak 15-20 sec. Rinse bottle with 15 mL acetonitrile and add to disk.
Organochlorine pesticides	5 mL acetone, soak 15-20 sec. Rinse bottle with 15 mL methylene chloride and add to disk.
Polychlorinated biphenyls (PCBs)	5 mL acetone, soak 15-20 sec. Rinse bottle with 20 mL acetonitrile and add to disk.
Organophosphorus pesticides	0.6 mL acetone, soak 1 min. Rinse bottle with 5 mL MTBE and add to disk. Repeat bottle rinse twice more.
Nitroaromatics and nitramines	5 mL acetonitrile, soak 3 min.
TCLP leachates containing organochlorine pesticides	Rinse bottle with 4 mL acetone and add to disk. Rinse glassware with 2 mL acetone and add to disk. Soak 1 min. Rinse bottle twice with 5 mL ethyl acetate and add to disk.
TCLP leachates containing semivolatiles	Rinse bottle with 4 mL acetone and add to disk. Rinse glassware with 2 mL acetone and add to disk. Soak 1 min. Rinse bottle twice with 5 mL ethyl acetate and add to disk.
TCLP leachates containing phenoxyacid herbicides	Rinse bottle with 5 mL acetonitrile and add to disk. Soak 1 min. Rinse bottle twice more with 5 mL acetonitrile and add to disk.

7.8.1 Remove the entire standard filter assembly (do not disassemble) from the manifold and insert a collection tube. The collection tube should have sufficient capacity to hold all of the elution solvents. The drip tip of the filtration apparatus should be seated sufficiently below the neck of the collection tube to prevent analyte loss due to splattering when vacuum is applied. When using a filter flask for single extractions, empty the water from the flask before inserting the collection tube.

7.8.2 An initial elution with a water-miscible solvent, i.e., acetone or acetonitrile, improves the recovery of analytes trapped in water-filled pores of the sorbent. Use of a water-miscible solvent is particularly critical when methylene chloride is used as the second elution solvent. With the collection tube in place, add the volume of elution solvent listed above to the extraction apparatus. Allow the solvent to spread out evenly across the disk (or inert filter) then quickly turn the vacuum on and off to pull the first drops of sovlent through the disk. Allow the disk to soak for 15 to 20 seconds before proceeding to Sec. 7.8.3

7.8.3 Rinse the sample bottle and/or glassware that held the sample with the second solvent listed above and transfer the solvent rinse to the extraction apparatus. As needed, use a disposable pipette to rinse the sides of the extraction apparatus with solvent from the bottle.

7.8.4 Draw about half of the solvent through the disk and then release the vacuum. Allow the remaining elution solvent to soak the disk and particulates for about one minute before drawing the remaining solvent through the disk under vacuum. When using a filtration aid, adjust the volume of elution solvent so that the entire filtration bed is initially submerged.

7.8.5 Repeat the bottle rinsing step as listed in the table above, continuing to apply vacuum and collecting the solvent in the tube.

7.9 Eluting the nitroaromatics and nitramines from the cartridge

Once the reagent water has passed through the column, place a collection tube under the cartridge. Add 5 mL of acetonitrile to the top of the cartridge and allow it to pass through the cartridge under gravity flow, collecting the solvent in the collection tube. Measure the volume of acetonitrile recovered from the cartridge.

7.10 K-D concentration technique

Where necessary to meet the sensitivity requirements, sample extracts may be concentrated to the final volume necessary for the determinative method and specific application, using the K-D technique or nitrogen evaporation.

7.10.1 Assemble a Kudema-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to an appropriately sized evaporation flask.

7.10.2 Dry the combined extracts in the collection tube (Secs. 7.8 and 7.9) by passing them through a drying column containing about 10 g of anhydrous sodium sulfate. Collect the dried extract in the K-D concentrator. Use acidified sodium sulfate (see Method 8151) if acidic analytes are to be measured.

7.10.3 Rinse the collection tube and drying column into the K-D flask with an additional 20-mL portion of solvent in order to achieve a quantitative transfer.

7.10.4 Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Attach the solvent vapor recovery glassware (condenser and collection device, see Sec. 4.6) to the Snyder column of the K-D apparatus, following the manufacturer's instructions. Pre-wet the Snyder column by adding about 1 mL of methylene chloride (or other suitable solvent) to the top of the column. Place the K-D apparatus on a hot water bath (15 - 20°C above the boiling point of the solvent) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 10 - 20 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

7.10.4.1 If a solvent exchange is required (as indicated in Table 1), momentarily remove the Snyder column, add 50 mL of the exchange solvent and a new boiling chip.

7.10.4.2 Reattach the Snyder column. Concentrate the extract, raising the temperature of the water bath, if necessary, to maintain a proper distillation rate.

7.10.5 Remove the Snyder column. Rinse the K-D flask and the lower joints of the Snyder column into the concentrator tube with 1 - 2 mL of solvent. The extract may be further concentrated by using one of the techniques outlined in Sec. 7.11, or adjusted to a final volume of 5.0 - 10.0 mL using an appropriate solvent (Table 1).

7.11 If further concentration is required, use either the micro-Snyder column technique (7.11.1) or nitrogen evaporation technique (7.11.2).

7.11.1 Micro-Snyder column technique

7.11.1.1 Add a fresh clean boiling chip to the concentrator tube and attach a two-ball micro-Snyder column directly to the concentrator tube. Attach the solvent vapor recovery glassware (condenser and collection device) to the micro-Snyder column of the K-D apparatus, following the manufacturer's instructions. Pre-wet the Snyder column by adding 0.5 mL of methylene chloride or the exchange solvent to the top of the column. Place the micro-concentration apparatus in a hot water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature, as necessary, to complete the concentration in 5 - 10 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood.

7.11.1.2 When the apparent volume of liquid reaches 0.5 mL, remove the apparatus from the water bath and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse its lower joints into the concentrator tube with 0.2 mL of solvent. Adjust the final extract volume to 1.0 - 2.0 mL.

7.11.2 Nitrogen evaporation technique

7.11.2.1 Place the concentrator tube in a warm bath (30°C) and evaporate the solvent volume to 0.5 mL using a gentle stream of clean, dry nitrogen (filtered through a column of activated carbon).

CAUTION: New plastic tubing must not be used between the carbon trap and the sample, since it may introduce phthalate interferences.

7.11.2.2 Rinse down the internal wall of the concentrator tube several times with solvent during the concentration. During evaporation, position the concentrator tube to avoid condensing water into the extract. Under normal procedures, the extract must not be allowed to become dry.

CAUTION: When the volume of solvent is reduced below 1 mL, some semivolatile analytes such as cresols may be lost.

7.12 The extract may now be subjected to cleanup procedures or analyzed for the target analytes using the appropriate determinative technique(s). If further handling of the extract will not be performed immediately, stopper the concentrator tube and store in a refrigerator. If the extract will be stored longer than 2 days, it should be transferred to a vial with a PTFE-lined screw-cap, and labeled appropriately.

8.0 QUALITY CONTROL

8.1 Any reagent blanks or matrix spike samples should be subjected to exactly the same analytical procedures as those used for actual samples.

8.2 Refer to Chapter One for general quality control procedures and Method 3500 for specific QC procedures for extraction and sample preparation.

9.0 METHOD PERFORMANCE

Refer to the determinative methods listed in Sec. 1.1 for performance data related to solid-phase extraction.

10.0 REFERENCES

1. Lopez-Avila, V., Beckert, W., et. al., "Single Laboratory Evaluation of Method 8060 - Phthalate Esters", EPA/600/4-89/039.

2. Tomkins, B.A., Merriweather, R., et. al., "Determination of Eight Organochlorine Pesticides at Low Nanogram/Liter Concentrations in Groundwater Using Filter Disk Extraction and Gas Chromatography", JAOAC International, 75(6), pp. 1091-1099 (1992).

3. Markell, C., "3M Data Submission to EPA," letter to B. Lesnik, June 27, 1995.

4. Jenkins, T. F., Thorne, P. G., Myers, K. F., McCormick, E. F., Parker, D. E., and B. L. Escalon (1995). Evaluation of Clean Solid Phases for Extraction of Nitroaromatics and Nitramines from Water. USACE Cold Regions Research and Engineering Laboratory, Special Report 95-22.

TABLE 1

SPECIFIC EXTRACTION CONDITIONS FOR VARIOUS DETERMINATIVE METHODS

Determinative Method	Extraction pH	Disk Medium[a]	Elution Solvent	Exchange Solvent	Final Extract Volume for Analysis (mL)[b]
8061 (phthalate esters)	5-7	C_{18}	acetonitrile	hexane	10.0
8081 (organochlorine pesticides)	5-9	C_{18}	methylene chloride	hexane	10.0
8082 (PCBs)	5-9	C_{18}	methylene chloride	hexane	10.0
8141 (organophosphorus pesticides)	as received	SDB-RPS	MTBE	hexane	10.0
8330 (nitroaromatics and nitramines)	as received	SDB-RPS	acetonitrile	acetonitrile	10.0
TCLP pesticides (8081)	as produced by TCLP	SDB-XC	ethyl acetate	hexane	10.0
TCLP semivolatiles (8270)	as produced by TCLP	SDB-XC	ethyl acetate	methylene chloride	1.0
TCLP phenoxyacid herbicides (8321)	1.0	SDB-XC	acetonitrile	hexane	10.0

[a] SDB has a greater capacity than C_{18} and a greater affinity for more analytes but they may be more difficult to elute.

[b] For methods where the suggested final extract volume is 10.0 mL, the volume may be reduced to as low as 1.0 mL to achieve lower detection limits.

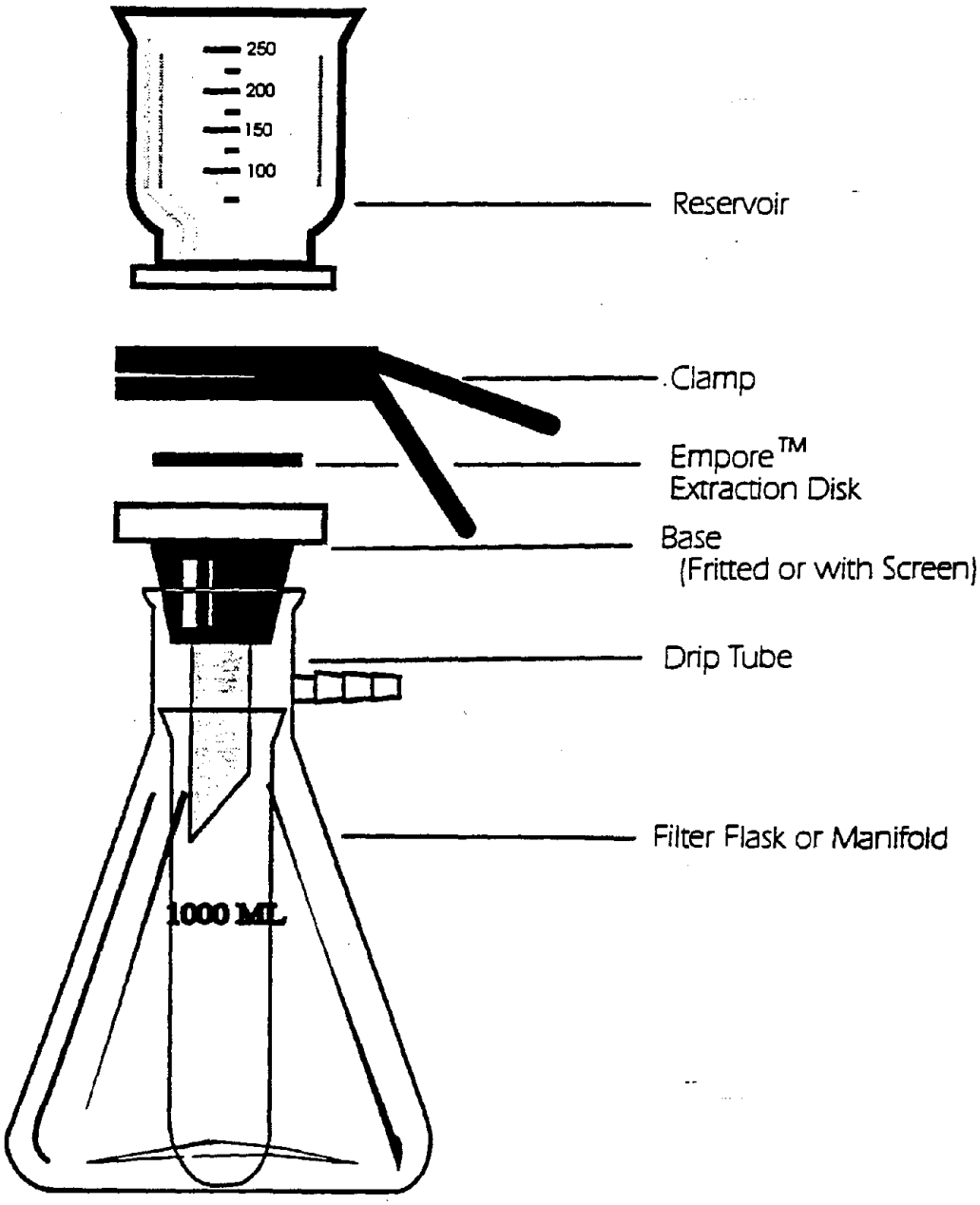
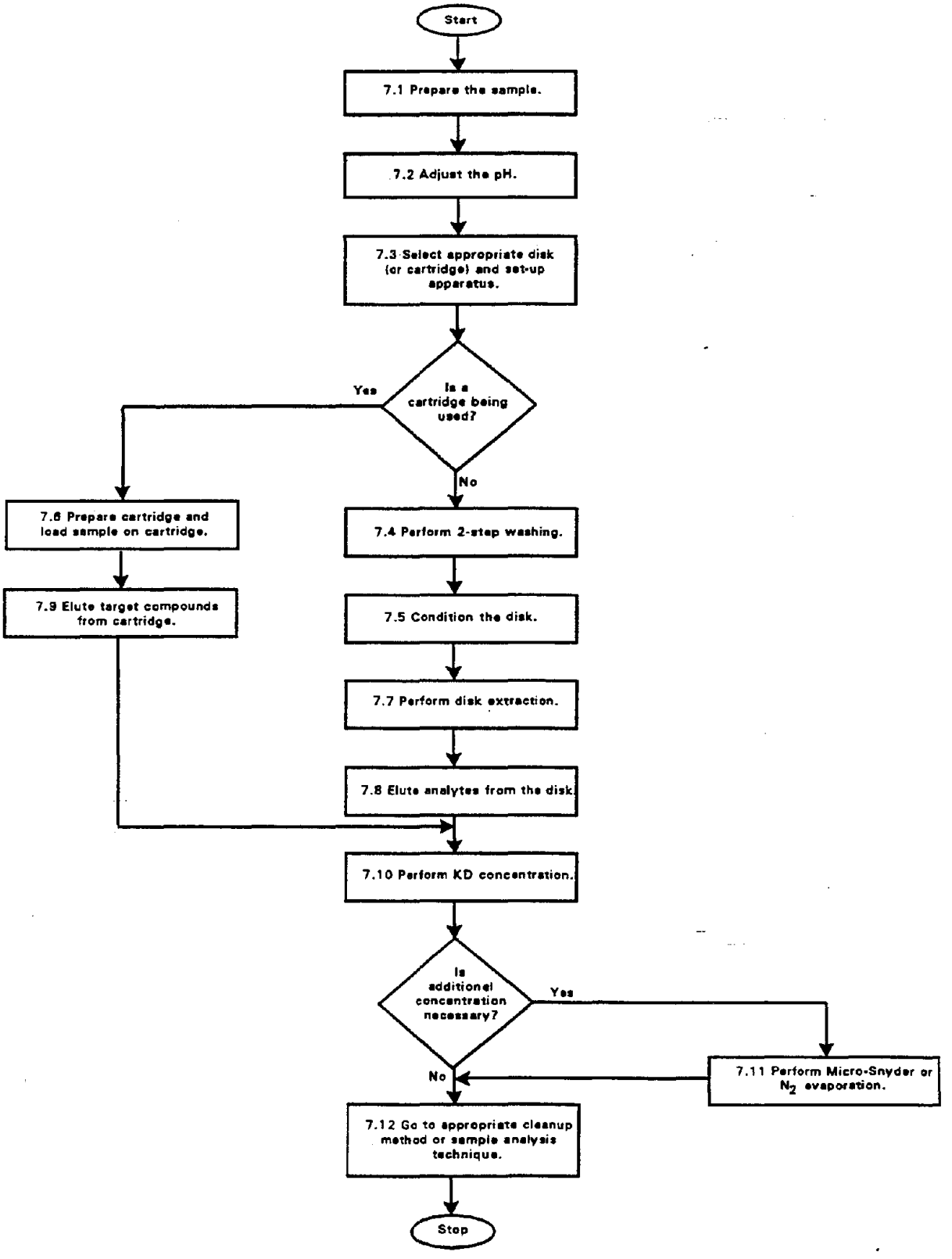


FIGURE 1
DISK EXTRACTION APPARATUS

METHOD 3535A
SOLID-PHASE EXTRACTION (SPE)



METHOD 3545A

PRESSURIZED FLUID EXTRACTION (PFE)

1.0 SCOPE AND APPLICATION

1.1 Method 3545 is a procedure for extracting water insoluble or slightly water soluble organic compounds from soils, clays, sediments, sludges, and waste solids. The method uses elevated temperature (100 - 180°C) and pressure (1500 - 2000 psi) to achieve analyte recoveries equivalent to those from Soxhlet extraction, using less solvent and taking significantly less time than the Soxhlet procedure. This procedure was developed and validated on a commercially-available, automated extraction system.

1.2 This method is applicable to the extraction of semivolatile organic compounds, organophosphorus pesticides, organochlorine pesticides, chlorinated herbicides, PCBs, and PCDDs/PCDFs, which may then be analyzed by a variety of chromatographic procedures.

1.3 This method has been validated for solid matrices containing 250 to 12,500 µg/kg of semivolatile organic compounds, 250 to 2500 µg/kg of organophosphorus pesticides, 5 to 250 µg/kg of organochlorine pesticides, 50 to 5000 µg/kg of chlorinated herbicides, 1 to 1400 µg/kg of PCBs, and 1 to 2500 ng/kg of PCDDs/PCDFs. The method may be applicable to samples containing these analytes at higher concentrations and may be employed after adequate performance has been demonstrated for the concentrations of interest (see Method 3500, Sec. 8.0).

1.4 This method is applicable to solid samples only, and is most effective on dry materials with small particle sizes. Therefore, waste samples must undergo phase separation, as described in Chapter Two, and only the solid phase material is to be extracted by this procedure. If possible, soil/sediment samples may be air-dried and ground to a fine powder prior to extraction. Alternatively, if the loss of analytes or during drying is a concern, soil/sediment samples may be mixed with anhydrous sodium sulfate or pelletized diatomaceous earth. (Drying and grinding samples containing PCDDs/PCDFs is *not* recommended, due to safety concerns). The total mass of material to be prepared depends on the specifications of the determinative method and the sensitivity required for the analysis, but 10 - 30 g of material are usually necessary and can be accommodated by this extraction procedure.

1.5 This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Samples are prepared for extraction either by air drying the sample, or by mixing the sample with anhydrous sodium sulfate or pelletized diatomaceous earth. The sample is then ground and loaded into the extraction cell. Drying and grinding samples containing PCDDs/PCDFs is *not* recommended, due to safety concerns. Grinding may also be a concern for other more volatile analytes. (See Sec. 7.1).

2.2 The extraction cell containing the sample is heated to the extraction temperature (see Sec. 7.8), pressurized with the appropriate solvent system, and extracted for 5 minutes (or as recommended by the instrument manufacturer). Multiple extractions are recommended for some

groups of analytes. The solvent systems used for this procedure vary with the analytes of interest and are described in Sec. 5.5.

2.3 The solvent is collected from the heated extraction vessel and allowed to cool.

2.4 The extract may be concentrated, if necessary, and, as needed, exchanged into a solvent compatible with the cleanup or determinative step being employed.

3.0 INTERFERENCES

3.1 Refer to Method 3500.

3.2 If necessary, Florisil and/or sulfur cleanup procedures may be employed. In such cases, proceed with Method 3620 and/or Method 3660.

3.3 Samples for PCDD/PCDF analysis should be subjected to the various cleanup procedures described in the determinative methods (8280 and 8290).

4.0 APPARATUS AND MATERIALS

4.1 Pressurized fluid extraction device

4.1.1 Dionex Accelerated Solvent Extractor or Supelco SFE-400 with appropriately-sized extraction cells. Currently, cells are available that will accommodate 10-g, 20-g and 30-g samples. Cells should be made of stainless steel or other material capable of withstanding the pressure requirements (2000+ psi) necessary for this procedure.

4.1.2 Other system designs may be employed, provided that adequate performance can be demonstrated for the analytes and matrices of interest.

4.2 Apparatus for determining percent dry weight

4.2.1 Oven - drying

4.2.2 Desiccator

4.2.3 Crucibles - porcelain or disposable aluminum

4.3 Apparatus for grinding - capable of reducing particle size to < 1 mm.

4.4 Analytical balance - capable of weighing to 0.01 g.

4.5 Vials for collection of extracts - 40-mL or 60-mL, pre-cleaned, open top screw-cap with PTFE-lined silicone septum (Dionex 049459, 049460, 049461, 049462 or equivalent).

4.6 Filter disk - 1.91 cm, Type D28 (Whatman 10289356, or equivalent).

4.7 Cell cap sealing disk (Dionex 49454, 49455, or equivalent).

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Organic-free reagent water. All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Drying agents

5.3.1 Sodium sulfate (granular anhydrous), Na_2SO_4.

5.3.2 Pelletized diatomaceous earth.

5.3.3 The drying agents should be purified by heating at 400°C for 4 hours in a shallow tray, or by extraction with methylene chloride. If extraction with methylene chloride is employed, then a reagent blank should be prepared to demonstrate that the drying agent is free of interferences.

5.3.4 Quartz sand. Although not strictly a drying agent, clean sand may be used to facilitate grinding of some sample matrices, to fill void volumes in the extraction cell, and to increase the flow of solvent through the sample. It may be prepared as described in Sec. 5.3.3.

5.4 Acids

5.4.1 Phosphoric acid solution (see Sec. 5.5.5). Prepare a 1:1 (v/v) solution of 85% phosphoric acid (H_3PO_4) in organic-free reagent water.

5.4.2 Trifluoroacetic acid solution (see Sec. 5.5.5). Prepare a 1% (v/v) solution of trifluoroacetic acid in acetonitrile.

5.4.3 Glacial acetic acid (see Sec. 5.5.6).

5.5 Extraction solvents

The extraction solvent to be employed depends on the analytes to be extracted, as described below. All solvents should be pesticide quality or equivalent. Solvents may be degassed prior to use.

5.5.1 Organochlorine pesticides may be extracted with acetone/hexane (1:1, v/v), CH_3COCH_3/C_6H_{14} or acetone/methylene chloride (1:1,v/v), CH_3COCH_3/CH_2Cl_2.

5.5.2 Semivolatile organics may be extracted with acetone/methylene chloride (1:1, v/v), CH_3COCH_3/CH_2Cl_2 or acetone/hexane (1:1, v/v), CH_3COCH_3/C_6H_{14}.

5.5.3 PCBs may be extracted with acetone/hexane (1:1, v/v), CH_3COCH_3/C_6H_{14} or acetone/methylene chloride (1:1, v/v), CH_3COCH_3/CH_2Cl_2 or hexane, C_6H_{14}.

5.5.4 Organophosphorus pesticides may be extracted with methylene chloride, CH_2Cl_2 or acetone/methylene chloride (1:1, v/v), CH_3COCH_3/CH_2Cl_2.

5.5.5 Chlorinated herbicides may be extracted with an acetone/methylene chloride/phosphoric acid solution (250:125:15, v/v/v), $CH_3COCH_3/CH_2Cl_2/H_3PO_4$, or an acetone/methylene chloride/trifluoroacetic acid solution (250:125:1, v/v/v), $CH_3COCH_3/CH_2Cl_2/CF_3COOH$. (If the second option is used, the trifluoroacetic acid solution should be prepared by mixing 1% trifluoroacetic acid in acetonitrile.) Make fresh solutions before each batch of extractions.

5.5.6 PCDDs/PCDFs may be extracted with toluene, $C_6H_5CH_3$. Fly ash samples to be extracted for PCDDs/PCDFs may be extracted with a toluene/acetic acid solution (5% v/v glacial acetic acid in toluene) in lieu of the HCl pretreatment described in Methods 8280 and 8290.

5.5.7 Other solvent systems may be employed, provided that the analyst can demonstrate adequate performance for the analytes of interest in the sample matrix (see Method 3500, Sec. 8.0).

CAUTION: For best results with very wet samples (e.g., ≥30% moisture), reduce or eliminate the quantity of hydrophilic solvent used.

5.6 High-purity gases such as nitrogen, carbon dioxide, or helium are used to purge and/or pressurize the extraction cell. Follow the instrument manufacturer's recommendation for the choice of gases.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

See the introductory material to this chapter, Organic Analysis, Sec. 4.1, and Method 3500.

7.0 PROCEDURE

7.1 Sample preparation

7.1.1 Sediment/soil samples - Decant and discard any water layer on a sediment sample. Mix the sample thoroughly, especially composited samples. Discard any foreign objects such as sticks, leaves, and rocks. Air dry the sample at room temperature for 48 hours in a glass tray or on hexane-rinsed aluminum foil. Alternatively, mix the sample with an equal volume of anhydrous sodium sulfate or pelletized diatomaceous earth until a free-flowing powder is obtained.

NOTE: Dry, finely-ground soil/sediment allows the best extraction efficiency for nonvolatile, nonpolar organics, e.g., 4,4'-DDT, PCBs, etc. Air-drying may not be appropriate for the analysis of the more volatile organochlorine pesticides (e.g., the BHCs) or the more volatile of the semivolatile organics because of losses during the drying process. *Drying of samples for PCDDs/PCDFs is not generally recommended, due to safety concerns with samples containing these analytes.* The use of sodium sulfate as a drying agent can lead to clogging of the frits in the cell with recrystallized sodium sulfate. (See "Caution" following Sec. 5.5.6.)

7.1.2 Waste samples - Multiphase waste samples must be prepared by the phase separation method in Chapter Two before extraction. *This extraction procedure is for solids only.*

7.1.3 Dry sediment/soil and dry waste samples amenable to grinding - Grind or otherwise reduce the particle size of the waste so that it either passes through a 1-mm sieve or can be extruded through a 1-mm hole. Disassemble grinder between samples, according to manufacturer's instructions, and decontaminate with soap and water, followed by acetone and hexane rinses. *Grinding of samples for PCDDs/PCDFs is not generally recommended, due to safety concerns with samples containing these analytes.*

NOTE: The note in Sec. 7.1.1 also applies to the grinding process.

7.1.4 Gummy, fibrous, or oily materials not amenable to grinding should be cut, shredded, or otherwise reduced in size to allow mixing and maximum exposure of the sample surfaces for the extraction. The analyst may add anhydrous sodium sulfate, pelletized diatomaceous earth, sand, or other clean, dry reagents to the sample to make it more amenable to grinding.

7.1.5 Solid samples for PCDD/PCDF analysis are generally carefully mixed with clean sand and a drying agent such as diatomaceous earth or sodium sulfate, breaking up lumps with a spatula or other suitable tool.

7.1.6 Fly ash samples may be pretreated with an HCl solution prior to extraction (See Sec. 7 of Method 8280 or 8290). Alternatively, they may be extracted with the toluene/acetic acid solution described in Sec. 5.5.6.

7.2 Determination of percent dry weight - When sample results are to be calculated on a dry weight basis, a second portion of sample should be weighed at the same time as the portion used for analytical determination.

WARNING: The drying oven should be contained in a hood or vented. Significant laboratory contamination may result from drying a heavily contaminated sample.

7.2.1 Immediately after weighing the sample for extraction, weigh 5 - 10 g of the sample into a tared crucible. Dry this aliquot overnight at 105°C. Allow to cool in a desiccator before weighing. Calculate the % dry weight as follows:

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

7.3 Grind a sufficient weight of the dried sample from Sec. 7.1 to yield the sample weight needed for the determinative method (usually 10 - 30 g). Grind the sample until it passes through a 10 mesh sieve. *Grinding of samples for PCDDs/PCDFs is not generally recommended, due to safety concerns with samples containing these analytes.*

7.4 Transfer the ground sample to an extraction cell of the appropriate size for the aliquot. Generally, an 11-mL cell will hold about 10 g of material, a 22-mL cell will hold about 20 g of material, and a 33-mL cell will hold about 30 g of material. The weight of a specific sample that a cell will contain depends on the bulk density of the sample and the amount of drying agent that must be added to the sample in order to make it suitable for extraction. Analysts should ensure that the

sample aliquot extracted is large enough to provide the necessary sensitivity and choose the extraction cell size accordingly. Use disposable cellulose or glass fiber filters in the cell outlets. Clean sand may be used to fill any void volume in the extraction cells.

7.5 Add the surrogates (or labeled internal standards for PCDDs/PCDFs) listed in the determinative method to each sample. Add the matrix spike/matrix spike duplicate compounds listed in the determinative method to the two additional aliquots of the sample selected for spiking.

7.6 Place the extraction cell into the instrument or autosampler tray, as described by the instrument manufacturer.

7.7 Place a precleaned collection vessel in the instrument for each sample, as described by the instrument manufacturer. The total volume of the collected extract will depend on the specific instrumentation and the extraction procedure recommended by the manufacturer and may range from 0.5 to 1.4 times the volume of the extraction cell. Ensure that the collection vessel is sufficiently large to hold the extract.

7.8 Recommended extraction conditions

7.8.1 Semivolatiles, organophosphorus pesticides, organochlorine pesticides, herbicides, and PCBs

Oven temperature:	100°C
Pressure:	1500 - 2000 psi
Static time:	5 min (after 5 min pre-heat equilibration)
Flush volume:	60% of the cell volume
Nitrogen purge:	60 sec at 150 psi (purge time may be extended for larger cells)
Static Cycles:	1

7.8.2 PCDDs/PCDFs

Oven temperature:	150 - 175°C
Pressure:	1500 - 2000 psi
Static time:	5-10 min (after 5 min pre-heat equilibration)
Flush volume:	60 - 75% of the cell volume
Nitrogen purge:	60 sec at 150 psi (purge time may be extended for larger cells)
Static Cycles:	2 or 3

7.8.3 Optimize the conditions, as needed, according to the manufacturer's instructions. In general, the pressure is not a critical parameter, as the purpose of pressurizing the extraction cell is to prevent the solvent from boiling at the extraction temperature and to ensure that the solvent remains in intimate contact with the sample. Any pressure in the range of 1500 - 2000 psi should suffice.

7.8.4 Once established, the same pressure should be used for all samples extracted for the same analysis type.

7.9 Begin the extraction according to the manufacturer's instructions. For PCDD/PCDF extraction, 2 to 3 static extractions are recommended.

7.10 Collect each extract in a clean vial (see Sec. 7.7). Allow the extracts to cool after the extractions are complete.

7.11 The extract is now ready for concentration, cleanup, or analysis, depending on the extent of interferants and the determinative method to be employed. Refer to Method 3600 for guidance on selecting appropriate cleanup methods. Excess water present in extracts may be removed by filtering the extract through a bed of anhydrous sodium sulfate. Certain cleanup and/or determinative methods may require a solvent exchange prior to cleanup and/or sample analysis.

7.12 If the phosphoric acid solution in Sec. 5.5.5 is used for the extraction of chlorinated herbicides, then the extractor should be rinsed by pumping acetone through all the lines of the system. The use of other solvents for these analytes may not require this rinse step.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for guidance on quality control procedures. Refer to Method 3500 for specific guidance on extraction and sample preparation procedures.

8.2 Before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a solid matrix method blank (e.g., clean sand). Each time samples are extracted, and when there is a change in reagents, a method blank needs to be extracted and analyzed for the compounds of interest. The method blank should be carried through all stages of the sample preparation and measurement.

8.3 Standard quality assurance practices should be used with this method. Field duplicates should be collected to validate the precision of the sampling procedures. A matrix spike/matrix spike duplicate, or matrix spike and duplicate sample analysis, and a laboratory control sample should be prepared and analyzed with each batch of samples prepared by this procedure, unless the determinative method provides other guidance.

8.4 When listed in the appropriate determinative method, surrogate standards should be added to all samples prior to extraction. For PCDDs/PCDFs, the labeled internal standards listed in the determinative methods should be added to all samples prior to extraction.

9.0 METHOD PERFORMANCE

9.1 Chlorinated pesticides and semivolatile organics

Single-laboratory accuracy data were obtained for chlorinated pesticides and semivolatile organics at three different spiking concentrations in three different soil types. Spiking concentrations ranged from 5 to 250 µg/kg for the chlorinated pesticides and from 250 to 12500 µg/kg for the semivolatiles. Spiked samples were extracted both by the Dionex Accelerated Solvent Extraction system and by a Perstorp Environmental Soxtec™ (automated Soxhlet). Extracts were analyzed either by Method 8270 or Method 8081. Method blanks, spikes and spike duplicates were included for the low concentration spikes; matrix spikes were included for all other concentrations. The data are reported in detail in Reference 1, and represent seven replicate extractions and analyses for each sample. Data summary tables are included in Methods 8270 and 8081.

9.2 Organophosphorus pesticides and chlorinated herbicides

Single-laboratory accuracy data were obtained for organophosphorus pesticides (OPPs) and chlorinated herbicides at two different spiking concentrations in three different soil types. Spiking

concentrations ranged from 250 to 2500 µg/kg for the OPPs and from 50 to 5000 µg/kg for the chlorinated herbicides. Chlorinated herbicides were spiked with a mixture of the free acid and the ester (1:1). Spiked samples were extracted both by the Dionex Accelerated Solvent Extractor and by Soxhlet for the OPPs. Extracts were analyzed by Method 8141. Spiked chlorinated herbicides were extracted by the Dionex Accelerated Solvent Extractor and by the shaking method described in Method 8151. Extracts were analyzed by Method 8151. Method blanks, spikes and spike duplicates were included for the low concentration spikes; matrix spikes were included for all other concentrations. The data are reported in detail in Reference 2, and represent seven replicate extractions and analyses for each sample. Data summary tables are included in Methods 8141 and 8151.

9.3 PCBs

Single-laboratory accuracy data were obtained for PCBs from a soil sample with PCB content certified by NIST (Standard Reference Material, SRM 1939, River Sediment). A PCB-contaminated soil was purchased from a commercial source. Spiking or certified concentrations ranged from 1 to 1400 µg/kg. Samples were extracted by the Dionex Accelerated Solvent Extractor and by Soxtec™ (Perstorp Environmental). Extracts were analyzed using Method 8082. Method blanks, spikes and spike duplicates were included. The data are reported in Reference 2, and represent seven replicate extractions and analyses for each sample. Data summary tables are included in Method 8082.

9.4 PCDDs/PCDFs

Single-laboratory data were obtained for PCDDs/PCDFs from ground chimney brick, urban dust, fly ash, a relatively highly contaminated soil sample (EC-2, National Water Research Institute, Burlington, Ontario, Canada), a low-level sediment sample (HS-2, National Research Council Institute of Marine Biosciences, Halifax, Nova Scotia, Canada) and various field-contaminated soils and sediments. Concentrations of PCDDs/PCDFs ranged from low ng/kg to mid µg/kg levels. Samples were extracted by the Dionex Accelerated Solvent Extractor and by traditional Soxhlet techniques. Extracts were analyzed by a high resolution mass spectrometric method employing isotope dilution quantitation. The data are reported in Reference 3. Data summary tables are included in Method 8290.

10.0 REFERENCES

1. B. Richter, Ezzell, J., and Felix, D., "Single Laboratory Method Validation Report. Extraction of TCL/PPL (Target Compound List/Priority Pollutant List) BNAs and Pesticides using Accelerated Solvent Extraction (ASE) with Analytical Validation by GC/MS and GC/ECD"; Document 116064.A, Dionex Corporation, June 16, 1994.

2. B. Richter, Ezzell, J., and Felix, D., "Single Laboratory Method Validation Report. Extraction of TCL/PPL (Target Compound List/Priority Pollutant List) OPPs, Chlorinated Herbicides and PCBs using Accelerated Solvent Extraction (ASE)". Document 101124, Dionex Corporation, December 2, 1994).

3. B. E. Richter *et al.*, "Extraction of Polychlorinated Dibenzo-*p*-Dioxins and Polychlorinated Dibenzofurans from Environmental Samples Using Accelerated Solvent Extraction (ASE)." *Chemosphere*, 34(5-7), pp. 975-987, 1997.

11.0 SAFETY

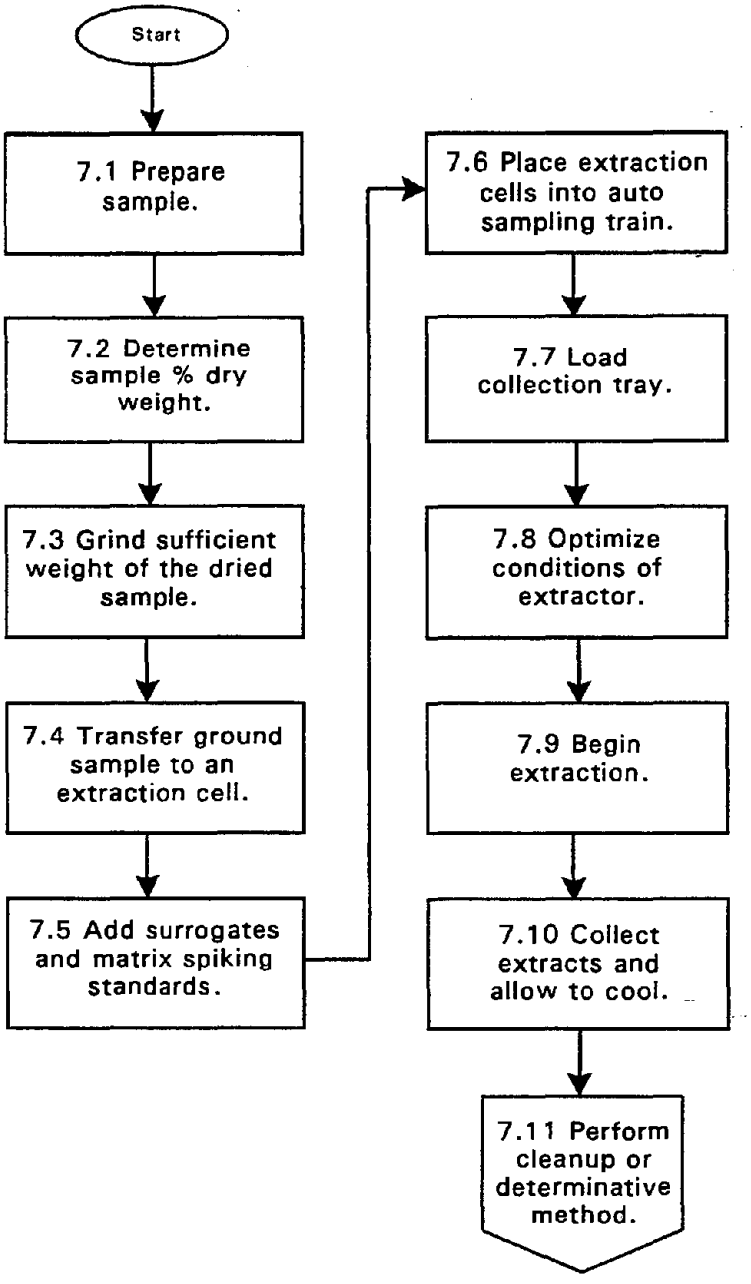
The use of organic solvents, elevated temperatures, and high pressures in Method 3545 present potential safety concerns in the laboratory. Common sense laboratory practices can be employed to minimize these concerns. However, the following sections describe additional steps that should be taken.

11.1 Extraction cells in the oven are hot enough to burn unprotected skin. Allow the cells to cool before removing them from the oven or use appropriate protective equipment (e.g., insulated gloves or tongs), as recommended by the manufacturer.

11.2 During the gas purge step, some solvent vapors may exit through a vent port in the instrument. Follow the manufacturer's directions regarding connecting this port to a fume hood or other means to prevent release of solvent vapors to the laboratory atmosphere.

11.3 The instrument may contain flammable vapor sensors and should be operated with all covers in place and doors closed to ensure proper operation of the sensors. If so equipped, follow the manufacturer's directions regarding replacement of extraction cell seals when frequent vapor leaks are detected.

PRESSURIZED FLUID EXTRACTION (PFE)



METHOD 3562

SUPERCRITICAL FLUID EXTRACTION OF POLYCHLORINATED BIPHENYLS (PCBs) AND ORGANOCHLORINE PESTICIDES

1.0 SCOPE AND APPLICATION

1.1 Method 3562 describes the extraction with supercritical fluids of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) from soils, sediments, fly ash, solid-phase extraction media, and other solid materials which are amenable to extraction with conventional solvents. The method is suitable for use with any supercritical fluid extraction (SFE) system that allows extraction conditions (e.g., pressure, temperature, flow rate) to be adjusted to achieve separation of the PCBs and OCPs from the matrices of concern. The following compounds have been extracted by this method during validation studies. Similar compounds not listed should also be amenable to this extraction.

Compound	CAS Registry No.	IUPAC No.
2,4,4'-Trichlorobiphenyl	7012-37-5	28
2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	52
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	101
2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4	105
2,3',4,4',5-Pentachlorobiphenyl	31508-00-6	118
2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	128
2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	138
2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0	149
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	153
2,3,3',4,4',5'-Hexachlorobiphenyl	38380-08-4	156
2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6	170
2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3	180
Aldrin	309-00-2	
β-Hexachlorocyclohexane (β-BHC)	319-85-7	
δ-Hexachlorocyclohexane (δ-BHC)	319-86-8	
γ-Hexachlorocyclohexane (γ-BHC, or Lindane)	58-89-9	
α-Chlordane	5103-71-9	
4,4'-DDD	72-54-8	
4,4'-DDE	72-55-9	
4,4'-DDT	50-29-3	
Dieldrin	60-57-1	
Endosulfan II	33213-65-9	
Endrin	72-20-8	
Endrin aldehyde	7421-93-4	
Heptachlor	76-44-8	
Heptachlor epoxide	1024-57-3	

1.2 Method 3562 is not suitable for the extraction of PCBs or organochlorine pesticides from liquid samples without some treatment of the liquid before introduction into the SFE to "stabilize" the liquid. Otherwise, the sample may be extruded through the end pieces of the extraction vessel without the benefit of SFE. The use of solid-phase extraction (SPE) media is one way to stabilize a liquid sample and it allows an easy coupling of two selective sample preparation techniques. The use of large diameter (ca. 90 mm) SPE disks coupled with SFE allows large volumes of aqueous samples to be prepared without the need for organic solvent elution. Furthermore, SFE may allow an in-line cleanup to be performed, thus eliminating the need for separate column cleanup and subsequent solvent concentration steps.

1.3 The extraction conditions listed in this procedure (Sec. 11.6) employed a variable restrictor and solid trapping media. Other extraction conditions and equipment are acceptable once appropriate method performance is demonstrated. The method applicability demonstration should be based on the extraction of a certified reference sample or an environmentally-contaminated sample, not on spiked soil/solids, whenever possible. It should be noted that there are currently no "certified" samples for organochlorine pesticides. An authentic, weathered, environmental sample which has been extracted by a traditional sample preparation technique should be used as the reference for these compounds.

1.4 This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 In order to assure a homogeneous sample and minimum subsampling errors, at least 100 g of sample are homogenized with an equal volume of solid CO_2 "snow". A 1 - 5 g aliquot of this mixture is packed into a stainless steel SFE extraction vessel. Copper powder may be added to the cell to remove sulfur from the sample extract. Surrogates and/or internal standards are added to the portion of the sample in the cell and the cell is placed in the SFE extraction device.

2.2 The sample is extracted using supercritical carbon dioxide with no modifiers. Samples to be analyzed for PCBs are subjected to a 10-minute static extraction, followed by a 40-minute dynamic extraction. Samples for organochlorine pesticides are subjected to a 20-minute static extraction, followed by a 30-minute dynamic extraction.

2.3 The sample extract is trapped on a solid-phase sorbent (Florisil for PCBs and octadecyl silane for pesticides). The trapping material is then rinsed with solvent to collect the analytes of interest and reactivate the trapping material for reuse.

2.4 The sample extracts may be subjected to additional cleanup steps (see Method 3600) and then analyzed by the appropriate determinative methods.

3.0 DEFINITIONS

Dynamic extraction - An application of SFE in which the supercritical extraction fluid flows through the sample and out of the extraction cell to a collection device during the extraction. Dynamic extraction is contrasted with static extraction (see below).

Modifier - A liquid or gaseous component added to the supercritical fluid to change its extraction capabilities, often through changes in the solvation power of the extraction fluid. Modifiers may be polar or nonpolar.

Supercritical fluid - A gas maintained above its critical temperature through the application of pressure.

Supercritical fluid extraction (SFE) - The use of a gas maintained above its critical temperature as an extraction fluid.

Static extraction - An application of SFE in which the supercritical extraction fluid is held in the extraction vessel during the entire procedure, and is then released to a collection device. Static extraction is contrasted with dynamic extraction (see above).

4.0 INTERFERENCES

4.1 The analyst must demonstrate through the analysis of method blanks that the supercritical fluid extraction system is free from interferants. To do this, perform a simulated extraction using an empty extraction vessel and a known amount of CO_2 under the same conditions as those used for sample extraction, and determine the background contamination by analyzing the extract by the determinative method that will be used for sample analysis.

4.2 The extraction vessel(s), the end-frits, the nozzle restrictor(s), and the multi-port valve(s) may retain solutes whenever high-concentration samples are extracted. Therefore, it is good practice to clean the extraction system after such extractions. Suspect parts of the system should be replaced when reagent blanks indicate carryover. At least one reagent blank should be prepared and analyzed daily when the instrument is in use. Furthermore, reagent blanks should be prepared and analyzed after each extraction of a high-concentration sample (high part per million range). If reagent blanks continue to indicate contamination, even after replacement of the extraction vessel (and the restrictor, if a fixed restrictor system is used), then the multi-port valve must be cleaned. The operator must be ever vigilant against impurities arising from liquid solvents and CO_2 itself. Avoid any apparatus, valves, solenoids, and other hardware that contain lubricants or chlorofluorohydrocarbon materials that can serve as background contaminant sources.

4.3 No modifier was employed in the development of this method for either PCBs or organochlorine pesticides. Use of a modifier may cause many other problems in these samples. If the method is modified by the user to include an on-line modifier, or pre-mixed tanks of CO_2 and modifier, considerable effort must be made to validate this change.

4.4 Refer to Method 3500, Section 3.0, for general extraction interference guidance.

5.0 SAFETY

5.1 SFE involves the use of high pressure gases. Typical SFE systems have maximum operating pressures of approximately 400 atm (6000 psi). Great care must be taken to ensure that *all* components of the system are capable of withstanding such pressures.

5.2 SFE also involves heating portions of the system above ambient temperature, resulting in further increases in pressure. The combined effects of the starting pressure and temperature increase must be taken into account when evaluating the capabilities of system components.

5.3 SFE devices typically employ gases at high pressure directly from a tank, with no pressure regulator. In addition to making it difficult to monitor the level of gas in the tank, the lack of a regulator means that system leaks may involve gases at 2000 psi or more.

5.4 When liquid CO_2 comes in contact with skin, it can cause "burns" because of its low temperature (-70°C). Burns are especially severe when CO_2 is modified with organic liquids.

5.5 The extraction fluid usually exhausts through an exhaust gas and liquid waste port on the rear of the panel of the extractor. This port must be connected to a chemical fume hood to prevent contamination of the laboratory atmosphere.

5.6 Combining modifiers with supercritical fluids requires an understanding and evaluation of the potential chemical interaction between the modifier and the supercritical fluid, and between the supercritical fluid and/or modifier and the analyte(s) or matrix.

5.7 When CO_2 is used for cryogenic cooling, typical coolant consumption is 5 L/min, which results in a CO_2 level of 900 ppm for a room of 4.5 m x 3.0 m x 2.5 m, assuming 10 air exchanges per hour.

6.0 EQUIPMENT AND SUPPLIES

6.1 Supercritical fluid extractor and associated hardware - Any supercritical fluid extraction system that can achieve the extraction conditions and performance specifications detailed in this procedure may be used.

WARNING: A safety feature to prevent over-pressurization is required on the extractor. This feature should be designed to protect the laboratory personnel and the instrument from possible injuries or damage resulting from equipment failure under high pressure.

6.1.1 Extraction vessel - Stainless-steel vessel with end fittings with 2 µm frits. Use the extraction vessel supplied by the manufacturer of the SFE system being used. Fittings used for the extraction vessel must be capable of withstanding the required extraction pressures. The maximum operating pressure for most extractors is 400 atm. Pressures above 400 atm, especially at elevated temperatures, are likely to exceed the ratings of standard chromatography tubing and fittings. Check with the manufacturer of the particular extraction system and especially the tubing manufacturer for the maximum operating pressure and temperature for that system. Make sure that the extraction vessels are rated for such pressures and temperatures.

6.1.2 Restrictor - This method was developed with continuously variable nozzle restrictors that do not require that the operator take steps to remove water from the sample. If a fixed restrictor is used, additional validation must be done to verify that moisture from the sample does not adversely affect the chromatography of the determinative step.

6.1.3 Collection device - This method is based on a solid trap used at sub-ambient and above ambient temperatures for the different classes of analytes (PCBs vs. OCPs). However, a liquid (solvent) trap may also be used.

6.1.3.1 Use Florisil, 30-40 µm particle diameter (commonly used in SPE cartridges), as a solid trap for the PCBs.

6.1.3.2 For organochlorine pesticides, octadecyl silane (ODS) may be used as a solid trap, although the use of Florisil is also possible.

6.1.3.3 Analytes may be collected in a small volume of solvent in a suitable vial, however, great care must be taken to recover the most volatile compounds. The use of a glass wool plug in the inner tube of the collection vial improves recoveries. Gas flow must not be so high as to evaporate the collection solvent to dryness. A 15-mL collection solvent volume is recommended.

6.2 CO_2 cylinder balance (optional) - Balances from Scott Specialty Gases, Model 5588D, or equivalent, may be used to monitor the fluid usage. Such a device is useful because CO_2 tanks used for SFE are not equipped with regulators, and it is difficult to determine when the tank needs to be replaced.

6.3 Glass microfiber filter paper disks - Cored out of Whitman QF/F filter paper (Whatman No. 1825021), or equivalent. A disk is placed at both ends of the sample. This ultra-fine filter paper has good retentive properties for particulate matter down to 0.7 µm and is easy to core. The normal background is insignificant, but blanks must be run on each batch.

7.0 REAGENTS AND STANDARDS

7.1 CO_2 - SFE-grade CO_2 is absolutely necessary for use in SFE. Aluminum cylinders are preferred to steel cylinders. The cylinders must be fitted with eductor tubes.

7.2 CO_2 for cryogenic cooling - Certain parts of some models of extractors (i.e., the high-pressure pump head and the analyte trap) must be cooled during use. The CO_2 used for this purpose must be supplied in tanks with a full-length eductor tube, but need not be SFE-grade. A low-cost industrial grade is acceptable.

7.3 Reconstitution solvents - The reconstitution solvents dispensed by the SFE instruments that use solid-phase trapping may be the same solvent that is used for liquid trapping. This method was developed with only sub-ambient solid trapping. Liquid trapping will work for this method as well, however the trapping volume is typically ten times larger than that with a solid trap. Further, the use of liquid trapping will likely require the use of manual Florisil or silica cleanup. These manual cleanup steps will also require the concentration of the solvent after the cleanup, a step that can be avoided through use of solid-phase trapping.

7.4 Internal Standards - Refer to the appropriate determinative method for information of the choice of internal standards, where applicable. However, note that for PCBs, certain ethers work well as internal standards, but do not survive the SFE extraction particularly well.

7.4.1 Internal standards for PCBs - Internal standards that have been evaluated using this method include PCB 35, PCB 36, PCB 169, 2,4-dichlorobenzyl hexyl ether, 2,4-dichlorobenzyl heptyl ether, 1,2,3,4-tetrachloronaphthalene, hexabromobenzene, and octachloronaphthalene.

7.4.2 Internal standard for organochlorine pesticides - Pentachloronitrobenzene

7.5. Surrogate standards - Refer to the appropriate determinative method for information of the choice of surrogates. Surrogates that have been evaluated using this method include hexabromobenzene, PCB 35, PCB 36, PCB 169, 1,2,3,4-tetrachloronaphthalene, and

octachloronaphthalene. Prepare a stock solution of 10 mg/mL. Apply 150-µL aliquots to the soil samples within the extraction vessels at the exit end of the flow-through vessels. It has been observed that a very small volume (10 µL) of a concentrated surrogate mixture often gives poor recoveries, while adding a larger volume of more dilute surrogate standard to the sample matrix achieved the expected recoveries.

7.6 Copper powder - Electrolytic grade. Added to samples that contain elemental sulfur. The powder is pretreated by rinsing 20 g with 150 mL organic-free reagent water, 150 mL acetone, 150 mL of hexane, and drying in a rotary evaporator. The powder is kept under argon or helium until used. Copper powder must have a shiny bright appearance to be effective. If it has oxidized and turned dark, it should not be used.

7.7 Sodium Sulfate - Anhydrous (12-60 mesh), Baker Analyzed grade, or equivalent.

7.8 Celite 545 - 60/80 mesh, J. T. Baker, or equivalent. Prepare a reagent blank to assure that no background contaminants are present.

7.9 Solvents - Used for eluting the analytes of interest from the solid trapping material and rinsing the trapping material prior to reuse. All solvents should be pesticide-grade or equivalent.

7.9.1 n-Heptane, C_7H_{16}

7.9.2 Methylene chloride, CH_2Cl_2

7.9.3 Acetone, CH_3COCH_3

7.10 Florisil - Pesticide residue grade.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

8.2 Solid samples to be extracted by this procedure should be collected and stored as any other solid samples containing semivolatile organics.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One and Method 8000 for specific Quality Control procedures and to Method 3500 for sample preparation quality control procedures.

9.2 Each time samples are extracted, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. Any method blanks, matrix spike samples, or replicate samples should be subjected to the same analytical procedures (Sec. 11) as those used on actual samples.

9.3 All instrument operating conditions should be recorded.

10.0 CALIBRATION AND STANDARDIZATION

There are no calibration or standardization steps associated with this sample extraction procedure other than establishing the extraction conditions in Sec. 11.6.

11.0 PROCEDURE

11.1 Sample handling - Decant and discard any water layer on a sediment sample. Discard any foreign objects such as pieces of wood, glass, leaves and rocks.

11.2 Determination of sample % dry weight - In certain cases, sample results are desired based on dry-weight basis. When such data are desired, a separate portion of sample for this determination should be weighed out at the same time as the portion used for analytical determination.

WARNING: The drying oven should be contained in a hood or vented. Significant laboratory contamination may result from a heavily contaminated hazardous waste sample.

11.2.1 Immediately after weighing the sample aliquot to be extracted, weigh an additional 5 - 10 g aliquot of the sample into a tared crucible. Determine the % dry weight of the sample by drying overnight at 105°C. Allow to cool in a desiccator before weighing.

11.2.2 Calculate the % dry weight as follows:

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

11.3 Sample grinding and homogenization

NOTE: Sample grinding is a critical step in the SFE process. The soil/solid must be a fine particle to ensure efficient extraction.

11.3.1 Mix at least 100 grams of sample with an equal volume of CO_2 solid "snow" prepared from the extraction grade CO_2. Place this mixture in a small food-type chopper, and grind for two minutes. Place the chopped sample on a clean surface and allow the CO_2 to sublime away. As soon as the sample appears free-flowing and solid CO_2 is no longer visible, weigh the sample and place it in the extraction vessel. This procedure will ensure the homogeneity of the sample without loss of the volatile analytes and also retains the original moisture content of the sample.

11.3.2 Weigh 1.0 to 5.0 g of the homogenized sample from Sec. 11.3.1 into a pre-cleaned aluminum dish. For samples in the mg/kg (ppm) concentration range, use a 0.1-gram sample after carefully homogenizing (Sec. 11.3.1) the bulk sample, to avoid sub-sampling errors.

11.4 For samples known to contain elemental sulfur, use copper powder (Sec. 7.6) to remove the dissolved sulfur from the sample and CO_2 eluant. The copper powder (1 to 2 grams per sample) can be mixed with the sample in the extraction vessel itself, or packed in a separate vessel between the extraction vessel and the nozzle (restrictor). The copper addition to samples is a useful precaution, whether or not one suspects the presence of elemental sulfur. In tests, no adverse

effect from the addition of copper was observed and it appears that finely divided copper may enhance the dispersion of CO_2. If copper powder is added to the samples, it must also be added to the method blank.

11.5 Packing the extraction cell

The procedure used for a 7.0-mL SFE extraction vessel with sample and copper powder is as follows:

11.5.1 Place a small disk of fiber glass filter paper at the bottom of the extraction vessel to protect the end frits from particulate matter (this makes the cleanup very easy between samples and lessens any chance of plugging of the frits).

11.5.2 Place approximately two grams of anhydrous sodium sulfate on top of this disk in the extraction vessel. Weigh 1.0 gram of solid waste sample into a weighing dish. Add two grams of electrolytic grade copper powder to the same weighing dish, followed by 7 grams of anhydrous sodium sulfate. Mix the weighed material. Transfer the entire homogeneous mixture to the extraction vessel on top of the existing small layer of sodium sulfate. Finally, place a top layer (2 grams) of sodium sulfate on top of the mixture. The densities of the respective materials are such that this still leaves a small volume at the top of a 7-mL vessel. These ratios may be adjusted for different sample sizes and vessel sizes, but should be kept consistent among samples and blanks.

11.5.3 If a surrogate is being added, transfer half the weighed sample to the extraction vessel. Add 150 µL of surrogate standard to the sample in the vessel and then add the remainder of the sample material.

11.5.4 To ensure efficient extraction, fill the extraction vessel completely, avoiding any dead volume. If any dead volume remains, fill the space with an inert, porous material, e.g., pre-cleaned Pyrex glass wool, Celite, etc.

11.6 Sample extraction conditions

11.6.1 Recommended conditions for PCBs

11.6.1.1 Extraction conditions

Pressure:	4417 psi (305 bar)
Extraction chamber temperature:	80°C
Density:	0.75 g/mL
Extraction fluid composition:	CO_2
Static equilibration time:	10 minutes
Dynamic extraction time:	40 minutes
Extraction fluid flow rate:	2.5 mL/minute

The resultant thimble volume swept is 17.6 times the volume of the cell at 1 bar (this is equivalent to 100 mL of liquid CO_2 at a reference temperature of 4.0°C and a density 0.92 g/mL, or 92 g of CO_2).

11.6.1.2 Collection conditions (during extraction)

Trap packing:	Florisil
Trap temperature:	15-20°C
Nozzle temperature:	45-55°C (variable restrictor)

11.6.1.3 Reconstitution conditions for collected extracts

The reconstitution process consists of four rinse steps. The first rinse is used to elute the analytes of interest from the trapping material. All four rinse steps are performed with a recommended trap temperature of 38°C, a nozzle temperature of 30°C, and a flow rate of 1.0 mL/min.

Rinse Substep 1:

Rinse solvent	n-Heptane
Collected rinse volume:	1.6 mL

Rinse Substep 2:

Rinse solvent	n-Heptane
Collected rinse volume:	1.6 mL

This second rinse step is an "insurance rinse". The vial is usually not analyzed unless there is a need or desire to assure that the entire sample rinsed in substep 1.

Rinse Substep 3:

Rinse solvent	Methylene chloride:Acetone (1:1)
Collected rinse volume:	4.0 mL (to waste)

This third rinse step provides a means of rinsing the solid Florisil trap to remove interfering compounds such as lipids, hydrocarbons, and PAHs. The rinse solvent is then discarded.

Rinse Substep 4:

Rinse solvent	n-Heptane
Collected rinse volume:	3.0 mL (to waste)

This fourth rinse step provides a means of regenerating the solid Florisil trap to prepare it (reactivate) for reuse.

11.6.2 Recommended conditions for organochlorine pesticides

11.6.2.1 Extraction conditions

Pressure:	4330 psi (299 bar)
Extraction chamber temperature:	50°C
Density:	0.87 g/mL
Extraction fluid composition:	CO_2

Static equilibration time:	20 minutes
Dynamic extraction time:	30 minutes
Extraction fluid flow rate:	1.0 mL/minute

The resultant thimble volume swept is 4.6 times the volume of the cell at 1 bar (this is equivalent to 30 mL of liquid CO_2 at a reference temperature of 4.0°C and a density 0.92 g/mL, or 28 g of CO_2).

11.6.2.2 Collection conditions (during extraction)

Trap packing:	ODS
Trap temperature:	20°C
Nozzle temperature:	50°C (variable restrictor)

11.6.2.3 Reconstitution conditions for collected extracts

The extraction of organochlorine pesticides requires only a single rinse step.

Rinse solvent:	n-Hexane
Collected fraction volume:	1.3 mL
Trap temperature:	50°C
Nozzle temperature:	30°C (variable restrictor)
Rinse solvent flow rate:	2 mL/minute

NOTE: If a fixed restrictor and liquid trapping are used, a restrictor temperature between 100 and 150°C is recommended.

11.7 Label the extract with the fraction designation and vial number.

11.8 If the copper powder was not added to the sample prior to loading the cell, additional sulfur cleanup of the extracts may be required prior to analysis.

11.9 SFE System Maintenance

11.9.1 Depressurize the system following the manufacturer's instructions.

11.9.2 After extraction of an especially "tarry" sample, the end-frits of the extraction vessel may require extensive cleanup or replacement to ensure adequate flow of extraction fluid without an excessive pressure drop. In addition, very fine particles may clog the exit frit, necessitating its replacement. By placing a layer of inert material such as Celite or sand between the sample and the exit frit (and placing disks of filter paper or glass fiber filter on top of the inert material), this maintenance may be delayed.

11.9.3 Clean the extraction vessel after each sample extraction. The cleaning procedure depends upon the type of sample. After removing the bulk of the extracted sample from the extraction vessel, the cell and end-frits should be scrubbed with a solution of detergent and water using a stiff brush. Placing the parts in an ultrasonic bath with a warm detergent solution may help. Rinse the parts with organic-free reagent water. Repeat the ultrasonic bath treatment with either methyl alcohol, or acetone, or both, followed by air drying.

12.0 DATA ANALYSIS AND CALCULATIONS

There are no calculations explicitly associated with this extraction procedure. See the appropriate determinative method for calculation of final sample results.

13.0 METHOD PERFORMANCE

13.1 Tables in Method 8081 contain single laboratory performance data for the organochlorine pesticides using supercritical fluid extraction Method 3562 on an HP 7680. Samples were analyzed using GC/ELCD. The method was performed using a variable restrictor and solid trapping material. Three different soil samples were spiked at 5 and 250 ug/kg. Soil 1 (Delphi) is described as loamy sand, with 2.4% clay, 94% sand, 0.9% organic matter, 3.4% silt, and 0.1% moisture. Soil 2 (McCarthy) is described as sandy-loam, with 11% clay, 56% sand, 22% organic matter, 33% silt, and 8.7% moisture. Soil 3 (Auburn) is described as clay loam, with 32% clay, 21% sand, 5.4% organic matter, 46% silt, and 2.2% moisture. Seven replicate extractions were made of each soil at the 2 concentrations.

13.2 Tables in Method 8082 contain laboratory performance data for several PCB congeners using supercritical fluid extraction Method 3562 on an HP 7680. Seven replicate extractions on each sample were performed. The method was performed using a variable restrictor and solid trapping material (Florisil). Sample analysis was performed by GC/ECD. The following soil samples were used for this study:

13.2.1 Two field-contaminated certified reference materials were extracted by a single laboratory. One of the materials was a lake sediment from Environment Canada (EC-5). The other material was soil from a dump site and was provided by the National Science and Engineering Research Council of Canada (EC-1). The average recoveries for EC-5 are based on the certified value for that sample. The average recoveries for EC-1 are based on the certified value of the samples or a Soxhlet value, if a certified value was unavailable for a specific analyte.

13.2.2 Four certified reference materials were extracted by two independent laboratories. The materials were: a marine sediment from NIST (SRM 1941), a fish tissue from NIST (SRM 2974), a sewage sludge from BCR European Union(CRM 392), and a soil sample from BCR European Union (CRM 481). The average recoveries are based on the certified value of the samples or a Soxhlet value, if a certified value was unavailable for a specific analyte.

13.2.3 A weathered sediment sample from Michigan (Saginaw Bay) was extracted by a single laboratory. Soxhlet extractions were carried out on this sample and the SFE recovery is relative to that for each congener. The average recoveries are based on the certified value of the samples. Additional data is shown in the tables for some congeners that were not certified.

14.0 POLLUTION PREVENTION

Extraction of organic compounds using supercritical fluid extraction conforms with EPA's pollution prevention goals. The volumes of solvent employed, if any, are significantly smaller than with other extraction procedures. Minimal waste is generated.

15.0 WASTE MANAGEMENT

Laboratory waste management procedures must be consistent with federal, state, and local regulations.

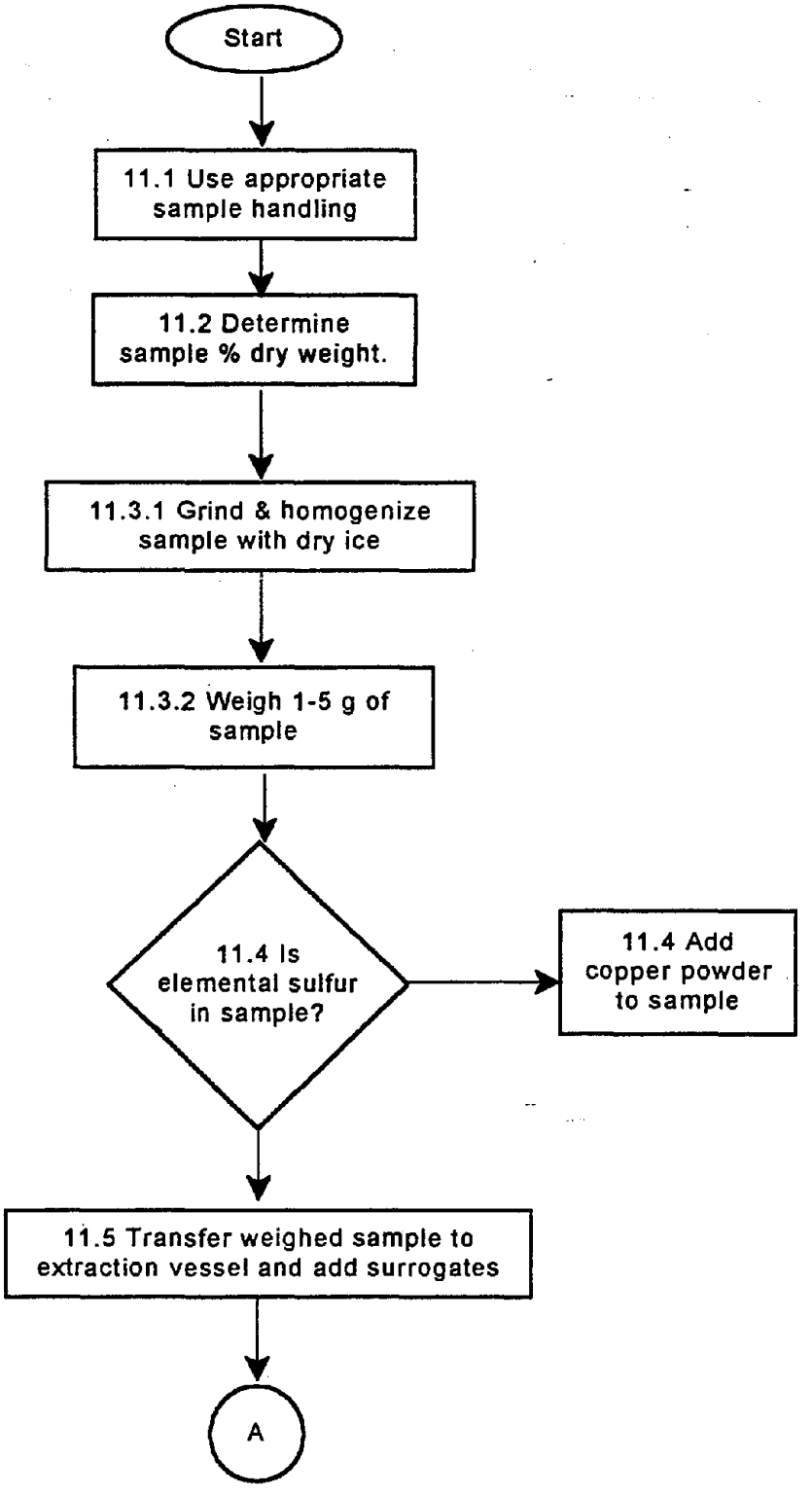
16.0 REFERENCES

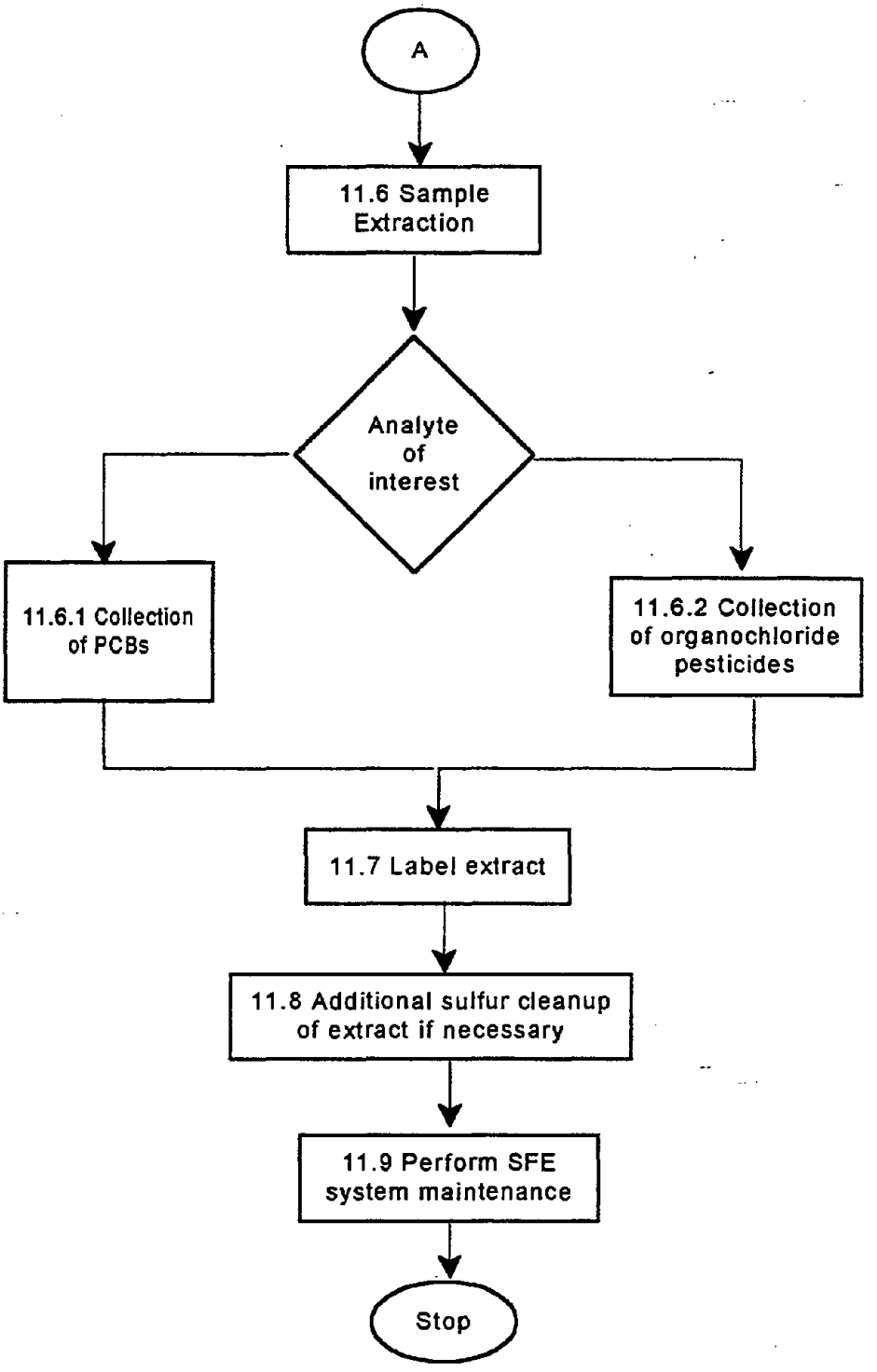
Gere, D, "Final Deliverables for PCB/OCP SFE Draft Method," letter to B. Lesnik, April 15, 1995.

17.0 TABLES, DIAGRAMS, FLOW CHARTS, AND VALIDATION DATA

A flow diagram for the method procedure follows.

SUPERCRITICAL FLUID EXTRACTION OF POLYCHLORINATED BIPHENYLS (PCBs) AND ORGANOCHLORINE PESTICIDES.




A
11.6 Sample Extraction
Analyte of interest
11.6.1 Collection of PCBs
11.6.2 Collection of organochloride pesticides
11.7 Label extract
11.8 Additional sulfur cleanup of extract if necessary
11.9 Perform SFE system maintenance
Stop

4.2 SAMPLE PREPARATION METHODS

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgements necessary to meet the data quality objectives or needs for the intended use of the data.

4.2.2 CLEANUP

The following methods are included in this section:

Method 3600C:	Cleanup
Method 3610B:	Alumina Cleanup
Method 3611B:	Alumina Column Cleanup and Separation of Petroleum Wastes
Method 3620B:	Florisil Cleanup
Method 3630C:	Silica Gel Cleanup
Method 3640A:	Gel-Permeation Cleanup
Method 3650B:	Acid-Base Partition Cleanup
Method 3660B:	Sulfur Cleanup
Method 3665A:	Sulfuric Acid/Permanganate Cleanup

4.3 DETERMINATION OF ORGANIC ANALYTES

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgements necessary to meet the data quality objectives or needs for the intended use of the data.

4.3.1 GAS CHROMATOGRAPHIC METHODS

The following methods are included in this section:

Method 8000B:	Determinative Chromatographic Separations
Method 8011:	1,2-Dibromoethane and 1,2-Dibromo-3-chloropropane by Microextraction and Gas Chromatography
Method 8015B:	Nonhalogenated Organics Using GC/FID
Method 8021B:	Aromatic and Halogenated Volatiles by Gas Chromatography Using Photoionization and/or Electrolytic Conductivity Detectors
Method 8031:	Acrylonitrile by Gas Chromatography
Method 8032A:	Acrylamide by Gas Chromatography
Method 8033:	Acetonitrile by Gas Chromatography with Nitrogen-Phosphorus Detection
Method 8041:	Phenols by Gas Chromatography
Method 8061A:	Phthalate Esters by Gas Chromatography with Electron Capture Detection (GC/ECD)
Method 8070A:	Nitrosamines by Gas Chromatography
Method 8081B:	Organochlorine Pesticides by Gas Chromatography
Method 8082A:	Polychlorinated Biphenyls (PCBs) by Gas Chromatography
Method 8091:	Nitroaromatics and Cyclic Ketones by Gas Chromatography
Method 8100:	Polynuclear Aromatic Hydrocarbons
Method 8111:	Haloethers by Gas Chromatography
Method 8121:	Chlorinated Hydrocarbons by Gas Chromatography: Capillary Column Technique
Method 8131:	Aniline and Selected Derivatives by Gas Chromatography
Method 8141B:	Organophosphorus Compounds by Gas Chromatography
Method 8151A:	Chlorinated Herbicides by GC Using Methylation or Pentafluorobenzylation Derivatization

METHOD 8081B

ORGANOCHLORINE PESTICIDES BY GAS CHROMATOGRAPHY

1.0 SCOPE AND APPLICATION

1.1 Method 8081 may be used to determine the concentrations of various organochlorine pesticides in extracts from solid and liquid matrices, using fused-silica, open-tubular, capillary columns with electron capture detectors (ECD). When compared to the packed columns, these columns offer improved resolution, better selectivity, increased sensitivity, and faster analysis. The compounds listed below may be determined by either a single- or dual-column analysis system.

Compound	CAS Registry No.
Aldrin	309-00-2
α-BHC	319-84-6
β-BHC	319-85-7
γ-BHC (Lindane)	58-89-9
δ-BHC	319-86-8
Chlorobenzilate	510-15-6
α-Chlordane	5103-71-9
γ-Chlordane	5103-74-2
Chlordane - not otherwise specified	57-74-9
DBCP	96-12-8
4,4'-DDD	72-54-8
4,4'-DDE	72-55-9
4,4'-DDT	50-29-3
Diallate	2303-16-4
Dieldrin	60-57-1
Endosulfan I	959-98-8
Endosulfan II	33213-65-9
Endosulfan sulfate	1031-07-8
Endrin	72-20-8
Endrin aldehyde	7421-93-4
Endrin ketone	53494-70-5
Heptachlor	76-44-8
Heptachlor epoxide	1024-57-3
Hexachlorobenzene	118-74-1
Hexachlorocyclopentadiene	77-47-4
Isodrin	465-73-6
Methoxychlor	72-43-5
Toxaphene	8001-35-2

1.2 This revision of Method 8081 no longer includes the PCBs as Aroclors in the list of target analytes. The analysis of PCBs should be undertaken using Method 8082, which includes specific cleanup and quantitation procedures designed for PCB analysis. This change was made to obtain PCB data of better quality and to eliminate the complications inherent in a combined organochlorine pesticide and PCB method. Therefore, if the presence of PCBs is expected, use Method 8082 for PCB analyses, and this method (8081) for the organochlorine pesticides. If there is no information of the likely presence of PCBs, either employ a PCB-specific screening procedure such as an immunoassay (e.g., Method 4020), or split the sample extract *prior to* any cleanup steps, and process part of the extract for organochlorine pesticide analysis and the other portion for PCB analysis using Method 8082.

1.3 The analyst must select columns, detectors and calibration procedures most appropriate for the specific analytes of interest in a study. Matrix-specific performance data must be established and the stability of the analytical system and instrument calibration must be established for each analytical matrix (e.g., hexane solutions from sample extractions, diluted oil samples, etc.).

1.4 Although performance data are presented for many of the target analytes, it is unlikely that all of them could be determined in a single analysis. The chemical and chromatographic behaviors of many of these chemicals can result in co-elution of some target analytes. Several cleanup/fractionation schemes are provided in this method and in Method 3600.

1.5 Several multi-component mixtures (i.e., Chlordane and Toxaphene) are listed as target analytes. When samples contain more than one multi-component analyte, a higher level of analyst expertise is required to attain acceptable levels of qualitative and quantitative analysis. The same is true of multi-component analytes that have been subjected to environmental degradation or degradation by treatment technologies. These result in "weathered" multi-component mixtures that may have significant differences in peak patterns than those of standards.

1.6 Compound identification based on single-column analysis should be confirmed on a second column, or should be supported by at least one other qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm the measurements made with the primary column. GC/MS Method 8270 is also recommended as a confirmation technique, if sensitivity permits (Sec. 8.0).

1.7 This method includes a dual-column option. The option allows a hardware configuration of two analytical columns joined to a single injection port. The option allows one injection to be used for dual-column analysis. Analysts are cautioned that the dual-column option may not be appropriate when the instrument is subject to mechanical stress, many samples are to be run in a short period, or when contaminated samples are analyzed.

1.8 This method is restricted to use by, or under the supervision of, analysts experienced in the use of gas chromatographs (GC) and skilled in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

1.9 Extracts suitable for analysis by this method may also be analyzed for organophosphorus pesticides (Method 8141). Some extracts may also be suitable for triazine herbicide analysis, if low recoveries (normally samples taken for triazine analysis must be preserved) are not a problem.

1.10 The following compounds may also be determined using this method:

Compound	CAS Registry No.
Alachlor	15972-60-8
Captafol	2425-06-1
Chloroneb	2675-77-6
Chloropropylate	5836-10-2
Chlorothalonil	1897-45-6
DCPA	1861-32-1
Dichlone	117-80-6
Dicofol	115-32-2
Etridiazole	2593-15-9
Halowax-1000	58718-66-4
Halowax-1001	58718-67-5
Halowax-1013	12616-35-2
Halowax-1014	12616-36-3
Halowax-1051	2234-13-1
Halowax-1099	39450-05-0
Mirex	2385-85-5
Nitrofen	1836-75-5
PCNB	82-68-8
Permethrin (*cis* + *trans*)	52645-53-1
Perthane	72-56-0
Propachlor	1918-16-7
Strobane	8001-50-1
trans-Nonachlor	39765-80-5
Trifluralin	1582-09-8

1.11 Kepone extracted from samples or standards exposed to water or methanol may produce peaks with broad tails that elute later than the standard by up to 1 minute. This shift is presumably the result of the formation of a hemi-acetal from the ketone functionality. As a result, Method 8081 is not recommended for determining Kepone. Method 8270 may be more appropriate for the analysis of Kepone.

2.0 SUMMARY OF METHOD

2.1 A measured volume or weight of sample (approximately 1 L for liquids, 2 g to 30 g for solids) is extracted using the appropriate matrix-specific sample extraction technique.

2.2 Liquid samples are extracted at neutral pH with methylene chloride using either Method 3510 (separatory funnel), Method 3520 (continuous liquid-liquid extractor), Method 3535 (solid-phase extraction), or other appropriate technique.

2.3 Solid samples are extracted with hexane-acetone (1:1) or methylene chloride-acetone (1:1) using Method 3540 (Soxhlet), Method 3541 (automated Soxhlet), Method 3545 (pressurized fluid extraction), Method 3550 (ultrasonic extraction), or other appropriate technique.

2.4 A variety of cleanup steps may be applied to the extract, depending on the nature of the matrix interferences and the target analytes. Suggested cleanups include alumina (Method 3610), Florisil (Method 3620), silica gel (Method 3630), gel permeation chromatography (Method 3640), and sulfur (Method 3660).

2.5 After cleanup, the extract is analyzed by injecting a 1-µL sample into a gas chromatograph with a narrow- or wide-bore fused-silica capillary column and electron capture detector (GC/ECD) or an electrolytic conductivity detector (GC/ELCD).

3.0 INTERFERENCES

3.1 Refer to Methods 3500 (Sec. 3.0, in particular), 3600, and 8000, for a discussion of interferences.

3.2 Sources of interference in this method can be grouped into three broad categories.

3.2.1 Contaminated solvents, reagents, or sample processing hardware.

3.2.2 Contaminated GC carrier gas, parts, column surfaces, or detector surfaces.

3.2.3 Compounds extracted from the sample matrix to which the detector will respond.

3.2.4 Interferences co-extracted from the samples will vary considerably from waste to waste. While general cleanup techniques are referenced or provided as part of this method, unique samples may require additional cleanup approaches to achieve desired degrees of discrimination and quantitation.

3.3 Interferences by phthalate esters introduced during sample preparation can pose a major problem in pesticide determinations.

3.3.1 These materials may be removed prior to analysis using Method 3640 (Gel Permeation Cleanup) or Method 3630 (Silica Gel Cleanup).

3.3.2 Common flexible plastics contain varying amounts of phthalate esters which are easily extracted or leached from such materials during laboratory operations.

3.3.3 Cross-contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled.

3.3.4 Interferences from phthalate esters can best be minimized by avoiding contact with any plastic materials and checking all solvents and reagents for phthalate contamination. Exhaustive cleanup of solvents, reagents and glassware may be required to eliminate background phthalate ester contamination.

3.4 Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used. This should be followed by detergent washing with

hot water, and rinses with tap water and organic-free reagent water. Drain the glassware and dry it in an oven at 130°C for several hours, or rinse with methanol and drain. Store dry glassware in a clean environment.

3.5 The presence of elemental sulfur will result in broad peaks that interfere with the detection of early-eluting organochlorine pesticides. Sulfur contamination should be expected with sediment samples. Method 3660 is suggested for removal of sulfur. Since the recovery of Endrin aldehyde (using the TBA procedure) is drastically reduced, this compound must be determined prior to sulfur cleanup.

3.6 Waxes, lipids, and other high molecular weight materials can be removed by Method 3640 (gel-permeation cleanup).

3.7 Other halogenated pesticides or industrial chemicals may interfere with the analysis of pesticides. Certain co-eluting organophosphorus pesticides are eliminated by Method 3640 (gel permeation cleanup - pesticide option). Co-eluting chlorophenols may be eliminated by using Method 3630 (silica gel), Method 3620 (Florisil), or Method 3610 (alumina). Polychlorinated biphenyls (PCBs) also may interfere with the analysis of the organochlorine pesticides. The problem may be most severe for the analysis of multicomponent analytes such as Chlordane, Toxaphene, and Strobane. If PCBs are known or expected to occur in samples, the analyst should consult Methods 3620 and 3630 for techniques that may be used to separate the pesticides from the PCBs.

3.8 Co-elution among the many target analytes in this method can cause interference problems. The following target analytes may coelute on the GC columns listed, when using the single-column analysis scheme:

DB 608	Trifluralin/Diallate isomers
	PCNP/Dichlone/Isodrin
DB 1701	Captafol/Mirex
	Methoxychlor/Endosulfan sulfate

3.9 The following compounds may coelute using the dual-column analysis scheme. In general, the DB-5 column resolves fewer compounds that the DB-1701.

DB-5	Permethrin/Heptachlor epoxide
	Endosulfan I/α-Chlordane
	Perthane/Endrin
	Endosulfan II/Chloropropylate/Chlorobenzilate
	4,4'-DDT/Endosulfan sulfate
	Methoxychlor/Dicofol
DB-1701	Chlorothalonil/β-BHC
	δ-BHC/DCPA/Permethrin
	α-Chlordane/*trans*-Nonachlor

Nitrofen, Dichlone, Carbophenothion, Dichloran exhibit extensive peak tailing on both columns. Simazine and Atrazine give poor responses on the ECD detector. Triazine compounds should be analyzed using Method 8141 (NPD option).

4.0 APPARATUS AND MATERIALS

4.1 Gas chromatograph - An analytical system complete with gas chromatograph suitable for on-column and split-splitless injection and all required accessories including syringes, analytical columns, gases, electron capture detectors (ECD), and recorder/integrator or data system.

4.2 GC columns

This method describes procedures for both single-column and dual-column analyses. The single-column approach involves one analysis to determine that a compound is present, followed by a second analysis to confirm the identity of the compound (Sec. 8.4 describes how GC/MS confirmation techniques may be employed). The single-column approach may employ either narrow-bore ($\leq$0.32 mm ID) columns or wide-bore (0.53 mm ID) columns. The dual-column approach involves a single injection that is split between two columns that are mounted in a single gas chromatograph. The dual-column approach employs only wide-bore (0.53 mm ID) columns.

The columns listed in this section were the columns used to develop the method performance data. The mention of these columns in this method is not intended to exclude the use of other columns that may be developed. Laboratories may use other capillary columns provided that they document method performance data (e.g., chromatographic resolution, analyte breakdown, and MDLs) that equals or exceeds the performance described in this method, or as appropriate for the intended application.

4.2.1 Narrow-bore columns for single-column analysis (use both columns to confirm compound identifications unless another confirmation technique such as GC/MS is employed).

4.2.1.1 30-m x 0.25 or 0.32 mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1 µm film thickness.

4.2.1.2 30-m x 0.25 mm ID fused-silica capillary column chemically bonded with 35 percent phenyl methylpolysiloxane (DB-608, SPB-608, or equivalent), 2.5 µm coating thickness, 1 µm film thickness.

4.2.1.3 Narrow-bore columns should be installed in split/splitless (Grob-type) injectors.

4.2.2 Wide-bore columns for single-column analysis (use two of the three columns listed to confirm compound identifications unless another confirmation technique such as GC/MS is employed).

4.2.2.1 30-m x 0.53 mm ID fused silica capillary column chemically bonded with 35 percent phenyl methylpolysiloxane (DB-608, SPB-608, RTx-35, or equivalent), 0.5 µm or 0.83 µm film thickness.

4.2.2.2 30-m x 0.53 mm ID fused silica capillary column chemically bonded with 50 percent phenyl methylpolysiloxane (DB-1701, or equivalent), 1.0 µm film thickness.

4.2.2.3 30-m x 0.53 mm ID fused silica capillary column chemically bonded with 95 percent dimethyl - 5 percent diphenyl polysiloxane (DB-5, SPB-5, RTx-5, or equivalent), 1.5 µm film thickness.

4.2.2.4 Wide-bore columns should be installed in 1/4 inch injectors, with deactivated liners designed specifically for use with these columns.

4.2.3 Wide-bore columns for dual-column analysis (choose one of the two pairs of columns listed below).

4.2.3.1 Column pair 1

30-m x 0.53 mm ID fused silica capillary column chemically bonded with SE-54 (DB-5, SPB-5, RTx-5, or equivalent), 1.5 µm film thickness.

30-m x 0.53 mm ID fused silica capillary column chemically bonded with 50 percent phenyl methylpolysiloxane (DB-1701, or equivalent), 1.0 µm film thickness.

Column pair 1 is mounted in a press-fit Y-shaped glass 3-way union splitter (J&W Scientific, Catalog No. 705-0733) or a Y-shaped fused-silica connector (Restek, Catalog No. 20405), or equivalent.

4.2.3.2 Column pair 2

30-m x 0.53 mm ID fused silica capillary column chemically bonded with SE-54 (DB-5, SPB-5, RTx-5, or equivalent), 0.83 µm film thickness.

30-m x 0.53 mm ID fused silica capillary column chemically bonded with 50 percent phenyl methylpolysiloxane (DB-1701, or equivalent), 1.0 µm film thickness.

Column pair 2 is mounted in an 8 in. deactivated glass injection tee (Supelco, Catalog No. 2-3665M), or equivalent.

4.3 Column rinsing kit - Bonded-phase column rinse kit (J&W Scientific, Catalog No. 430-3000), or equivalent.

4.4 Volumetric flasks, 10-mL and 25-mL, for preparation of standards.

5.0 REAGENTS

5.1 Reagent grade or pesticide grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

NOTE: Store the standard solutions (stock, composite, calibration, internal, and surrogate) at 4°C in polytetrafluoroethylene (PTFE)-sealed containers in the dark. When a lot of standards is prepared, it is recommended that aliquots of that lot be stored in individual small vials. All stock standard solutions must be replaced after one year or sooner if routine QC tests (Sec. 8.0) indicate a problem. All other standard solutions must be replaced after six months or sooner if routine QC (Sec. 8.0) indicates a problem.

5.2 Solvents used in the extraction and cleanup procedures (appropriate 3500 and 3600 series methods) include n-hexane, diethyl ether, methylene chloride, acetone, ethyl acetate, and

isooctane (2,2,4-trimethylpentane) and must be exchanged to n-hexane or isooctane prior to analysis.

Therefore, n-hexane and isooctane will be required in this procedure. Acetone or toluene may be required for the preparation of some standard solutions (see Sec. 5.4.2). All solvents should be pesticide quality or equivalent, and each lot of solvent should be determined to be phthalate free.

5.3 Organic-free reagent water - All references to water in this method refer to organic-free reagent water as defined in Chapter One.

5.4 Stock standard solutions (1000 mg/L) - May be prepared from pure standard materials or can be purchased as certified solutions.

5.4.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure compound. Dissolve the compound in isooctane or hexane and dilute to volume in a 10-mL volumetric flask. If compound purity is 96 percent or greater, the weight can be used without correction to calculate the concentration of the stock standard solution. Commercially prepared stock standard solutions can be used at any concentration if they are certified by the manufacturer or by an independent source.

5.4.2 β-BHC, Dieldrin, and some other standards may not be adequately soluble in isooctane. A small amount of acetone or toluene should be used to dissolve these compounds during the preparation of the stock standard solutions.

5.5 Composite stock standard - May be prepared from individual stock solutions.

5.5.1 For composite stock standards containing less than 25 components, take exactly 1 mL of each individual stock solution at a concentration of 1000 mg/L, add solvent, and mix the solutions in a 25-mL volumetric flask. For example, for a composite containing 20 individual standards, the resulting concentration of each component in the mixture, after the volume is adjusted to 25 mL, will be 1 mg/25 mL. This composite solution can be further diluted to obtain the desired concentrations.

5.5.2 For composite stock standards containing more than 25 components, use volumetric flasks of the appropriate volume (e.g., 50 mL, 100 mL), and follow the procedure described above.

5.6 Calibration standards should be prepared at a minimum of five different concentrations by dilution of the composite stock standard with isooctane or hexane. The concentrations should correspond to the expected range of concentrations found in real samples and should bracket the linear range of the detector.

5.6.1 Although all single component analytes can be resolved on a new 35 percent phenyl methyl silicone column (e.g., DB-608), two calibration mixtures should be prepared for the single component analytes of this method. This procedure is established to minimize potential resolution and quantitation problems on confirmation columns or on older 35 percent phenyl methyl silicone (e.g. DB-608) columns and to allow determination of Endrin and DDT breakdown for method QC (Sec. 8.0).

5.6.2 Separate calibration standards are required for each multi-component target analyte (e.g., Toxaphene and Chlordane). Analysts should evaluate the specific Toxaphene standard carefully. Some Toxaphene components, particularly the more heavily chlorinated

components, are subject to dechlorination reactions. As a result, standards from different vendors may exhibit marked differences which could lead to possible false negative results or to large differences in quantitative results.

5.7 Internal standard (optional)

5.7.1 Pentachloronitrobenzene is suggested as an internal standard for the single-column analysis, when it is not considered to be a target analyte. 1-bromo-2-nitrobenzene may also be used. Prepare a solution of 5000 mg/L (5000 ng/µL) of pentachloronitrobenzene or 1-bromo-2-nitrobenzene. Spike 10 µL of this solution into each 1 mL sample extract.

5.7.2 1-bromo-2-nitrobenzene is suggested as an internal standard for the dual-column analysis. Prepare a solution of 5000 mg/L (5000 ng/µL) of 1-bromo-2-nitrobenzene. Spike 10 µL of this solution into each 1 mL of sample extract.

5.8 Surrogate standards

The performance of the method should be monitored using surrogate compounds. Surrogate standards are added to all samples, method blanks, matrix spikes, and calibration standards. The following compounds are recommended as possible surrogates.

5.8.1 Decachlorobiphenyl and tetrachloro-m-xylene have been found to be a useful pair of surrogates for both the single-column and dual-column configurations. Method 3500, Sec. 5.0, describes the procedures for preparing these surrogates.

5.8.2 4-Chloro-3-nitrobenzotrifluoride may also be useful as a surrogate if the chromatographic conditions of the dual-column configuration cannot be adjusted to preclude co-elution of a target analyte with either of the surrogates in Sec. 5.8.1. However, this compound elutes early in the chromatographic run and may be subject to other interference problems. A recommended concentration for this surrogate is 500 ng/µL. Use a spiking volume of 100 µL for a 1-L aqueous sample.

5.8.3 Store surrogate spiking solutions at 4°C in PTFE-sealed containers in the dark.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See Chapter Four, Organic Analytes, Sec. 4.0, for sample collection and preservation instructions.

6.2 Extracts must be stored under refrigeration in the dark and analyzed within 40 days of extraction.

7.0 PROCEDURE

7.1 Sample extraction

Refer to Chapter Two and Method 3500 for guidance in choosing the appropriate extraction procedure. In general, water samples are extracted at a neutral pH with methylene chloride using a separatory funnel (Method 3510), a continuous liquid-liquid extractor (Method

3520), solid-phase extraction (Method 3535), or other appropriate technique. Solid samples are extracted with hexane-acetone (1:1) or methylene chloride-acetone (1:1) using one of the Soxhlet extraction (Method 3540 or 3541), pressurized fluid extraction (Method 3545), ultrasonic extraction (Method 3550), supercritical fluid extraction (Method 3562), or other appropriate technique.

NOTE: Hexane-acetone (1:1) may be more effective as an extraction solvent for organochlorine pesticides in some environmental and waste matrices than is methylene chloride-acetone (1:1). Relative to the methylene chloride-acetone mixture, use of hexane-acetone generally reduces the amount of interferences that are extracted and improves signal-to-noise.

Spiked samples are used to verify the applicability of the chosen extraction technique to each new sample type. Each sample type must be spiked with the compounds of interest to determine the percent recovery and the limit of detection for that sample (see Chapter One). See Method 8000 for guidance on demonstration of initial method proficiency as well as guidance on matrix spikes for routine sample analysis.

7.2 Extract cleanup

Cleanup procedures may not be necessary for a relatively clean sample matrix, but most extracts from environmental and waste samples will require additional preparation before analysis. The specific cleanup procedure used will depend on the nature of the sample to be analyzed and the data quality objectives for the measurements. General guidance for sample extract cleanup is provided in this section and in Method 3600.

7.2.1 If a sample is of biological origin, or contains high molecular weight materials, the use of Method 3640 (GPC cleanup - pesticide option) is recommended. Frequently, one of the adsorption chromatographic cleanups (alumina, silica gel, or Florisil) may also be required following the GPC cleanup.

7.2.2 Method 3610 (alumina) may be used to remove phthalate esters.

7.2.3 Method 3620 (Florisil) may be used to separate organochlorine pesticides from aliphatic compounds, aromatics, and nitrogen-containing compounds.

7.2.4 Method 3630 (silica gel) may be used to separate single component organochlorine pesticides from some interferants.

7.2.5 Elemental sulfur, which may be present in certain sediments and industrial wastes, interferes with the electron capture gas chromatography of certain pesticides. Sulfur should be removed by the technique described in Method 3660.

7.3 GC conditions

This method allows the analyst to choose between a single-column or a dual-column configuration in the injector port. Either wide- or narrow-bore columns may be used. Identifications based on retention times from a single-column must be confirmed on a second column or with an alternative qualitative technique.

7.3.1 Single-column analysis

This capillary GC/ECD method allows the analyst the option of using 0.25-0.32 mm ID capillary columns (narrow-bore) or 0.53 mm ID capillary columns (wide-bore). Performance data are provided for both options. Figures 1 - 6 provide example chromatograms.

7.3.1.1 The use of narrow-bore (≤0.32 mm ID) columns is recommended when the analyst requires greater chromatographic resolution. Use of narrow-bore columns is suitable for relatively clean samples or for extracts that have been prepared with one or more of the clean-up options referenced in the method. Wide-bore columns (0.53 mm ID) are suitable for more complex environmental and waste matrices.

7.3.1.2 Table 1 lists average retention times for the target analytes using wide-bore capillary columns. Table 2 lists average retention times for the target analytes using narrow-bore capillary columns.

7.3.1.3 Table 4 lists the GC operating conditions for the single-column method of analysis.

7.3.2 Dual-column analysis

The dual-column/dual-detector approach involves the use of two 30-m x 0.53 mm ID fused-silica open-tubular columns of different polarities, thus, different selectivities towards the target analytes. The columns are connected to an injection tee and separate electron capture detectors.

7.3.2.1 Retention times for the organochlorine analytes on dual-columns are in Table 6. The GC operating conditions for the compounds in Table 6 are given in Table 7.

7.3.2.2 Multi-component mixtures of Toxaphene and Strobane were analyzed separately (Figures 5 and 6) using the operating conditions found in Table 7.

7.3.2.3 Figure 6 is a sample chromatogram for a mixture of organochlorine pesticides. The retention times of the individual components detected in these mixtures are given in Tables 6 and 7.

7.3.2.4 Operating conditions for a more heavily loaded DB-5/DB-1701 pair are given in Table 8. This column pair was used for the detection of multi-component organochlorine compounds.

7.3.2.5 Operating conditions for a DB-5/DB-1701 column pair with thinner films, a different type of splitter, and a slower temperature programming rate are provided in Table 7. These conditions gave better peak shapes for Nitrofen and Dicofol. Table 6 lists the retention times for the compounds on this column pair.

7.4 Calibration

7.4.1 Prepare calibration standards using the procedures in Sec. 5.0. Refer to Method 8000 (Sec. 7.0) for proper calibration techniques for both initial calibration and calibration verification. The procedure for either internal or external calibration may be used.

In most cases, external standard calibration is used with Method 8081 because of the sensitivity of the electron capture detector and the probability of the internal standard being affected by interferences. Because several of the pesticides may co-elute on any single-column, analysts should use two calibration mixtures (see Sec. 3.8). The specific mixture should be selected to minimize the problem of peak overlap.

NOTE: Because of the sensitivity of the electron capture detector, the injection port and column should always be cleaned prior to performing the initial calibration.

7.4.1.1 Unless otherwise necessary for a specific project, the analysis of the multi-component analytes employs a single-point calibration. A single calibration standard near the mid-point of the expected calibration range of each multi-component analyte is included with the initial calibration of the single component analytes for pattern recognition, so that the analyst is familiar with the patterns and retention times on each column.

7.4.1.2 For calibration verification (each 12-hour shift) all target analytes required in the project plan must be injected.

7.4.2 Establish the GC operating conditions appropriate for the configuration (single-column or dual column, Sec. 7.3) using Tables 4, 5, 7, or 8 as guidance. Optimize the instrumental conditions for resolution of the target analytes and sensitivity. An initial oven temperature $\leq$140 -150°C is required to resolve the four BHC isomers. A final temperature of 240 - 270°C is required to elute decachlorobiphenyl. Use of injector pressure programming will improve the chromatography of late eluting peaks.

NOTE: Once established, the same operating conditions must be used for both calibrations and sample analyses.

7.4.3 A 2-µL injection volume of each calibration standard is recommended. Other injection volumes may be employed, provided that the analyst can demonstrate adequate sensitivity for the compounds of interest.

7.4.4 Because of the low concentration of pesticide standards injected on a GC/ECD, column adsorption may be a problem when the GC has not been used for a day or more. Therefore, the GC column should be primed (or deactivated) by injecting a pesticide standard mixture approximately 20 times more concentrated than the mid-concentration standard. Inject this standard mixture prior to beginning the initial calibration or calibration verification.

CAUTION: Several analytes, including Aldrin, may be observed in the injection just following this system priming. Always run an acceptable blank prior to running any standards or samples.

7.4.5 Calibration factors

When external standard calibration is employed, calculate the calibration factor for each analyte at each concentration, the mean calibration factor, and the relative standard deviation (RSD) of the calibration factors, using the formulae below. If internal standard calibration is employed, refer to Method 8000 for the calculation of response factors.

7.4.5.1 Calculate the calibration factor for each analyte at each concentration as:

$$CF = \frac{\text{Peak Area (or Height) of the Compound in the Standard}}{\text{Mass of the Compound Injected (in nanograms)}}$$

7.4.5.2 Calculate the mean calibration factor for each analyte as:

$$\text{mean CF} = \overline{CF} = \frac{\sum_{i=1}^{n} CF_i}{n}$$

where n is the number of standards analyzed.

7.4.5.3 Calculate the standard deviation (SD) and the RSD of the calibration factors for each analyte as:

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (CF_i - \overline{CF})^2}{n-1}} \qquad RSD = \frac{SD}{\overline{CF}} \times 100$$

If the RSD for each analyte is ≤ 20%, then the response of the instrument is considered linear and the mean calibration factor can be used to quantitate sample results. If the RSD is greater than 20%, then linearity through the origin cannot be assumed. The analyst must use a calibration curve or a non-linear calibration model (e.g., a polynomial equation) for quantitation. See Method 8000 for information on non-linear calibrations.

7.4.6 Retention time windows

Absolute retention times are used for compound identification. Retention time windows are crucial to the identification of target compounds, and should be established by one of the approaches described in Method 8000.

7.4.6.1 Before establishing the retention time windows, make sure the gas chromatographic system is operating within optimum conditions.

7.4.6.2 The widths of the retention time windows are defined as described in Method 8000. However, the experience of the analyst should weigh heavily in the interpretation of the chromatograms.

7.5 Gas chromatographic analysis of sample extracts

7.5.1 The same GC operating conditions used for the initial calibration must be employed for samples analyses.

7.5.2 Verify calibration each 12-hour shift by injecting calibration verification standards prior to conducting any sample analyses. Analysts should alternate the use of high and low concentration mixtures of single-component analytes and multi-component analytes for calibration verification. A calibration standard must also be injected at intervals of not less than once every twenty samples (after every 10 samples is *recommended* to minimize the number of samples requiring re-injection when QC limits are exceeded) and at the end of the analysis sequence. See Sec. 8.4.4 for additional guidance on the frequency of the standard injections.

7.5.2.1 The calibration factor for each analyte should not exceed a ± 15 percent difference from the mean calibration factor calculated for the initial calibration. If a non-linear calibration model or a linear model not through the origin has been employed for the initial calibration, consult Sec. 7 of Method 8000 for the specifics of calibration verification.

$$\% \text{ Difference} = \frac{CF - \overline{CF_v}}{\overline{CF}} \times 100$$

7.5.2.2 If this criterion is exceeded for any analyte, use the approach described in Sec. 7 of Method 8000 to calculate the average percent difference across all analytes. If the average of the responses for all analytes is within ±15%, then the calibration has been verified. However, the conditions in Sec. 7 of Method 8000 also apply, e.g., the average must include all analytes in the calibration, regardless of whether they are target analytes for a specific project, and the data user must be provided with the calibration verification data or a list of those analytes that exceeded the ±15% limit.

7.5.2.3 If the calibration does not meet the ±15% limit (either on the basis of each compound or the average across all compounds), check the instrument operating conditions, and if necessary, restore them to the original settings, and inject another aliquot of the calibration verification standard. If the response for the analyte is still not within ±15%, then a new initial calibration must be prepared. The effects of a failing calibration verification standard on sample results are discussed in Sec. 7.5.7.

7.5.3 Compare the retention time of each analyte in the calibration standard with the absolute retention time windows established in Sec. 7.4.6. As described in Method 8000, the center of the absolute retention time window for each analyte is its retention time in the mid-concentration standard analyzed during the initial calibration. Each analyte in each standard must fall within its respective retention time window. If not, the gas chromatographic system must either be adjusted so that a second analysis of the standard does result in all analytes falling within their retention time windows, or a new initial calibration must be performed and new retention time windows established.

7.5.4 Inject a 2-µL aliquot of the concentrated sample extract. Record the volume injected to the nearest 0.05 µL and the resulting peak size in area units.

7.5.5 Tentative identification of an analyte occurs when a peak from a sample extract falls within the absolute retention time window. Each tentative identification must be confirmed using either a second GC column of dissimilar stationary phase or using another technique such as GC/MS (see Sec. 7.7).

When results are confirmed using a second GC column of dissimilar stationary phase, the analyst should check the agreement between the quantitative results on both columns once the identification has been confirmed. See Sec. 7 of Method 8000 for a discussion of such a comparison. Unless otherwise specified in an approved project plan, the higher result should be reported, as this is a conservative approach relative to protection of the environment. If the relative percent difference of the results exceeds 40%, consult Method 8000 for steps that may be taken to address the discrepancy.

7.5.6 When using the external calibration procedure (Method 8000), determine the quantity of each component peak in the sample chromatogram which corresponds to the compounds used for calibration purposes, as follows. Proper quantitation requires the appropriate selection of a baseline from which the peak area or height can be determined.

7.5.6.1 For aqueous samples

$$\text{Concentration } (\mu g/L) = \frac{(A_x)(V_t)(D)}{(\overline{CF})(V_i)(V_s)}$$

where:

A_x = Area (or height) of the peak for the analyte in the sample.

V_t = Total volume of the concentrated extract (µL).

D = Dilution factor, if the sample or extract was diluted prior to analysis. If no dilution was made, D = 1. The dilution factor is always dimensionless.

$\overline{CF}$ = Mean calibration factor from the initial calibration (area/ng).

V_i = Volume of the extract injected (µL). The injection volume for samples and calibration standards must be the same. For purge-and-trap analysis, V_i is not applicable and therefore is set at 1.

V_s = Volume of the aqueous sample extracted in mL. If units of liters are used for this term, multiply the results by 1000.

Using the units specified here for these terms will result in a concentration in units of ng/mL, which is equivalent to µg/L.

7.5.6.2 For non-aqueous samples

$$\text{Concentration } (\mu g/kg) = \frac{(A_x)(V_t)(D)}{(\overline{CF})(V_i)(W_s)}$$

where A_x, V_t, D, $\overline{CF}$, and V_i are the same as for aqueous samples, and

W_s = Weight of sample extracted (g). The wet weight or dry weight may be used, depending upon the specific application of the data. If units of kilograms are used for this term, multiply the results by 1000.

Using the units specified here for these terms will result in a concentration in units of ng/g, which is equivalent to µg/kg.

7.5.6.3 See Method 8000 for the equation used for internal standard quantitation.

7.5.6.4 If the responses exceed the calibration range of the system, dilute the extract and reanalyze. Peak height measurements are recommended over peak area integration when overlapping peaks cause errors in area integration.

7.5.6.5 If partially overlapping or coeluting peaks are found, change GC columns or try GC/MS quantitation (see Sec. 8.0 and Method 8270).

7.5.7 Each sample analysis must be bracketed with an acceptable initial calibration, calibration verification standard(s) (each 12-hour analytical shift), or calibration standards interspersed within the samples.

Although analysis of a single mid-concentration standard (standard mixture or multi-component analyte) will satisfy the minimum requirements, analysts are urged to use different calibration verification standards during organochlorine pesticide analyses. Also, multi-level standards (mixtures or multi-component analytes) are highly recommended to ensure that the detector response remains stable for all the analytes over the calibration range.

The results from these bracketing standards must meet the calibration verification criteria in Sec. 7.5.2. When a calibration verification standard fails to meet the QC criteria, all samples that were injected after the last standard that last met the QC criteria must be evaluated to prevent mis-quantitations and possible false negative results, and re-injection of the sample extracts may be required. More frequent analyses of standards will minimize the number of sample extracts that would have to be reinjected if the QC limits are violated for the standard analysis.

However, if the standard analyzed after a group of samples exhibits a response for an analyte that is above the acceptance limit, i.e., >15%, and the analyte was not detected in the specific samples analyzed during the analytical shift, then the extracts for those samples do not need to be reanalyzed, as the verification standard has demonstrated that the analyte would have been detected were it present. In contrast, if an analyte above the QC limits was detected in a sample extract, then re-injection is necessary to ensure accurate quantitation. If an analyte was not detected in the sample and the standard response is more than 15% below the initial calibration response, then re-injection is necessary to ensure that the detector response has not deteriorated to the point that the analyte would not have been detected even though it was present (i.e., a false negative result).

7.5.8 Sample injections may continue for as long as the calibration verification standards and standards interspersed with the samples meet instrument QC requirements. It is *recommended* that standards be analyzed after every 10 samples (*required* after every 20 samples and at the end of a set) to minimize the number of samples that must be re-injected when the standards fail the QC limits. The sequence ends when the set of samples has been injected or when qualitative and/or quantitative QC criteria are exceeded.

7.5.9 If the peak response is less than 2.5 times the baseline noise level, the validity of the quantitative result may be questionable. The analyst should consult with the source of the sample to determine whether further concentration of the sample is warranted.

7.5.10 Validation of GC system qualitative performance

7.5.10.1 Use the calibration standards analyzed during the sequence to evaluate retention time stability. The retention time windows are established using the absolute retention time of each analyte as described in Method 8000.

7.5.10.2 Each subsequent injection of a standard during the 12-hour analytical shift (i.e., those standards injected every 20 samples, or more frequently) must be checked against the retention time windows. If any of these subsequent standards fall outside their absolute retention time windows, the GC system is out of control. Determine the cause of the problem and correct it. If the problem cannot be corrected, a new initial calibration must be performed.

7.5.11 Identification of mixtures (i.e. Chlordane and Toxaphene) is based on the characteristic "fingerprint" retention time and shape of the indicator peak(s); and quantitation is based on the area under the characteristic peaks as compared to the area under the corresponding calibration peak(s) of the same retention time and shape generated using either internal or external calibration procedures.

7.5.12 If compound identification or quantitation is precluded due to interference (e.g., broad, rounded peaks or ill-defined baselines are present) cleanup of the extract or replacement of the capillary column or detector is warranted. Rerun the sample on another instrument to determine if the problem results from analytical hardware or the sample matrix. Refer to Method 3600 for the procedures to be followed in sample cleanup.

7.6 Quantitation of multi-component analytes - Multi-component analytes present problems in measurement. Suggestions are offered in the following sections for handling Toxaphene, Strobane, Chlordane, BHC, and DDT.

7.6.1 Toxaphene and Strobane - Toxaphene is manufactured by the chlorination of camphenes, whereas Strobane results from the chlorination of a mixture of camphenes and pinenes. Quantitation of Toxaphene or Strobane is difficult, but reasonable accuracy can be obtained. To calculate Toxaphene from GC/ECD results:

7.6.1.1. Adjust the sample size so that the major Toxaphene peaks are 10-70% of full-scale deflection (FSD).

7.6.1.2 Inject a Toxaphene standard that is estimated to be within ± 10 ng of the sample amount.

7.6.1.3 Quantitate Toxaphene using the total area of the Toxaphene pattern or using 4 to 6 major peaks.

7.6.1.3.1 While Toxaphene contains a large number of compounds that will produce well resolved peaks in a GC/ECD chromatogram, it also contains many other components that are not chromatographically resolved. This unresolved complex mixture results in the "hump" in the chromatogram that is characteristic of this mixture. Although the resolved peaks are important for the identification of the mixture, the area of the unresolved complex mixture contributes a significant portion of the area of the total response.

7.6.1.3.2 To measure total area, construct the baseline of Toxaphene in the sample chromatogram between the retention times of the first and last eluting Toxaphene components in the standard. In order to use the total area approach, the pattern in the sample chromatogram must be compared to that of the standard to ensure that all of the major components in the standard are present in the sample. Otherwise, the sample concentration may be significantly underestimated.

7.6.1.3.3 Toxaphene may also be quantitated on the basis of 4 to 6 major peaks. A collaborative study of a series of Toxaphene residues evaluated several approaches to quantitation of this compound, including the use of the total area of the peaks in the Toxaphene chromatogram and the use of a subset of 4 to 6 peaks. That study indicated that the use of 4 to 6 peaks provides results that agree well with the total peak area approach and may avoid difficulties when interferences with Toxaphene peaks are present in the early portion of the chromatogram from compounds such as DDT. Whichever approach is employed should be documented and available to the data user, if necessary.

7.6.1.3.4 When Toxaphene is determined using the 4 to 6 peaks approach, the analyst must take care to evaluate the relative areas of the peaks chosen in the sample and standard chromatograms. It is highly unlikely that the peaks will match exactly, but the analyst should not employ peaks from the sample chromatogram whose relative sizes or areas appear to be disproportionally larger or smaller in the sample compared to the standard.

7.6.1.3.5 The heights or areas of the 4 to 6 peaks should be summed together and used to determine the Toxaphene concentration. Alternatively, use each peak in the standard to calculate a calibration factor for that peak, using the total mass of Toxaphene in the standard. These calibration factors are then used to calculate the concentration of each corresponding peak in the sample chromatogram and the 4 to 6 resulting concentrations are averaged to provide the final result for the sample.

7.6.2 Chlordane - Technical Chlordane is a mixture of at least 11 major components and 30 or more minor components that is used to prepare specific pesticide formulations. The CAS Registry number for Technical Chlordane is properly given as 12789-03-6. *Trans*-Chlordane (or α-Chlordane, CAS RN 5103-71-9) and *cis*-Chlordane (γ-Chlordane, CAS RN 5103-74-2), are the two most prevalent major components of Technical Chlordane. However, the exact percentage of each in the technical material is not completely defined, and is not consistent from batch to batch. Moreover, changes may occur when the technical material is used to prepare specific pesticide formulations. The approach used for evaluating and reporting Chlordane results will often depend on the end use of the results and the analyst's skill in interpreting this multi-component pesticide residue. The following sections discuss three specific options: reporting Technical Chlordane (12789-03-6), reporting Chlordane (not otherwise specified, 57-74-9), and reporting the individual Chlordane components that can be identified under their individual CAS numbers.

7.6.2.1 When the GC pattern of the residue resembles that of Technical Chlordane, the analyst may quantitate Chlordane residues by comparing the total area of the Chlordane chromatogram using three to five major peaks or the total area. If the

Heptachlor epoxide peak is relatively small, include it as part of the total Chlordane area for calculation of the residue. If Heptachlor and/or Heptachlor epoxide are much out of proportion, calculate these separately and subtract their areas from the total area to give a corrected Chlordane area.

NOTE: Octachloro epoxide, a metabolite of Chlordane, can easily be mistaken for Heptachlor epoxide on a nonpolar GC column.

To measure the total area of the Chlordane chromatogram, inject an amount of a Technical Chlordane standard which will produce a chromatogram in which the major peaks are approximately the same size as those in the sample chromatograms. Construct the baseline of Technical Chlordane in the standard chromatogram between the retention times of the first and last eluting toxaphene components. Use this area and the mass of Technical Chlordane in the standard to calculate a calibration factor. Construct a similar baseline in the sample chromatogram, measure the area, and use the calibration factor to calculate the concentration in the sample.

7.6.2.2 The GC pattern of a Chlordane residue in a sample may differ considerably from that of the Technical Chlordane standard. In such instances, it may not be practical to relate a sample chromatogram back to the pesticide active ingredient Technical Chlordane. Therefore, depending on the objectives of the analysis, the analyst may choose to report the sum of all the identifiable Chlordane components as "Chlordane (n.o.s.)" under the CAS number 57-74-9.

7.6.2.3 The third option is to quantitate the peaks of α-Chlordane, γ-Chlordane, and Heptachlor separately against the appropriate reference materials, and report these individual components under their respective CAS numbers.

7.6.2.4 To measure the total area of the Chlordane chromatogram, inject an amount of a Technical Chlordane standard which will produce a chromatogram in which the major peaks are approximately the same size as those in the sample chromatograms.

7.6.3 Hexachlorocyclohexane - Hexachlorocyclohexane is also known as BHC, from the former name, benzene hexachloride. Technical grade BHC is a cream-colored amorphous solid with a very characteristic musty odor. It consists of a mixture of six chemically distinct isomers and one or more heptachlorocyclohexanes and octachlorocyclohexanes. Commercial BHC preparations may show a wide variance in the percentage of individual isomers present. Quantitate each isomer (α, β, γ, and δ) separately against a standard of the respective pure isomer.

7.6.4 DDT - Technical DDT consists primarily of a mixture of 4,4'-DDT (approximately 75%) and 2,4'-DDT (approximately 25%). As DDT weathers, 4,4'-DDE, 2,4'-DDE, 4,4'-DDD, and 2,4'-DDD are formed. Since the 4,4'-isomers of DDT, DDE, and DDD predominate in the environment, these are the isomers normally regulated by EPA. Therefore, sample extracts should be quantitated against standards of the respective pure isomers of 4,4'-DDT, 4,4'-DDE, and 4,4'-DDD.

7.7 GC/MS confirmation may be used in conjunction with either single-column or dual-column analysis if the concentration is sufficient for detection by GC/MS.

7.7.1 Full-scan GC/MS will normally require a concentration of approximately 10 ng/µL in the final extract for each single-component compound. Ion trap or selected ion monitoring will normally require a concentration of approximately 1 ng/µL.

7.7.2 The GC/MS must be calibrated for the specific target pesticides when it is used for quantitative analysis. If GC/MS is used only for confirmation of the identification of the target analytes, then the analyst must demonstrate that those pesticides identified by GC/ECD can be confirmed by GC/MS. This demonstration may be accomplished by analyzing a single-point standard containing the analytes of interest at or below the concentrations reported in the GC/EC analysis.

7.7.3 GC/MS is not recommended for confirmation when concentrations are below 1 ng/µL in the extract, unless a more sensitive masss spectrometer is employed.

7.7.4 GC/MS confirmation should be accomplished by analyzing the same extract that is used for GC/ECD analysis and the extract of the associated method blank.

7.7.5 The base/neutral/acid extract and the associated blank may be used for GC/MS confirmation if the surrogates and internal standards do not interfere and if it is demonstrated that the analyte is stable during acid/base partitioning. However, if the compounds are *not* detected in the base/neutral/acid extract, then GC/MS analysis of the pesticide extract should be performed.

7.8 Suggested chromatographic system maintenance - When system performance does not meet the established QC requirements, corrective action is required, and may include one or more of the following.

7.8.1 Splitter connections - For dual-columns which are connected using a press-fit Y-shaped glass splitter or a Y-shaped fused-silica connector, clean and deactivate the splitter port insert or replace with a cleaned and deactivated splitter. Break off the first few centimeters (up to 30 cm) of the injection port side of the column. Remove the columns and solvent backflush according to the manufacturer's instructions. If these procedures fail to eliminate the degradation problem, it may be necessary to deactivate the metal injector body and/or replace the columns.

7.8.2 GC injector ports can be of critical concern, especially in the analysis of DDT and Endrin. Injectors that are contaminated, chemically active, or too hot can cause the degradation ("breakdown") of the analytes. Endrin and DDT breakdown to endrin aldehyde, endrin ketone, DDD, or DDE. When such breakdown is observed, clean and deactivate the injector port, break off at least 30 cm of the column and remount it. Check the injector temperature and lower it to 205°C, if required. Endrin and DDT breakdown are less of a problem when ambient on-column injectors are used.

7.8.3 Metal injector body - Turn off the oven and remove the analytical columns when the oven has cooled. Remove the glass injection port insert (instruments with on-column injection). Lower the injection port temperature to room temperature. Inspect the injection port and remove any noticeable foreign material.

7.8.3.1 Place a beaker beneath the injector port inside the oven. Using a wash bottle, serially rinse the entire inside of the injector port with acetone and then toluene, catching the rinsate in the beaker.

7.8.3.2 Prepare a solution of a deactivating agent (Sylon-CT or equivalent) following manufacturer's directions. After all metal surfaces inside the injector body have been thoroughly coated with the deactivation solution, rinse the injector body with toluene, methanol, acetone, then hexane. Reassemble the injector and replace the columns.

7.8.4 Column rinsing - The column should be rinsed with several column volumes of an appropriate solvent. Both polar and nonpolar solvents are recommended. Depending on the nature of the sample residues expected, the first rinse might be water, followed by methanol and acetone. Methylene chloride is a good final rinse and in some cases may be the only solvent required. The column should then be filled with methylene chloride and allowed to stand flooded overnight to allow materials within the stationary phase to migrate into the solvent. The column is then flushed with fresh methylene chloride, drained, and dried at room temperature with a stream of ultrapure nitrogen.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Quality control procedures to ensure the proper operation of the various sample preparation techniques can be found in Method 3500. If an extract cleanup procedure was performed, refer to Method 3600 for the appropriate quality control procedures. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures necessary to evaluate the GC system operation are found in Method 8000, Sec. 7.0 and include evaluation of retention time windows, calibration verification, and chromatographic analysis of samples.

8.3 Initial Demonstration of Proficiency

8.3.1 Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made.

8.3.2 It is suggested that the quality control (QC) reference sample concentrate (as discussed in Sec. 8.0 of Methods 8000 and 3500) contain each analyte of interest at 10 mg/L. If this method is to be used for analysis of Chlordane or Toxaphene only, the QC reference sample concentrate should contain the most representative multi-component mixture at a suggested concentration of 50 mg/L in acetone. See Method 8000, Sec. 8.0 for additional information on how to accomplish this demonstration.

8.3.3 Calculate the average recovery and the standard deviation of the recoveries of the analytes in each of the four QC reference samples. Refer to Sec. 8.0 of Method 8000 for procedures for evaluating method performance.

8.4 Sample Quality Control for Preparation and Analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples, including a method

blank, a matrix spike, a duplicate, and a laboratory control sample (LCS), and the addition of surrogates to each field sample and QC sample.

8.4.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, the laboratories should use a matrix spike and matrix spike duplicate pair.

8.4.2 In-house method performance criteria should be developed using the guidance found in Sec. 8.0 of Method 8000 for procedures for evaluating method performance.

8.4.3 A Laboratory Control Sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicates a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

8.4.4 Include a calibration standard after each group of 20 samples (it is *recommended* that a calibration standard be included after every 10 samples to minimize the number of repeat injections) in the analysis sequence as a calibration check. Thus, injections of method blank extracts, matrix spike samples, and other non-standards are counted in the total. Solvent blanks, injected as a check on cross-contamination, need not be counted in the total. The response factors for the calibration should be within ±15% of the initial calibration (see Sec. 7.5.2). When this calibration verification standard falls out of this acceptance window, the laboratory should stop analyses and take corrective action.

8.4.5 Whenever quantitation is accomplished using an internal standard, internal standards must be evaluated for acceptance. The measured area of the internal standard must be no more than 50 percent different from the average area calculated during calibration. When the internal standard peak area is outside the limit, all samples that fall outside the QC criteria must be reanalyzed.

8.4.6 DDT and endrin are easily degraded in the injection port. Breakdown occurs when the injection port liner is contaminated with high boiling residue from sample injection or when the injector contains metal fittings. Check for degradation problems by injecting a standard containing only 4,4'-DDT and endrin. Presence of 4,4'-DDE, 4,4'-DDD, endrin ketone or endrin indicates breakdown. If degradation of either DDT or endrin exceeds 15%, take corrective action before proceeding with calibration.

8.4.6.1 Calculate percent breakdown as follows:

$$\% \text{ breakdown of DDT} = \frac{\text{sum of degradation peak areas (DDD + DDE)}}{\text{sum of all peak areas (DDT + DDE + DDD)}} \times 100$$

$$\% \text{ breakdown of endrin} = \frac{\text{sum of degradation peak areas (aldehyde + ketone)}}{\text{sum of all peak areas (endrin + aldehyde + ketone)}} \times 100$$

8.4.6.2 The breakdown of DDT and endrin should be measured before samples are analyzed and at the beginning of each 12-hour shift. Injector maintenance and recalibration should be completed if the breakdown is greater than 15% for either compound (Sec. 7.8.2).

8.4.7 Whenever silica gel (Method 3630) or Florisil (Method 3620) cleanups are used, the analyst must demonstrate that the fractionation scheme is reproducible. Batch to batch variation in the composition of the silica gel or Florisil or overloading the column may cause a change in the distribution patterns of the organochlorine pesticides. When compounds are found in two fractions, add the concentrations found in the fractions, and correct for any additional dilution.

8.4.8 See Method 8000, Sec. 8.0 for the details on carrying out sample quality control procedures for preparation and analysis.

8.5 Surrogate recoveries

The laboratory must evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000, Sec. 8.0, for information on evaluating surrogate data and developing and updating surrogate limits.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 The method detection limit (MDL) is defined in Chapter One. Each laboratory should develop its own matrix-specific MDLs using the guidance found in Chapter One. Estimated quantitation limits (EQLs) may be determined using the factors in Table 3.

9.2 The chromatographic separations in this method have been tested in a single laboratory by using clean hexane and liquid and solid waste extracts that were spiked with the test compounds at three concentrations. Single-operator precision, overall precision, and method accuracy were found to be related to the concentration of the compound and the type of matrix.

9.3 The accuracy and precision that can be acheived with this method depend on the sample matrix, sample preparation technique, optional cleanup techniques, and calibration procedures used.

9.4 Tables 9 and 10 contain precision and recovery data generated for sewage sludge and dichloroethane stillbottoms. Table 11 contains recovery data for a clay soil, taken from Reference 10. The spiking concentration was for the clay soil was 500 µg/kg. The spiking solution was mixed into the soil and then immediately transferred to the extraction device and immersed in the extraction solvent. The spiked sample was then extracted by Method 3541 (Automated Soxhlet). The data represent a single determination. Analysis was by capillary column gas chromatography/electron capture detector following Method 8081 for the organochlorine pesticides.

9.5 Table 12 contains single-laboratory precision and accuracy data for solid-phase extraction of TCLP buffer solutions spiked at two levels and extracted using Method 3535.

9.6 Table 13 contains multiple-laboratory data for solid-phase extraction of spiked TCLP soil leachates extracted using Method 3535.

9.7 Table 14 contains single-laboratory data on groundwater and wastewater samples extracted by solid-phase extraction, using Method 3535.

9.8 Tables 15 and 16 contain single-laboratory performance data using supercritical fluid extraction Method 3562. Samples were analyzed by GC/ELCD. The method was performed using a variable restrictor and solid trapping material (octadecyl silane [ODS]). Three different soil samples were spiked at 5 and 250 µg/kg. Soil 1 (Delphi) is described as loamy sand, with 2.4% clay, 94% sand, 0.9% organic matter, 3.4% silt, and 0.1% moisture. Soil 2 (McCarthy) is described as sandy-loam, with 11% clay, 56% sand, 22% organic matter, 33% silt, and 8.7% moisture. Soil 3 (Auburn) is described as clay loam, with 32% clay, 21% sand, 5.4% organic matter, 46% silt, and 2.2% moisture. Seven replicate extractions were made of each soil at the two concentrations.

10.0 REFERENCES

1. Lopez-Avila, V., Baldin, E., Benedicto, J, Milanes, J., Beckert. W.F., "Application of Open-Tubular Columns to SW-846 GC Methods", report to the U.S. Environmental Protection Agency on Contract 68-03-3511; Mid-Pacific Environmental Laboratory, Mountain View, CA, 1990.

2. Development and Application of Test Procedures for Specific Organic Toxic Substances in Wastewaters. Category 10 - Pesticides and PCB Report for the U.S. Environmental Protection Agency on Contract 68-03-2606.

3. Goerlitz, D.F., Law, L.M., "Removal of Elemental Sulfur Interferences from Sediment Extracts for Pesticide Analysis", *Bull. Environ. Contam. Toxicol.*, 1971, 6, 9.

4. Jensen, S., Renberg, L., Reutergardth, L., "Residue Analysis of Sediment and Sewage Sludge for Organochlorines in the Presence of Elemental Sulfur", *Anal. Chem.*, 1977, 49, 316-318.

5. Wise, R.H., Bishop, D.F., Williams, R.T., Austem, B.M., "Gel Permeation Chromatography in the GC/MS Analysis of Organics in Sludges", U.S. Environmental Protection Agency, Cincinnati, OH, 45268.

6. Pionke, H.B., Chesters, G., Armstrong, D.E., "Extraction of Chlorinated Hydrocarbon Insecticides from Soil", *Agron. J.*, 1968, 60, 289.

7. Burke, J.A., Mills, P.A., Bostwick, D.C., "Experiments with Evaporation of Solutions of Chlorinated Pesticides", *J. Assoc. Off. Anal. Chem.*, 1966, 49, 999.

8. Glazer, J.A., et al., "Trace Analyses for Wastewaters", *Environ. Sci. and Technol.*, 1981, 15, 1426.

9. Marsden, P.J., "Performance Data for SW-846 Methods 8270, 8081, and 8141", U.S. Environmental Protection Agency, EMSL-Las Vegas, EPA/600/4-90/015.

10. Lopez-Avila, V. (Beckert, W., Project Officer), "Development of a Soxtec Extraction Procedure for Extracting Organic Compounds from Soils and Sediments", EPA 600/X-91/140, US Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Las Vegas, NV, October 1991.

11. Markell, C., "Solid-Phase Extraction of TCLP Leachates," Proceedings of the Tenth Annual Waste Testing and Quality Assurance Symposium, Arlington, VA, July, 1994.

12. Bennett, D., Lesnik, B., Lee, S.M., "Supercritical Fluid Extraction of Organochlorine Pesticide Residues from Soils," Proceedings of the Tenth Annual Waste Testing and Quality Assurance Symposium, Arlington, VA, July, 1994.

13. Markell, C., "3M Data Submission to EPA," letter to B. Lesnik, June 27, 1995.

TABLE 1

GAS CHROMATOGRAPHIC RETENTION TIMES FOR THE ORGANOCHLORINE PESTICIDES USING WIDE-BORE CAPILLARY COLUMNS, SINGLE-COLUMN METHOD OF ANALYSIS

	Retention Time (min)	
Compound	DB-608[a]	DB-1701[a]
Aldrin	11.84	12.50
α-BHC	8.14	9.46
β-BHC	9.86	13.58
δ-BHC	11.20	14.39
γ-BHC (Lindane)	9.52	10.84
α-Chlordane	15.24	16.48
γ-Chlordane	14.63	16.20
4,4'-DDD	18.43	19.56
4,4'-DDE	16.34	16.76
4,4'-DDT	19.48	20.10
Dieldrin	16.41	17.32
Endosulfan I	15.25	15.96
Endosulfan II	18.45	19.72
Endosulfan sulfate	20.21	22.36
Endrin	17.80	18.06
Endrin aldehyde	19.72	21.18
Heptachlor	10.66	11.56
Heptachlor epoxide	13.97	15.03
Methoxychlor	22.80	22.34
Toxaphene	MR	MR

MR = Multiple response compound.

[a] See Table 4 for GC operating conditions.

TABLE 2

GAS CHROMATOGRAPHIC RETENTION TIMES FOR THE ORGANOCHLORINE PESTICIDES USING NARROW-BORE CAPILLARY COLUMNS, SINGLE-COLUMN METHOD OF ANALYSIS

	Retention Time (min)	
Compound	DB-608[a]	DB-5[a]
Aldrin	14.51	14.70
α-BHC	11.43	10.94
β-BHC	12.59	11.51
δ-BHC	13.69	12.20
γ-BHC (Lindane)	12.46	11.71
α-Chlordane	NA	NA
γ-Chlordane	17.34	17.02
4,4'-DDD	21.67	20.11
4,4'-DDE	19.09	18.30
4,4'-DDT	23.13	21.84
Dieldrin	19.67	18.74
Endosulfan I	18.27	17.62
Endosulfan II	22.17	20.11
Endosulfan sulfate	24.45	21.84
Endrin	21.37	19.73
Endrin aldehyde	23.78	20.85
Heptachlor	13.41	13.59
Heptachlor epoxide	16.62	16.05
Methoxychlor	28.65	24.43
Toxaphene	MR	MR

NA = Data not available.

MR = Multiple response compound.

[a] See Table 4 for GC operating conditions.

TABLE 3

FACTORS FOR DETERMINATION OF ESTIMATED QUANTITATION LIMITS[a] (EQLs) FOR VARIOUS MATRICES

Matrix	Factor
Ground water	10
Low-concentration soil by sonication with GPC cleanup	670
High-concentration soil and sludges by sonication	10,000
Non-water miscible waste	100,000

[a] Laboratories may estimate the quantitation limits of the target analytes in environmental and waste media by generating MDLs in organic-free reagent water and using the following equation (see Sec. 5.0 of Chapter One for information on generating MDL data):

EQL = [MDL in water] × [factor in this table]

For nonaqueous samples, the factor is on a wet-weight basis. Sample EQLs are highly matrix-dependent. EQLs determined using these factors are provided as guidance and may not always be achievable.

TABLE 4

GC OPERATING CONDITIONS FOR ORGANOCHLORINE COMPOUNDS
SINGLE-COLUMN ANALYSIS USING NARROW-BORE COLUMNS

Column 1 - 30 m x 0.25 or 0.32 mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1 µm film thickness.

Carrier gas	Helium
Carrier gas pressure	16 psi
Injector temperature	225°C
Detector temperature	300°C
Initial temperature	100°C, hold 2 minutes
Temperature program	100°C to 160°C at 15°C/min, followed by 160°C to 270°C at 5°C/min
Final temperature	270°C

Column 2 - 30 m x 0.25 mm ID fused-silica capillary column chemically bonded with 35 percent phenyl methylpolysiloxane (DB-608, SPB-608, or equivalent), 25 µm coating thickness, 1 µm film thickness.

Carrier gas	Nitrogen
Carrier gas pressure	20 psi
Injector temperature	225°C
Detector temperature	300°C
Initial temperature	160°C, hold 2 minutes
Temperature program	160°C to 290°C at 5°C/min
Final temperature	290°C, hold 1 min

TABLE 5

GC OPERATING CONDITIONS FOR ORGANOCHLORINE COMPOUNDS
SINGLE-COLUMN ANALYSIS USING WIDE-BORE COLUMNS

Column 1 - 30 m x 0.53 mm ID fused-silica capillary column chemically bonded with 35 percent phenyl methylpolysiloxane (DB-608, SPB-608, RTx-35, or equivalent), 0.5 µm or 0.83 µm film thickness.

Column 2 - 30 m x 0.53 mm ID fused-silica capillary column chemically bonded with 50 percent phenyl methylpolysiloxane (DB-1701, or equivalent), 1.0 µm film thickness.

Both Column 1 and Column 2 use the same GC operating conditions.

Carrier gas	Helium
Carrier gas flow rate	5-7 mL/minute
Makeup gas	argon/methane (P-5 or P-10) or nitrogen
Makeup gas flow rate	30 mL/min
Injector temperature	250°C
Detector temperature	290°C
Initial temperature	150°C, hold 0.5 minute
Temperature program	150°C to 270°C at 5°C/min
Final temperature	270°C, hold 10 min

Column 3 - 30 m x 0.53 mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5, SPB-5, RTx-5, or equivalent), 1.5 µm film thickness.

Carrier gas	Helium
Carrier gas flow rate	6 mL/minute
Makeup gas	argon/methane (P-5 or P-10) or nitrogen
Makeup gas flow rate	30 mL/min
Injector temperature	205°C
Detector temperature	290°C
Initial temperature	140°C, hold 2 min
Temperature program	140°C to 240°C at 10°C/min, hold 5 minutes at 240°C, 240°C to 265°C at 5°C/min
Final temperature	265°C, hold 18 min

TABLE 6

RETENTION TIMES OF THE ORGANOCHLORINE PESTICIDES[a]
DUAL-COLUMN METHOD OF ANALYSIS

Compound	DB-5 RT (min)	DB-1701 RT (min)
DBCP	2.14	2.84
Hexachlorocyclopentadiene	4.49	4.88
Etridiazole	6.38	8.42
Chloroneb	7.46	10.60
Hexachlorobenzene	12.79	14.58
Diallate	12.35	15.07
Propachlor	9.96	15.43
Trifluralin	11.87	16.26
α-BHC	12.35	17.42
PCNB	14.47	18.20
γ-BHC	14.14	20.00
Heptachlor	18.34	21.16
Aldrin	20.37	22.78
Alachlor	18.58	24.18
Chlorothalonil	15.81	24.42
Alachlor	18.58	24.18
β-BHC	13.80	25.04
Isodrin	22.08	25.29
DCPA	21.38	26.11
δ-BHC	15.49	26.37
Heptachlor epoxide	22.83	27.31
Endosulfan-I	25.00	28.88
γ-Chlordane	24.29	29.32
α-Chlordane	25.25	29.82
trans-Nonachlor	25.58	30.01
4,4'-DDE	26.80	30.40
Dieldrin	26.60	31.20
Perthane	28.45	32.18
Endrin	27.86	32.44
Chloropropylate	28.92	34.14
Chlorobenzilate	28.92	34.42
Nitrofen	27.86	34.42
4,4'-DDD	29.32	35.32
Endosulfan II	28.45	35.51
4,4'-DDT	31.62	36.30
Endrin aldehyde	29.63	38.08

TABLE 6
(continued)

Compound	DB-5 RT (min)	DB-1701 RT (min)
Mirex	37.15	38.79
Endosulfan sulfate	31.62	40.05
Methoxychlor	35.33	40.31
Captafol	32.65	41.42
Endrin ketone	33.79	42.26
Permethrin	41.50	45.81
Kepone	31.10	[b]
Dicofol	35.33	[b]
Dichlone	15.17	[b]
α,α′-Dibromo-m-xylene	9.17	11.51
2-Bromobiphenyl	8.54	12.49

[a] See Table 7 for GC operating conditions.

[b] Not detected at 2 ng per injection.

TABLE 7

GC OPERATING CONDITIONS FOR ORGANOCHLORINE PESTICIDES
FOR DUAL-COLUMN METHOD OF ANALYSIS, LOW TEMPERATURE, THIN FILM

Column 1:	DB-1701 or equivalent 30-m x 0.53 mm ID 1.0 µm film thickness
Column 2:	DB-5 or equivalent 30-m x 0.53 mm ID 0.83 µm film thickness
Carrier gas	Helium
Carrier gas flow rate	6 mL/minute
Makeup gas	Nitrogen
Makeup gas flow rate	20 mL/min
Injector temperature	250°C
Detector temperature	320°C
Initial temperature	140°C, hold 2 minutes
Temperature program	140°C to 270°C at 2.8°C/min
Final temperature	270°C, hold 1 minute

TABLE 8

GC OPERATING CONDITIONS FOR ORGANOCHLORINE PESTICIDES
FOR THE DUAL COLUMN METHOD OF ANALYSIS
HIGH TEMPERATURE, THICK FILM

Column 1:	DB-1701 (J&W) or equivalent 30 m x 0.53 mm ID 1.0 µm film thickness
Column 2:	DB-5 (J&W) or equivalent 30 m x 0.53 mm ID 1.5 µm film thickness
Carrier gas:	Helium
Carrier gas flow rate:	6 mL/minute
Makeup gas:	Nitrogen
Makeup gas flow rate:	20 (mL/min)
Injector temperature:	250°C
Detector temperature:	320°C
Initial temperature:	150°C, hold 0.5 min
Temperature program:	150°C to 190°C at 12°C/min, hold 2 min190°C to 275°C at 4°C/min
Final temperature	275°C, hold 10 min

TABLE 9

ANALYTE RECOVERY FROM SEWAGE SLUDGE

Compound	Ultrasonic Extraction %Recovery	%RSD	Soxhlet %Recovery	%RSD
Hexachloroethane	80	7	79	1
2-Chloronapthalene	50	56	67	8
4-Bromodiphenyl ether	118	4	nd	nd
α-BHC	88	25	265	18
γ-BHC	55	9	155	29
Heptachlor	60	13	469	294
Aldrin	92	33	875	734
β-BHC	351	71	150	260
δ-BHC	51	11	57	2
Heptachlor epoxide	54	11	70	3
Endosulfan I	52	11	70	4
γ-Chlordane	50	9	65	1
α-Chlordane	49	8	66	0
DDE	52	11	74	1
Dieldrin	89	19	327	7
Endrin	56	10	92	15
Endosulfan II	52	10	88	11
DDT	57	10	95	17
Endrin aldehyde	45	6	42	10
DDD	57	11	99	8
Tetrachloro-m-xylene	71	19	82	1
Decachlorobiphenyl	26	23	28	48

Concentration spiked in the sample: 500-1000 ng/g, three replicates analyses.

Soxhlet extraction by Method 3540 with methylene chloride.

Ultrasonic extraction by Method 3550 with methylene chloride/acetone (1:1).

Cleanup by Method 3640.

GC column: DB-608, 30 m x 0.53 mm ID.

TABLE 10

ANALYTE RECOVERY FROM DICHLOROETHANE STILLBOTTOMS

Compound	Ultrasonic Extraction %Recovery	%RSD	Soxhlet %Recovery	%RSD
Hexachloroethane	70	2	50	30
2-Chloronapthalene	59	3	35	35
4-Bromodiphenyl ether	159	14	128	137
α-BHC	55	7	47	25
β-BHC	43	6	30	30
Heptachlor	48	6	55	18
Aldrin	48	5	200	258
β-BHC	51	7	75	42
δ-BHC	43	4	119	129
Heptachlor epoxide	47	6	66	34
Endosulfan I	47	4	41	18
γ-Chlordane	48	5	47	13
α-Chlordane	45	5	37	21
DDE	45	4	70	40
Dieldrin	45	5	58	24
Endrin	50	6	41	23
Endosulfan II	49	5	46	17
DDT	49	4	40	29
Endrin aldehyde	40	4	29	20
DDD	48	5	35	21
Tetrachloro-m-xylene	49	2	176	211
Decachlorobiphenyl	17	29	104	93

Concentration spiked in the sample: 500-1000 ng/g, three replicates analyses.

Soxhlet extraction by Method 3540 with methylene chloride.

Ultrasonic extraction by Method 3550 with methylene chloride/acetone (1:1).

Cleanup by Method 3640.

GC column: DB-608, 30 m x 0.53 mm ID.

TABLE 11

SINGLE LABORATORY ACCURACY DATA FOR THE EXTRACTION OF ORGANOCHLORINE PESTICIDES FROM SPIKED CLAY SOIL BY METHOD 3541 (AUTOMATED SOXHLET)[a]

Compound Name	% Recovery DB-5	DB-1701
α-BHC	89	94
β-BHC	86	ND
Heptachlor	94	95
Aldrin	ND	92
Heptachlor epoxide	97	97
trans-Chlordane	94	95
Endosulfan I	92	92
Dieldrin	ND	113
Endrin	111	104
Endosulfan II	104	104
4,4'-DDT	ND	ND
Mirex	108	102

[a] The operating conditions for the automated Soxhlet were:

Immersion time 45 min; extraction time 45 min; 10 g sample size; extraction solvent, 1:1 acetone/hexane. No equilibration time following spiking.

ND = Not able to determine because of interference.

All compounds were spiked at 500 µg/kg.

Data taken from Reference 10.

TABLE 12

SINGLE LABORATORY RECOVERY DATA FOR SOLID-PHASE EXTRACTION OF ORGANOCHLORINE PESTICIDES FROM TCLP BUFFERS SPIKED AT TWO LEVELS

Compound	Spike Level (µg/L)	Buffer 1 (pH = 2.886)		Buffer 2 (pH = 4.937)	
		Recovery (%)	RSD	Recovery (%)	RSD
Low Level Spike					
Toxaphene	250	86	13	77	17
Chlordane	15	88	7	95	6
γ-BHC (Lindane)	200	115	7	98	5
Heptachlor	4	95	11	77	23
Heptachlor epoxide	4	107	9	104	12
Endrin	10	89	5	100	6
Methoxychlor	5000	97	8	95	6
High Level Spike					
Toxaphene	1000	106	7	85	15
Chlordane	60	116	12	107	12
γ-BHC (Lindane)	800	109	19	112	5
Heptachlor	16	113*	18*	93	3
Heptachlor epoxide	16	82	17	91	7
Endrin	40	84	19	82	4
Methoxychlor	20,000	100	4	87	8

Results were from seven replicate spiked buffer samples, except where noted with *, which indciates that only three replicates were analyzed.

TABLE 13

RECOVERY DATA FROM THREE LABORATORIES FOR SOLID-PHASE EXTRACTION OF ORGANOCHLORINE PESTICIDES FROM SPIKED TCLP LEACHATES FROM SOIL SAMPLES

Compound	Spike Level (µg/L)*	Lab 1			Lab 2			Lab 3		
		%R	RSD	n	%R	RSD	n	%R	RSD	n
Buffer 1 pH = 2.886										
Toxaphene	500	75	25	7	95.4	2.4	3	86.0	4.3	3
Chlordane	30	80	15	7	57.8	12.0	3	73.8	0.9	3
γ-BHC (Lindane)	400	104	11	7	99.3	0.6	3	86.6	6.4	3
Heptachlor	8	88	13	7	70.8	20.4	3	88.0	9.1	3
Heptachlor epoxide	8	92	13	7	108.7	6.9	3	75.0	2.8	3
Endrin	20	106	12	7	110	0	3	78.3	4.6	3
Methoxychlor	10,000	107	12	7	86.7	2.2	3	84.8	8.5	3
Buffer 2 pH = 4.937										
Toxaphene	500	87	9	7	98	4.1	3	88.8	4.1	3
Chlordane	30	91	8	7	66.7	5.0	3	73.7	11.5	3
γ-BHC (Lindane)	400	74	20	7	102.7	2.2	3	89.3	3.1	3
Heptachlor	8	71	21	7	62.5	20	3	85.0	1.5	3
Heptachlor epoxide	8	118	1	3	113	0	3	81.3	2.7	3
Endrin	20	124	7	3	111.7	2.6	3	83.0	3.4	3
Methoxychlor	10,000	73	22	7	88.8	2.7	3	89.6	2.7	3

* 250-mL aliquots of leachate were spiked by Labs 2 and 3 at the levels show. Lab 1 spiked at one-half these levels.

TABLE 14

SINGLE LABORATORY ACCURACY AND PRECISION DATA FOR SOLID-PHASE EXTRACTION BY METHOD 3535[1]

	Bias (%)				Precision (%)				MDL	
Compound	Ground water (low)	Ground water (high)	Waste water (low)	Waste water (high)	Ground water (low)	Ground water (high)	Waste water (low)	Waste water (high)	Ground water (µg/L)	Waste water (µg/L)
Aldrin	37.3	93.5	79.3	94.0	23.7	5.5	6.7	3.4	1.4	0.83
β-BHC	89.2	107.8	79.7	82.3	6.5	2.5	1.6	4.2	0.91	0.20
δ-BHC	106.2	86.0	88.9	83.4	5.6	2.4	2.5	4.2	0.93	0.35
α-Chlordane	75.4	112.3	78.9	89.5	12.8	2.7	4.7	2.4	1.5	0.58
γ-Chlordane	70.7	98.9	79.9	93.9	15.8	2.7	4.6	2.9	1.8	0.58
Dieldrin	83.4	96.1	81.2	93.3	7.1	2.3	3.8	3.6	0.9	0.49
Endosulfan I	79.6	99.1	79.6	87.9	10.6	2.3	4.1	3.8	1.3	0.51
Endosulfan II	94.5	101.6	82.7	93.5	5.8	2.8	4.2	4.1	0.9	0.54
Endrin	88.3	98.4	85.1	89.6	6.2	2.3	3.1	2.9	1.7	0.82
Endrin aldehyde	87.5	99.9	69.0	80.2	6.0	4.0	3.3	5.9	0.8	0.36
Heptachlor	43.1	95.4	71.8	78.6	19.2	3.9	5.0	2.8	1.3	0.56
Heptachlor epoxide	76.4	97.6	75.3	83.4	12.1	2.4	2.9	3.3	1.5	0.34
Lindane	81.3	115.2	82.1	85.3	11.1	3.2	2.4	3.1	1.4	0.32
p,p'-DDE	80.3	96.0	85.1	97.9	8.3	2.5	4.4	2.4	1.0	0.59
p,p'-DDT	86.6	105.4	105	111	4.4	2.7	4.3	4.7	0.6	0.71
p,p'-TDE (DDD)	90.5	101.1	74.9	79.6	4.8	2.4	4.6	2.9	1.4	0.85

[1]All results determined from seven replicates of each sample type. Two spiking levels were used. "Low" samples were spiked at 5-10 µg/L for each analyte, while "high" samples were spiked at 250 - 500 µg/L. MDL values were determined from the "low" samples without further consideration of the spiking level.

TABLE 15

RECOVERY (BIAS) OF ORGANOCHLORINE PESTICIDES USING SFE METHOD 3562
(Seven replicates)

Compounds	Delphi[a] 250 ug/kg	Delphi-5 [a] 5 ug/kg	McCarthy[b] 250 ug/kg	McCarthy[b] 5 ug/kg	Auburn[c] 250 ug/kg	Auburn[c] 5 ug/kg	Mean Recovery
γ-BHC	102.6	66.4	80.7	82.7	86.0	86.1	84.1
β-BHC	101.9	73.0	86.1	85.1	87.4	86.3	86.6
Heptachlor	101.3	61.6	78.0	79.1	83.3	80.4	80.6
δ-BHC	120.9	82.3	90.4	89.6	92.9	89.4	94.2
Aldrin	56.7	28.7	52.1	77.1	42.1	74.6	55.2
Heptachlor epoxide	102.3	71.9	87.1	87.4	89.6	91.1	88.2
α-Chlordane	106.4	87.1	88.1	105.9	91.7	97.1	96.1
4,4'DDE	110.9	75.7	88.4	118.7	83.6	110.9	98.0
Dieldrin	106.9	80.4	88.1	140.8	90.6	80.1	97.8
Endrin	211.0	87.0	111.7	98.7	90.5	87.6	114.4
4,4'-DDD	93.0	80.4	85.0	88.1	83.7	90.4	86.8
Endosulfan II	105.6	89.9	92.1	88.6	87.7	92.9	92.5
4,4'-DDT	126.7	81.3	110.9	199.7	83.6	124.3	121.1
Endrin aldehyde	64.3	74.0	63.0	86.7	21.0	38.3	37.9
Matrix Mean Recovery	107.9	74.3	85.9	102.0	79.8	87.8	89.5

[a] Delphi: Loamy sand soil
[b] McCarthy: Sandy loamy-organic rich soil
[c] Auburn: Clay-loamy soil

TABLE 16

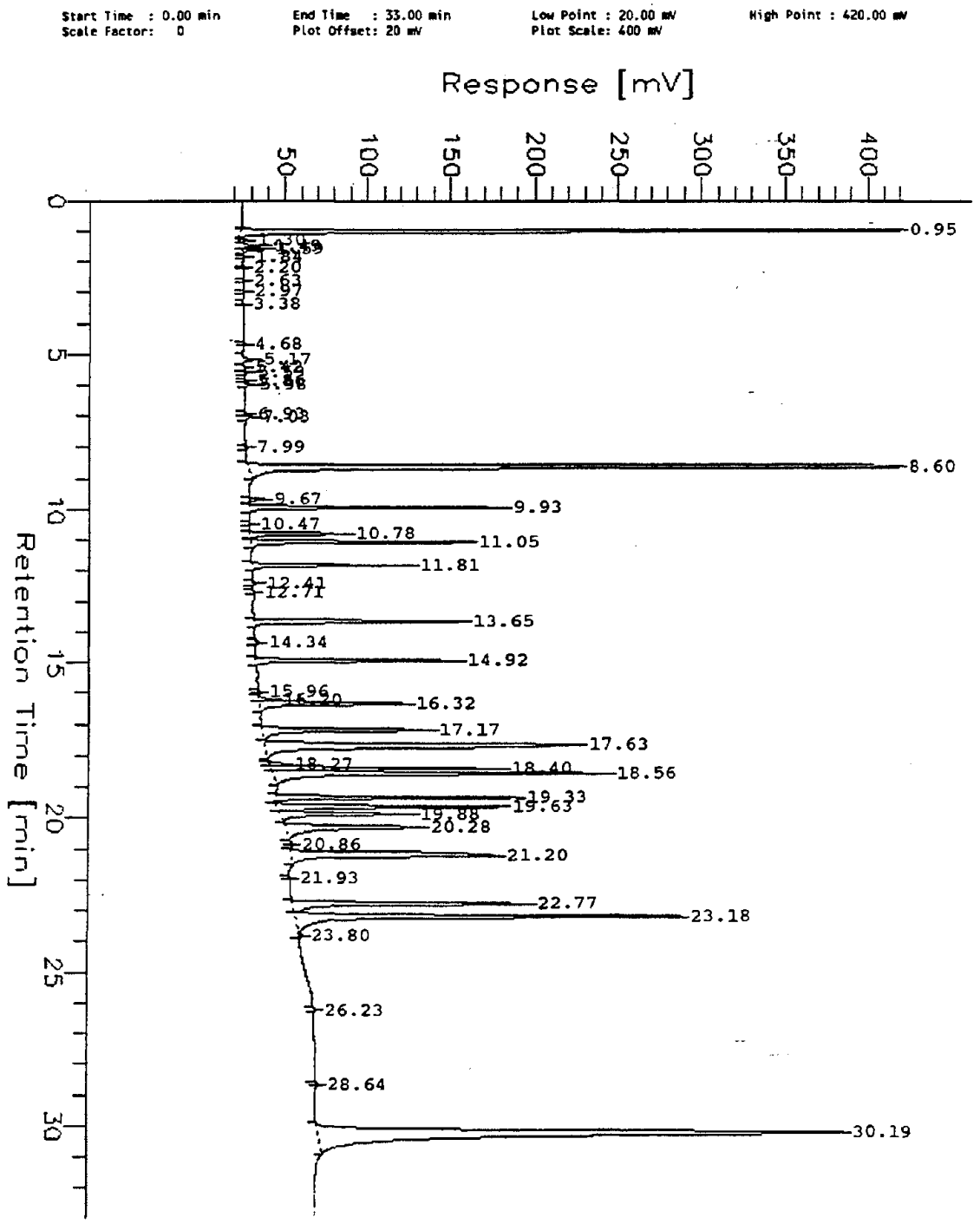
RELATIVE STANDARD DEVIATION (PRECISION) OF ORGANOCHLORINE PESTICIDES USING SFE METHOD 3562
(Seven replicates)

Compounds	Delphi[a] 250 ug/kg	Delphi[a] 5 ug/kg	McCarthy[b] 250 ug/kg	McCarthy[b] 5 ug/kg	Auburn[c] 250 ug/kg	Auburn[c] 5 ug/kg	Mean
γ-BHC	3.9	3.3	3.3	6.5	4.0	1.6	3.7
β-BHC	6.5	3.0	3.0	4.3	4.6	2.0	3.9
Heptachlor	4.4	2.1	4.3	5.0	4.4	2.6	3.8
δ-BHC	5.3	3.1	3.3	7.1	4.1	3.5	4.4
Aldrin	2.9	5.5	2.8	4.6	1.6	1.9	3.2
Heptachlor epoxide	3.0	2.7	3.6	4.3	4.7	4.2	3.8
α-Chlordane	3.6	5.7	4.8	13.8	4.2	2.5	5.8
4,4'DDE	5.2	15.3	4.8	4.2	7.7	3.4	6.8
Dieldrin	4.3	4.5	2.9	23.9	5.0	3.1	7.3
Endrin	7.2	6.0	4.5	6.0	4.3	10.5	6.4
4,4'-DDD	6.9	3.1	3.7	3.5	4.3	7.4	4.8
Endosulfan II	5.1	4.7	3.2	3.3	5.5	4.6	4.4
4,4'-DDT	12.5	6.2	6.6	5.9	4.9	3.4	6.6
Endrin aldehyde	3.9	7.5	4.7	11.6	1.9	26.0	9.3
Matrix Mean Recovery	5.3	5.2	4.0	7.4	4.4	5.5	5.3

[a] Delphi: Loamy sand soil
[b] McCarthy: Sandy loamy-organic rich soil
[c] Auburn: Clay-loamy soil

FIGURE 1

GAS CHROMATOGRAM OF THE MIXED ORGANOCHLORINE PESTICIDE STANDARD

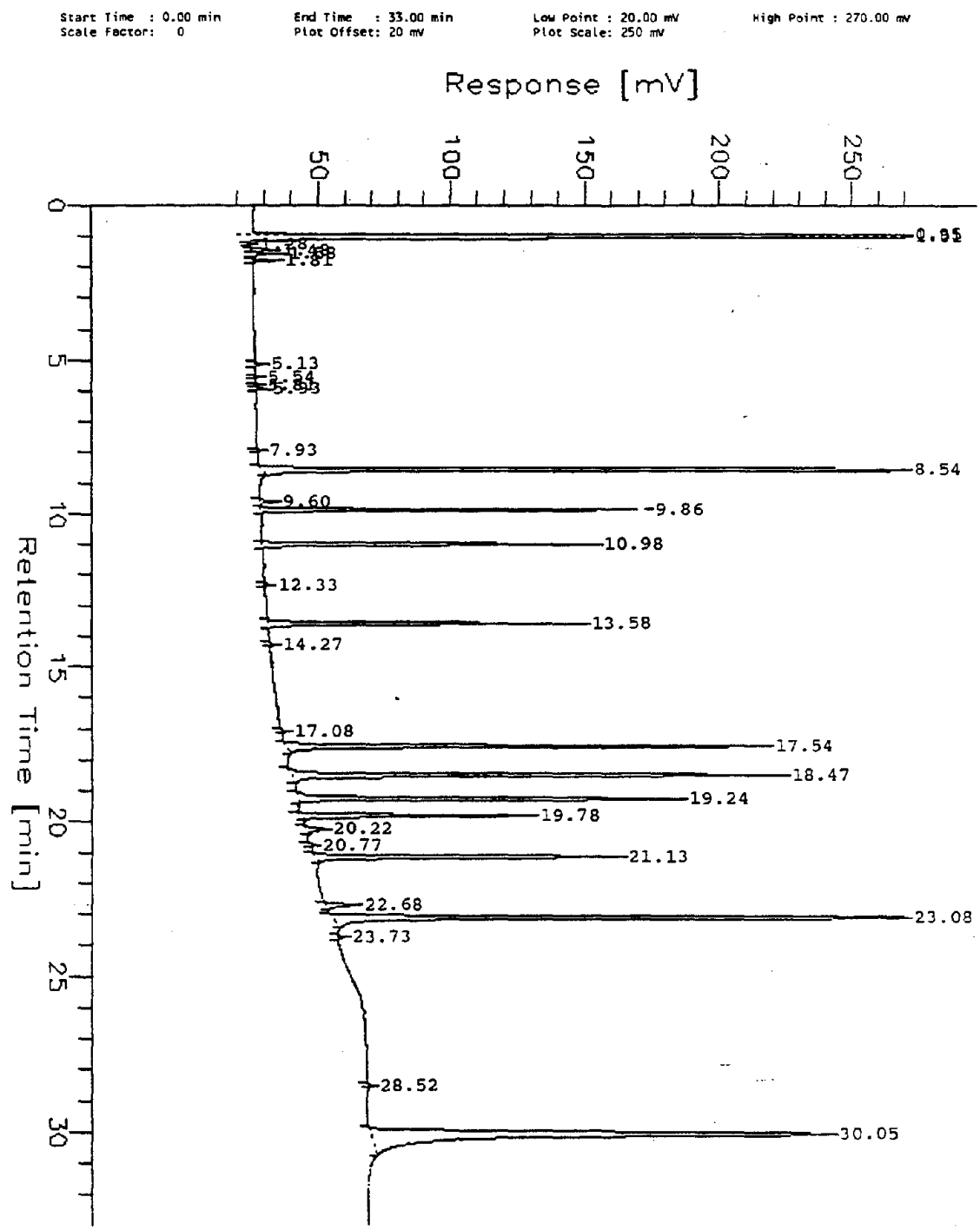


Column: 30 m x 0.25 mm ID, DB-5

Temperature program: 100°C (hold 2 minutes) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier He at 16 psi

FIGURE 2

GAS CHROMATOGRAM OF INDIVIDUAL ORGANOCHLORINE PESTICIDE STANDARD MIX A

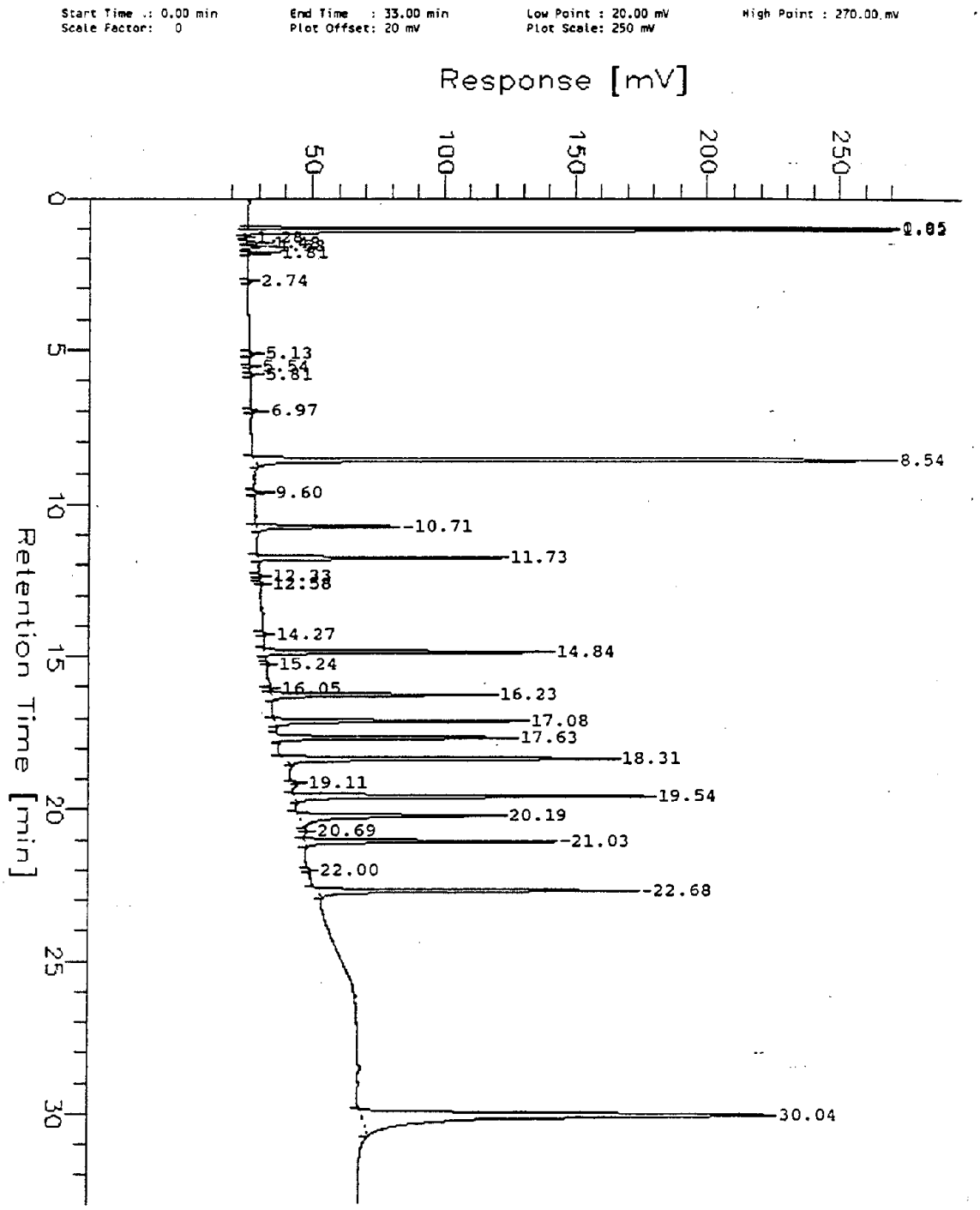


Column: 30 m x 0.25 mm ID, DB-5

Temperature program: 100°C (hold 2 minutes) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier He at 16 psi.

FIGURE 3

GAS CHROMATOGRAM OF INDIVIDUAL ORGANOCHLORINE PESTICIDE STANDARD MIX B

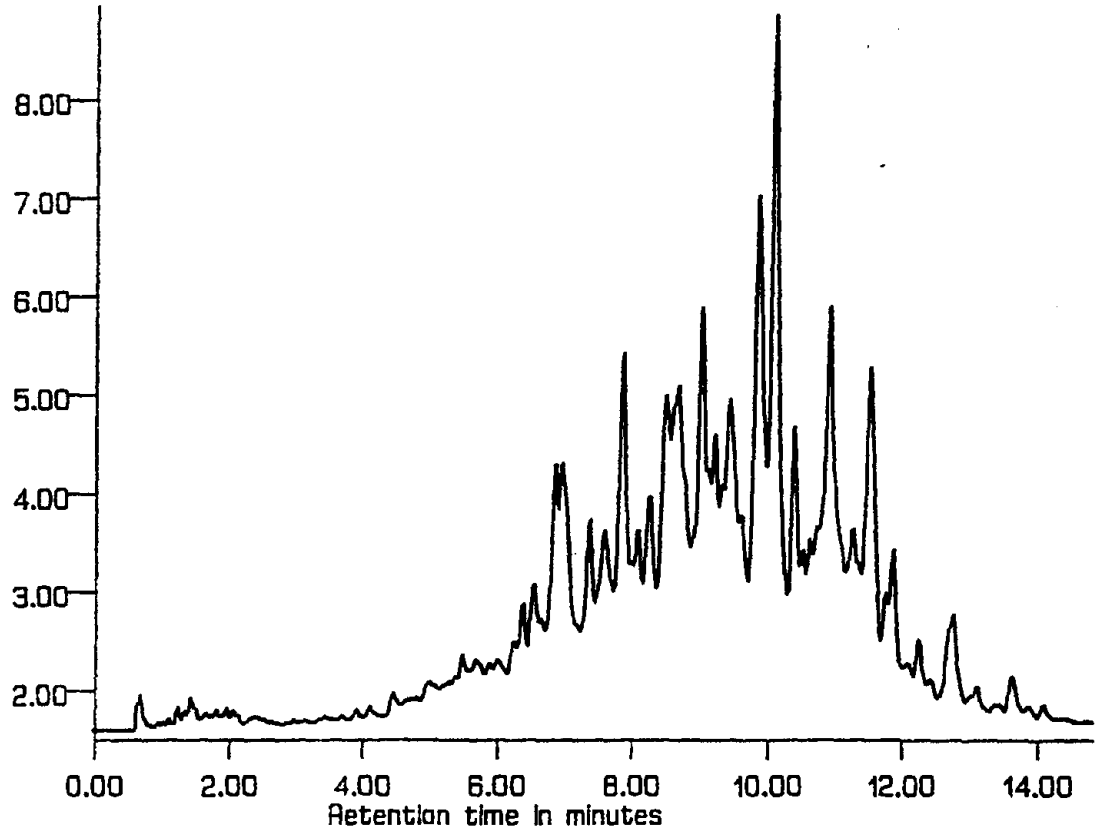


Column: 30 m x 0.25 mm ID, DB-5

Temperature program: 100°C (hold 2 minutes) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier He at 16 psi.

FIGURE 4

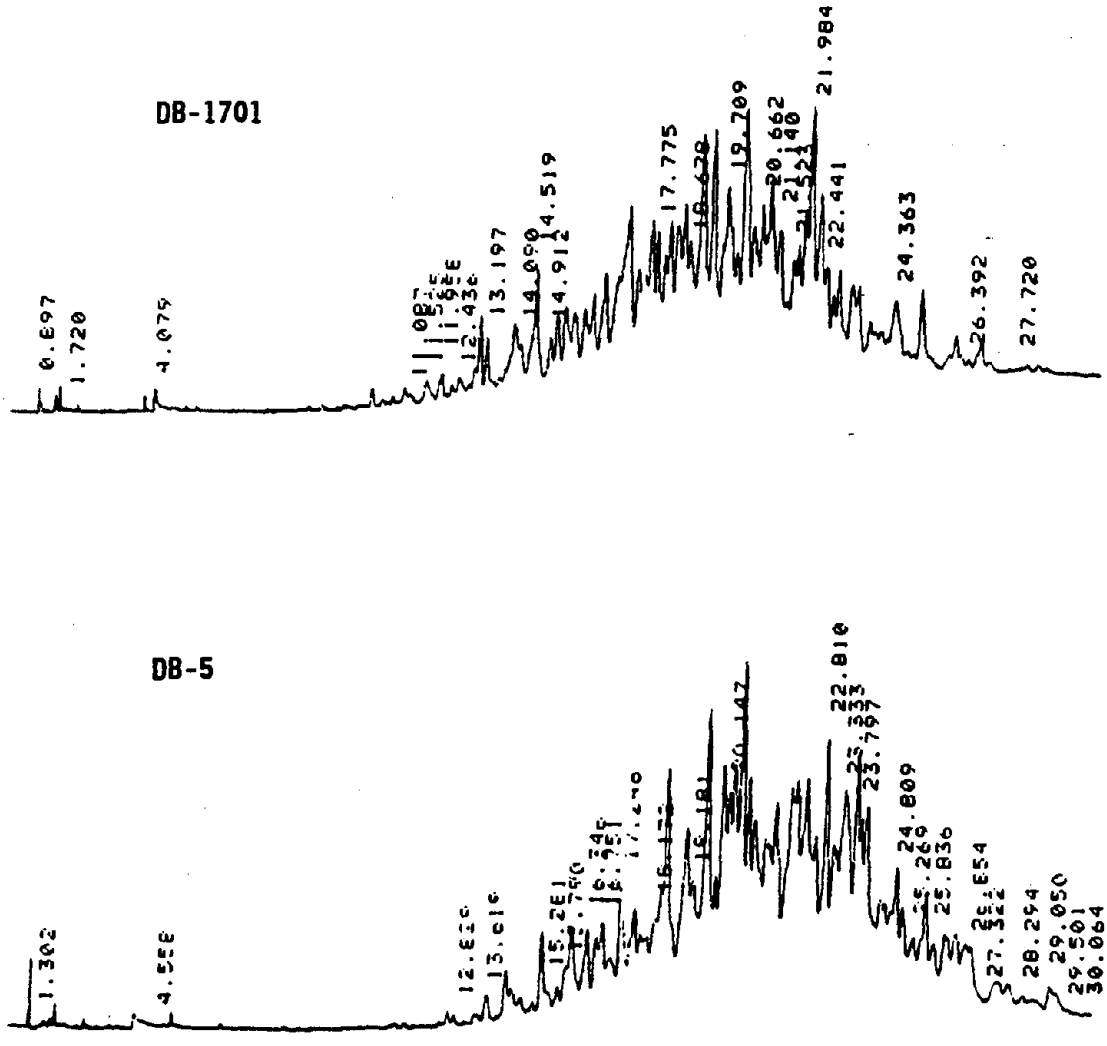
GAS CHROMATOGRAM OF TOXAPHENE



Toxaphene analyzed on an SPB-608 fused-silica open-tubular column. The GC operating conditions were as follows: 30 m x 0.53 mm ID SPB-608. Temperature program: 200°C (2 min hold) to 290°C at 6°C/min.

FIGURE 5

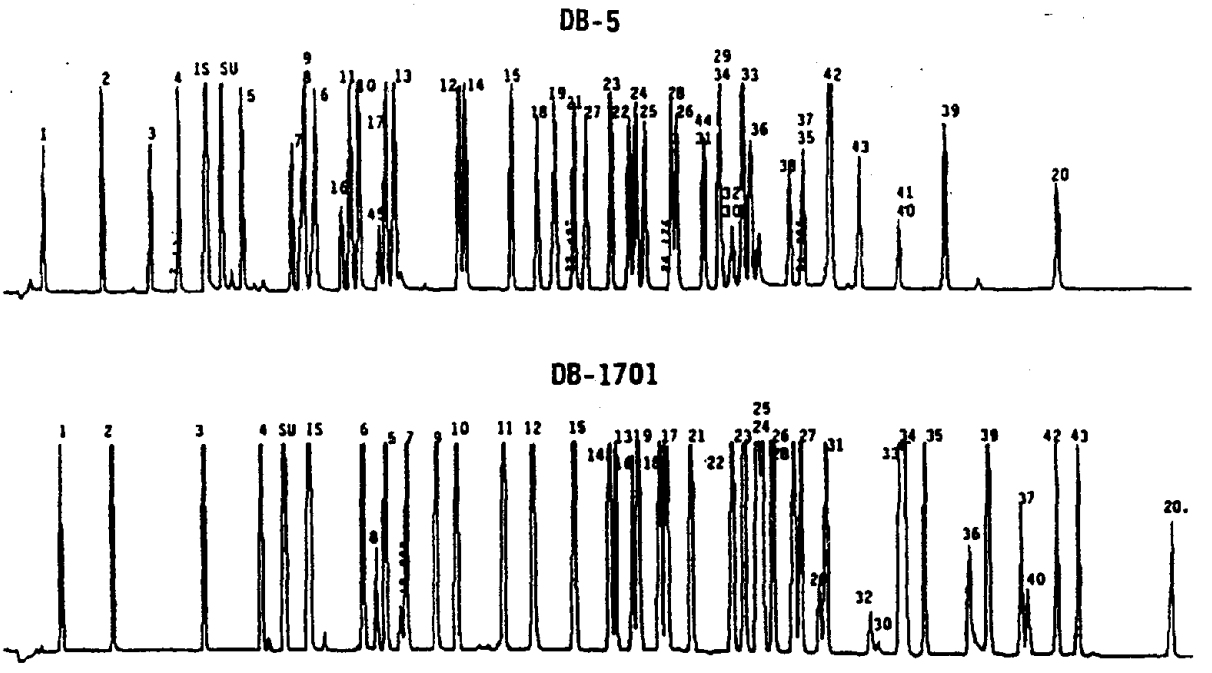
GAS CHROMATOGRAM OF STROBANE



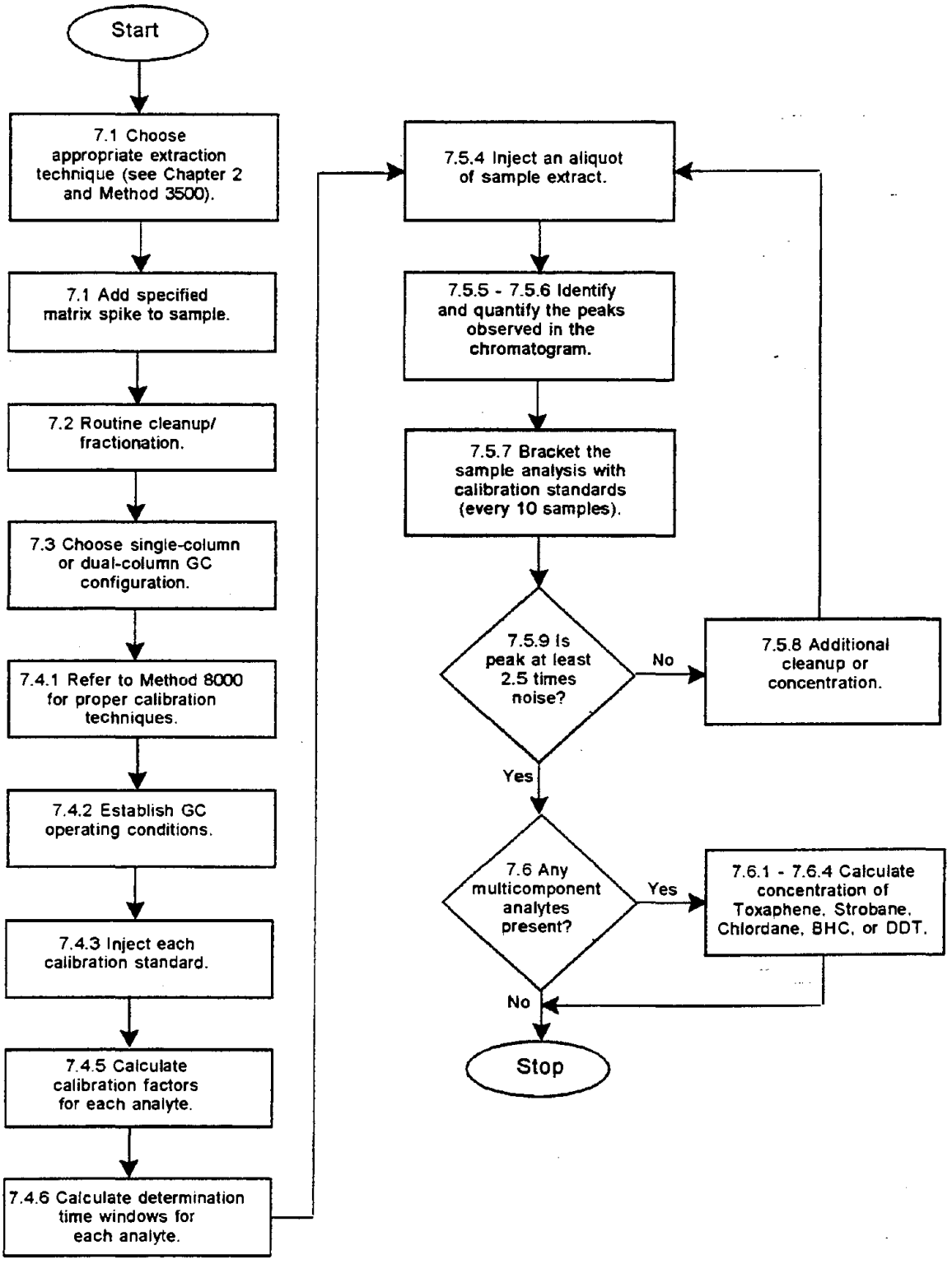
Strobane analyzed on a DB-5/DB-1701 fused-silica open-tubular column pair. The GC operating conditions were as follows: 30 m x 0.53 mm ID DB-5 (1.5-µm film thickness) and 30 m x 0.53 mm ID DB-1701 (1.0-µm film thickness) connected to a J&W Scientific press-fit Y-shaped inlet splitter. Temperature program: 150°C (0.5 min hold) to 190°C (2 min hold) at 12°C/min then to 275°C (10 min hold) at 4°C/min.

FIGURE 6

GAS CHROMATOGRAM OF ORGANOCHLORINE PESTICIDES



Organochlorine pesticides analyzed on a DB-5/DB-1701 fused-silica open-tubular column pair. The GC operating conditions were as follows: 30 m x 0.53 mm ID DB-5 (0.83-µm film thickness) and 30 m x 0.53 mm ID DB-1701 (1.0-µm film thickness) connected to an 8 in. injection tee (Supelco Inc.). Temperature program: 140°C (2 min hold) to 270°C (1 min hold) at 2.8°C/min.


Start
7.1 Choose appropriate extraction technique (see Chapter 2 and Method 3500).
7.1 Add specified matrix spike to sample.
7.2 Routine cleanup/ fractionation.
7.3 Choose single-column or dual-column GC configuration.
7.4.1 Refer to Method 8000 for proper calibration techniques.
7.4.2 Establish GC operating conditions.
7.4.3 Inject each calibration standard.
7.4.5 Calculate calibration factors for each analyte.
7.4.6 Calculate determination time windows for each analyte.
7.5.4 Inject an aliquot of sample extract.
7.5.5 - 7.5.6 Identify and quantify the peaks observed in the chromatogram.
7.5.7 Bracket the sample analysis with calibration standards (every 10 samples).
7.5.9 Is peak at least 2.5 times noise?
No
7.5.8 Additional cleanup or concentration.
Yes
7.6 Any multicomponent analytes present?
Yes
7.6.1 - 7.6.4 Calculate concentration of Toxaphene, Strobane, Chlordane, BHC, or DDT.
No
Stop

METHOD 8082A

POLYCHLORINATED BIPHENYLS (PCBs) BY GAS CHROMATOGRAPHY

1.0 SCOPE AND APPLICATION

1.1 Method 8082 is used to determine the concentrations of polychlorinated biphenyls (PCBs) as Aroclors or as individual PCB congeners in extracts from solid and aqueous matrices. Open-tubular, capillary columns are employed with electron capture detectors (ECD) or electrolytic conductivity detectors (ELCD). When compared to packed columns, these fused-silica, open-tubular columns offer improved resolution, better selectivity, increased sensitivity, and faster analysis. The target compounds listed below may be determined by either a single- or dual-column analysis system. The PCB congeners listed below have been tested by this method, and the method may be appropriate for additional congeners.

Compound	CAS Registry No.	IUPAC #
Aroclor 1016	12674-11-2	-
Aroclor 1221	11104-28-2	-
Aroclor 1232	11141-16-5	-
Aroclor 1242	53469-21-9	-
Aroclor 1248	12672-29-6	-
Aroclor 1254	11097-69-1	-
Aroclor 1260	11096-82-5	-
2-Chlorobiphenyl	2051-60-7	1
2,3-Dichlorobiphenyl	16605-91-7	5
2,2',5-Trichlorobiphenyl	37680-65-2	18
2,4',5-Trichlorobiphenyl	16606-02-3	31
2,2',3,5'-Tetrachlorobiphenyl	41464-39-5	44
2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	52
2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	66
2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8	87
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	101
2,3,3',4',6-Pentachlorobiphenyl	38380-03-9	110
2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	138
2,2',3,4,5,5'-Hexachlorobiphenyl	52712-04-6	141
2,2',3,5,5',6-Hexachlorobiphenyl	52663-63-5	151
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	153
2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6	170
2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3	180
2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1	183
2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	187
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	40186-72-9	206

1.2 Aroclors are multi-component mixtures. When samples contain more than one Aroclor, a higher level of analyst expertise is required to attain acceptable levels of qualitative and quantitative analysis. The same is true of Aroclors that have been subjected to environmental degradation ("weathering") or degradation by treatment technologies. Such weathered multi-component mixtures may have significant differences in peak patterns than those of Aroclor standards.

1.3 Quantitation of PCBs as Aroclors is appropriate for many regulatory compliance determinations, but is particularly difficult when the Aroclors have been weathered by long exposure in the environment. Therefore, this method provides procedures for the determination of selected individual PCB congeners. The 19 PCB congeners listed above have been tested by this method.

1.4 The PCB congener approach potentially affords greater quantitative accuracy when PCBs are known to be present. As a result, this method may be used to determine Aroclors, some PCB congeners, or "total PCBs," depending on regulatory requirements and project needs. The congener method is of particular value in determining weathered Aroclors. However, analysts should use caution when using the congener method when regulatory requirements are based on Aroclor concentrations.

1.5 Compound identification based on single-column analysis should be confirmed on a second column, or should be supported by at least one other qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm the measurements made with the primary column. GC/MS Method 8270 is also recommended as a confirmation technique when sensitivity permits (Sec. 8.0).

1.6 This method also describes a dual-column option. The option allows a hardware configuration of two analytical columns joined to a single injection port. The option allows one injection to be used for dual-column analysis. Analysts are cautioned that the dual-column option may not be appropriate when the instrument is subject to mechanical stress, many samples are to be run in a short period, or when highly contaminated samples are analyzed.

1.7 The analyst must select columns, detectors and calibration procedures most appropriate for the specific analytes of interest in a study. Matrix-specific performance data must be established and the stability of the analytical system and instrument calibration must be established for each analytical matrix (e.g., hexane solutions from sample extractions, diluted oil samples, etc.). Example chromatograms and GC conditions are provided as guidance.

1.8 The MDLs for Aroclors vary in the range of 0.054 to 0.90 µg/L in water and 57 to 70 µg/kg in soils. Estimated quantitation limits may be determined using the data in Table 1.

1.9 This method is restricted to use by, or under the supervision of, analysts experienced in the use of gas chromatographs (GC) and skilled in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 A measured volume or weight of sample (approximately 1 L for liquids, 2 g to 30 g for solids) is extracted using the appropriate matrix-specific sample extraction technique.

2.2 Aqueous samples are extracted at neutral pH with methylene chloride using a separatory funnel (Method 3510), a continuous liquid-liquid extractor (Method 3520), solid-phase extraction (Method 3535), or other appropriate technique.

2.3 Solid samples are extracted with hexane-acetone (1:1) or methylene chloride-acetone (1:1) using a Soxhlet extractor (Method 3540), an automated Soxhlet (Method 3541), supercritical fluid extraction (Method 3562), or other appropriate technique.

2.4 Extracts for PCB analysis may be subjected to a sulfuric acid/potassium permanganate cleanup (Method 3665) designed specifically for these analytes. This cleanup technique will remove (destroy) many single component organochlorine or organophosphorus pesticides. Therefore, Method 8082 is not applicable to the analysis of those compounds. Instead, use Method 8081.

2.5 After cleanup, the extract is analyzed by injecting a 2-µL aliquot into a gas chromatograph with a narrow- or wide-bore fused-silica capillary column and electron capture detector (GC/ECD).

2.6 The chromatographic data may be used to determine the seven Aroclors in Sec. 1.1, individual PCB congeners, or total PCBs.

3.0 INTERFERENCES

3.1 Refer to Methods 3500 (Sec. 3.0, in particular), 3600, and 8000 for a discussion of interferences.

3.2 Interferences co-extracted from the samples will vary considerably from matrix to matrix. While general cleanup techniques are referenced or provided as part of this method, unique samples may require additional cleanup approaches to achieve desired degrees of discrimination and quantitation. Sources of interference in this method can be grouped into three broad categories.

3.2.1 Contaminated solvents, reagents, or sample processing hardware.

3.2.2 Contaminated GC carrier gas, parts, column surfaces, or detector surfaces.

3.2.3 Compounds extracted from the sample matrix to which the detector will respond.

3.3 Interferences by phthalate esters introduced during sample preparation can pose a major problem in PCB determinations.

3.3.1 Common flexible plastics contain varying amounts of phthalate esters which are easily extracted or leached from such materials during laboratory operations. Interferences from phthalate esters can best be minimized by avoiding contact with any plastic materials and checking all solvents and reagents for phthalate contamination.

3.3.2 Exhaustive cleanup of solvents, reagents and glassware may be required to eliminate background phthalate ester contamination.

3.3.3 These materials can be removed through the use of Method 3665 (sulfuric acid/permanganate cleanup).

3.4 Cross-contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Glassware must be scrupulously cleaned.

Clean all glassware as soon as possible after use by rinsing with the last solvent used. This should be followed by detergent washing with hot water, and rinses with tap water and organic-free reagent water. Drain the glassware, and dry it in an oven at 130°C for several hours, or rinse with methanol and drain. Store dry glassware in a clean environment.

NOTE: Oven-drying of glassware used for PCB analysis can increase contamination because PCBs are readily volatilized in the oven and spread to other glassware. Therefore, exercise caution, and do not dry glassware from samples containing high concentrations of PCBs with glassware that may be used for trace analyses.

3.5 Elemental sulfur (S_8) is readily extracted from soil samples and may cause chromatographic interferences in the determination of PCBs. Sulfur can be removed through the use of Method 3660.

4.0 APPARATUS AND MATERIALS

4.1 Gas chromatograph - An analytical system complete with gas chromatograph suitable for on-column and split-splitless injection and all required accessories including syringes, analytical columns, gases, electron capture detectors (ECD), and recorder/integrator or data system.

4.2 GC columns

This method describes procedures for both single-column and dual-column analyses. The single-column approach involves one analysis to determine that a compound is present, followed by a second analysis to confirm the identity of the compound (Sec. 8.4 describes how GC/MS confirmation techniques may be employed). The single-column approach may employ either narrow-bore (≤ 0.32 mm ID) columns or wide-bore (0.53 mm ID) columns. The dual-column approach involves a single injection that is split between two columns that are mounted in a single gas chromatograph. The dual-column approach employs only wide-bore (0.53 mm ID) columns. A third alternative is to employ dual columns mounted in a single GC, but with each column connected to a separate injector and a separate detector.

The columns listed in this section were the columns used to develop the method performance data. Listing these columns in this method is not intended to exclude the use of other columns that may be developed. Laboratories may use other capillary columns provided that they document method performance (e.g., chromatographic resolution, analyte breakdown, and MDLs) that equals or exceeds the performance specified in this method.

4.2.1 Narrow-bore columns for single-column analysis (use both columns to confirm compound identifications unless another confirmation technique such as GC/MS is employed). Narrow bore columns should be installed in split/splitless (Grob-type) injectors.

4.2.1.1 30 m x 0.25 or 0.32 mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1 µm film thickness.

4.2.1.2 30 m x 0.25 mm ID fused-silica capillary column chemically bonded with 35 percent phenyl methylpolysiloxane (DB-608, SPB-608, or equivalent), 2.5 µm coating thickness, 1 µm film thickness.

4.2.2 Wide-bore columns for single-column analysis (use two of the three columns listed to confirm compound identifications unless another confirmation technique such as GC/MS is employed). Wide-bore columns should be installed in 1/4 inch injectors, with deactivated liners designed specifically for use with these columns.

4.2.2.1 30 m x 0.53 mm ID fused-silica capillary column chemically bonded with 35 percent phenyl methylpolysiloxane (DB-608, SPB-608, RTx-35, or equivalent), 0.5 µm or 0.83 µm film thickness.

4.2.2.2 30 m x 0.53 mm ID fused-silica capillary column chemically bonded with 14% cyanopropylmethylpolysiloxane (DB-1701, or equivalent), 1.0 µm film thickness.

4.2.2.3 30 m x 0.53 mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5, SPB-5, RTx-5, or equivalent), 1.5 µm film thickness.

4.2.3 Wide-bore columns for dual-column analysis (choose one of the two pairs of columns listed below).

4.2.3.1 Column pair 1

30 m x 0.53 mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5, SPB-5, RTx-5, or equivalent), 1.5 µm film thickness.

30 m x 0.53 mm ID fused-silica capillary column chemically bonded with 14% cyanopropylmethylpolysiloxane (DB-1701, or equivalent), 1.0 µm film thickness.

Column pair 1 is mounted in a press-fit Y-shaped glass 3-way union splitter (J&W Scientific, Catalog No. 705-0733) or a Y-shaped fused-silica connector (Restek, *Catalog No. 20405), or equivalent.*

4.2.3.2 Column pair 2

30 m x 0.53 mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5, SPB-5, RTx-5, or equivalent), 0.83 µm film thickness.

30 m x 0.53 mm ID fused-silica capillary column chemically bonded with 14% cyanopropylmethylpolysiloxane (DB-1701, or equivalent), 1.0 µm film thickness.

Column pair 2 is mounted in an 8 in. deactivated glass injection tee (Supelco, Catalog No. 2-3665M), or equivalent.

4.3 Column rinsing kit - Bonded-phase column rinse kit (J&W Scientific, Catalog No. 430-3000), or equivalent.

4.4 Volumetric flasks - 10-mL and 25-mL, for preparation of standards.

5.0 REAGENTS

5.1 Reagent grade or pesticide grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

NOTE: Store the standard solutions (stock, composite, calibration, internal, and surrogate standards) at 4°C in polytetrafluoroethylene (PTFE)-sealed containers in the dark. When a lot of standards is prepared, it is recommended that aliquots of that lot be stored in individual small vials. All stock standard solutions must be replaced after one year or sooner if routine QC (Sec. 8.0) indicates a problem. All other standard solutions must be replaced after six months or sooner if routine QC (Sec. 8.0) indicates a problem.

5.2 Sample extracts prepared by Methods 3510, 3520, 3540, 3541, 3545, or 3550 need to undergo a solvent exchange step prior to analysis. The following solvents are necessary for dilution of sample extracts. All solvent lots should be pesticide quality or equivalent and should be determined to be phthalate-free.

5.2.1 n-Hexane, C_6H_{14}

5.2.2 Isooctane, $(CH_3)_3CCH_2CH(CH_3)_2$

5.3 The following solvents may be necessary for the preparation of standards. All solvent lots must be pesticide quality or equivalent and should be determined to be phthalate-free.

5.3.1 Acetone, $(CH_3)_2CO$

5.3.2 Toluene, $C_6H_5CH_3$

5.4 Organic-free reagent water - All references to water in this method refer to organic-free reagent water as defined in Chapter One.

5.5 Stock standard solutions (1000 mg/L) - May be prepared from pure standard materials or can be purchased as certified solutions.

5.5.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure compound. Dissolve the compound in isooctane or hexane and dilute to volume in a 10-mL volumetric flask. If compound purity is 96 percent or greater, the weight may be used without correction to calculate the concentration of the stock standard solution.

5.5.2 Commercially-prepared stock standard solutions may be used at any concentration if they are certified by the manufacturer or by an independent source.

5.6 Calibration standards for Aroclors

5.6.1 A standard containing a mixture of Aroclor 1016 and Aroclor 1260 will include many of the peaks represented in the other five Aroclor mixtures. As a result, a multi-point initial calibration employing a mixture of Aroclors 1016 and 1260 at five concentrations should be sufficient to demonstrate the linearity of the detector response without the necessity of performing initial calibrations for each of the seven Aroclors. In addition, such a mixture can

be used as a standard to demonstrate that a sample does not contain peaks that represent any one of the Aroclors. This standard can also be used to determine the concentrations of either Aroclor 1016 or Aroclor 1260, should they be present in a sample. Prepare a minimum of five calibration standards containing equal concentrations of both Aroclor 1016 and Aroclor 1260 by dilution of the stock standard with isooctane or hexane. The concentrations should correspond to the expected range of concentrations found in real samples and should bracket the linear range of the detector.

5.6.2 Single standards of each of the other five Aroclors are required to aid the analyst in pattern recognition. Assuming that the Aroclor 1016/1260 standards described in Sec. 5.6.1 have been used to demonstrate the linearity of the detector, these single standards of the remaining five Aroclors are also used to determine the calibration factor for each Aroclor. Prepare a standard for each of the other Aroclors. The concentrations should correspond to the mid-point of the linear range of the detector.

5.7 Calibration standards for PCB congeners

5.7.1 If results are to be determined for individual PCB congeners, then standards for the pure congeners must be prepared. The table in Sec. 1.1 lists 19 PCB congeners that have been tested by this method along with the IUPAC numbers designating these congeners. This procedure may be appropriate for other congeners as well.

5.7.2 Stock standards may be prepared in a fashion similar to that described for the Aroclor standards, or may be purchased as commercially-prepared solutions. Stock standards should be used to prepare a minimum of five concentrations by dilution of the stock standard with isooctane or hexane. The concentrations should correspond to the expected range of concentrations found in real samples and should bracket the linear range of the detector.

5.8 Internal standard

5.8.1 When PCB congeners are to be determined, the use of an internal standard is highly recommended. Decachlorobiphenyl may be used as an internal standard, added to each sample extract prior to analysis, and included in each of the initial calibration standards.

5.8.2 When PCBs are to be determined as Aroclors, an internal standard is not used, and decachlorobiphenyl is employed as a surrogate (see Sec. 5.8).

5.9 Surrogate standards

5.9.1 When PCBs are to be determined as Aroclors, decachlorobiphenyl is used as a surrogate, and is added to each sample prior to extraction. Prepare a solution of decachlorobiphenyl at a concentration of 5 mg/L in acetone.

5.9.2 When PCB congeners are to be determined, decachlorobiphenyl is recommended for use as an internal standard, and therefore, cannot also be used as a surrogate. Therefore, tetrachloro-meta-xylene may be used as a surrogate for PCB congener analysis. Prepare a solution of tetrachloro-meta-xylene at a concentration of 5 mg/L in acetone.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See Chapter Four, Organic Analytes for sample collection and preservation instructions.

6.2 Extracts should be stored under refrigeration in the dark and analyzed within 40 days of extraction.

NOTE: The holding times listed in Sec. 6.2 are recommendations. PCBs are very stable in a variety of matrices, and holding times under the conditions listed in Sec. 6.2 may be as high as a year for some matrices.

7.0 PROCEDURE

7.1 Sample extraction

7.1.1 Refer to Chapter Two and Method 3500 for guidance in choosing the appropriate extraction procedure. In general, water samples are extracted at a neutral pH with methylene chloride using a separatory funnel (Method 3510) or a continuous liquid-liquid extractor (Method 3520) or other appropriate procedure. Solid samples are extracted with hexane-acetone (1:1) or methylene chloride-acetone (1:1) using one of the Soxhlet extraction (Method 3540 or 3541) procedures, ultrasonic extraction (Method 3550), supercritical fluid extraction (3562), or other appropriate procedure. Oils and other organic liquids may be amenable to the waste dilution procedure in Method 3580.

NOTE: Use of hexane-acetone generally reduces the amount of interferences that are extracted and improves signal-to-noise.

7.1.2 Reference materials, field-contaminated samples, or spiked samples should be used to verify the applicability of the selected extraction technique to each new sample type. Such samples should contain or be spiked with the compounds of interest in order to determine the percent recovery and the limit of detection for that sample type (see Chapter One). When other materials are not available and spiked samples are used, they should be spiked with the analytes of interest, either specific Aroclors or PCB congeners. When the presence of specific Aroclors is not anticipated, the Aroclor 1016/1260 mixture may be an appropriate choice for spiking. See Methods 3500 and 8000 for guidance on demonstration of initial method proficiency as well as guidance on matrix spikes for routine sample analysis.

7.2 Extract cleanup

Refer to Methods 3660 and 3665 for information on extract cleanup.

7.3 GC conditions

This method allows the analyst to choose between a single-column or a dual-column configuration in the injector port. Either wide- or narrow-bore columns may be used. See Sec. 7.7 for information on techniques for making positive identifications of multi-component analytes.

7.3.1 Single-column analysis

This capillary GC/ECD method allows the analyst the option of using 0.25-0.32 mm ID capillary columns (narrow-bore) or 0.53 mm ID capillary columns (wide-bore). The use of narrow-bore (0.25-0.32 mm ID) columns is recommended when the analyst requires greater chromatographic resolution. Use of narrow-bore columns is suitable for relatively clean samples or for extracts that have been prepared with one or more of the clean-up options referenced in the method. Wide-bore columns (0.53 mm ID) are suitable for more complex environmental and waste matrices.

7.3.2 Dual-column analysis

The dual-column/dual-detector approach involves the use of two 30 m x 0.53 mm ID fused-silica open-tubular columns of different polarities, thus different selectivities towards the target compounds. The columns are connected to an injection tee and ECD detectors.

7.3.3 GC temperature programs and flow rates

7.3.3.1 Table 2 lists GC operating conditions for the analysis of PCBs as Aroclors for single-column analysis, using either narrow-bore or wide-bore capillary columns. Table 3 lists GC operating conditions for the dual-column analysis. Use the conditions in these tables as guidance and establish the GC temperature program and flow rate necessary to separate the analytes of interest.

7.3.3.2 When determining PCBs as congeners, difficulties may be encountered with coelution of congener 153 and other sample components. When determining PCBs as Aroclors, chromatographic conditions should be adjusted to give adequate separation of the characteristic peaks in each Aroclor (see Sec. 7.4.6).

7.3.3.3 Tables 4 and 5 summarize the retention times of up to 73 Aroclor peaks determined during dual-column analysis using the operating conditions listed in Table 2. These retention times are provided as guidance as to what may be achieved using the GC columns, temperature programs, and flow rates described in this method. Note that the peak numbers used in these tables are *not* the IUPAC congener numbers, but represent the elution order of the peaks on these GC columns.

7.3.3.4 Once established, the same operating conditions must be used for the analysis of samples and standards.

7.4 Calibration

7.4.1 Prepare calibration standards as described in Sec. 5.0. Refer to Method 8000 (Sec. 7.0) for proper calibration techniques for both initial calibration and calibration verification. When PCBs are to be determined as congeners, the use of internal standard calibration is highly recommended. Therefore, the calibration standards must contain the internal standard (see Sec. 5.7) at the same concentration as the sample extracts. When PCBs are to be determined as Aroclors, external standard calibration is generally used.

NOTE: Because of the sensitivity of the electron capture detector, the injection port and column should always be cleaned prior to performing the initial calibration.

7.4.2 When PCBs are to be quantitatively determined as congeners, an initial multi-point calibration must be performed that includes standards for all the target analytes (congeners).

7.4.3 When PCBs are to be quantitatively determined as Aroclors, the initial calibration consists of two parts, described below.

7.4.3.1 As noted in Sec. 5.6.1, a standard containing a mixture of Aroclor 1016 and Aroclor 1260 will include many of the peaks represented in the other five Aroclor mixtures. Thus, such a standard may be used to demonstrate the linearity of the detector and that a sample does not contain peaks that represent any one of the Aroclors. This standard can also be used to determine the concentrations of either Aroclor 1016 or Aroclor 1260, should they be present in a sample. Therefore, an initial multi-point calibration is performed using the mixture of Aroclors 1016 and 1260 described in Sec. 5.6.1. See Sec. 7.0 of Method 8000 for guidance on the use of linear and non-linear calibrations.

7.4.3.2 Standards of the other five Aroclors are necessary for pattern recognition. These standards are also used to determine a single-point calibration factor for each Aroclor, assuming that the Aroclor 1016/1260 mixture in Sec. 7.4.3.1 has been used to describe the detector response. The standards for these five Aroclors should be analyzed before the analysis of any samples, and may be analyzed before or after the analysis of the five 1016/1260 standards in Sec. 7.4.3.1.

7.4.3.3 In situations where only a few Aroclors are of interest for a specific project, the analyst may employ a multi-point initial calibration of each of the Aroclors of interest (e.g., five standards of Aroclor 1232 if this Aroclor is of concern and linear calibration is employed) and not use the 1016/1260 mixture described in Sec. 7.4.3.1 or the pattern recognition standards described in 7.4.3.2.

7.4.4 Establish the GC operating conditions appropriate for the configuration (single-column or dual column, Sec. 7.3). Optimize the instrumental conditions for resolution of the target compounds and sensitivity. A final temperature of 240-270°C may be required to elute decachlorobiphenyl. Use of injector pressure programming will improve the chromatography of late eluting peaks.

NOTE: Once established, the same operating conditions must be used for both calibrations and sample analyses.

7.4.5 A 2-µL injection of each calibration standard is recommended. Other injection volumes may be employed, provided that the analyst can demonstrate adequate sensitivity for the compounds of interest.

7.4.6 Record the peak area (or height) for each congener or each characteristic Aroclor peak to be used for quantitation.

7.4.6.1 A minimum of 3 peaks must be chosen for each Aroclor, and preferably 5 peaks. The peaks must be characteristic of the Aroclor in question. Choose peaks in the Aroclor standards that are at least 25% of the height of the largest Aroclor peak. For each Aroclor, the set of 3 to 5 peaks should include at least one peak that is unique to that Aroclor. Use at least five peaks for the Aroclor 1016/1260 mixture, none of which should be found in both of these Aroclors.

7.4.6.2 Late-eluting Aroclor peaks are generally the most stable in the environment. Table 6 lists diagnostic peaks in each Aroclor, along with their retention times on two GC columns suitable for single-column analysis. Table 7 lists 13 specific PCB congeners found in Aroclor mixtures. Table 8 lists PCB congeners with corresponding retention times on a DB-5 wide-bore GC column. Use these tables as guidance in choosing the appropriate peaks.

7.4.7 When determining PCB congeners by the internal standard procedure, calculate the response factor (RF) for each congener in the calibration standards relative to the internal standard, decachlorobiphenyl, using the equation that follows.

$$RF = \frac{A_s \times C_{is}}{A_{is} \times C_s}$$

where:

A_s = Peak area (or height) of the analyte or surrogate.
A_{is} = Peak area (or height) of the internal standard.
C_s = Concentration of the analyte or surrogate, in µg/L.
C_{is} = Concentration of the internal standard, in µg/L.

7.4.8 When determining PCBs as Aroclors by the external standard technique, calculate the calibration factor (CF) for each characteristic Aroclor peak in each of the initial calibration standards (from either Sec. 7.4.3.1 or 7.4.3.2) using the equation below.

$$CF = \frac{\text{Peak Area (or Height) in the Standard}}{\text{Total Mass of the Standard Injected (in nanograms)}}$$

Using the equation above, a calibration factor will be determined for each characteristic peak, using the total mass of the Aroclor injected. These individual calibration factors are used to quantitate sample results by applying the factor for each individual peak to the area of that peak, as described in Sec. 7.9.

For a five-point calibration, five sets of calibration factors will be generated for the Aroclor 1016/1260 mixture, each set consisting of the calibration factors for each of the five (or more) peaks chosen for this mixture, e.g., there will be at least 25 separate calibration factors for the mixture. The single standard for each of the other Aroclors (see Sec. 7.4.3.1) will generate at least three calibration factors, one for each selected peak. If a non-linear calibration model is employed, as described in Method 8000, then additional standards containing the Aroclor 1016/1260 mixture will be employed, with a corresponding increase in the total number of calibration factors (e.g., at least 30 for a 6-point curve and 35 for a 7-point curve).

7.4.9 The response factors or calibration factors from the initial calibration are used to evaluate the linearity of the initial calibration, if a linear calibration model is used. This involves the calculation of the mean response or calibration factor, the standard deviation, and the relative standard deviation (RSD) for each congener or Aroclor peak. See Method 8000 for the specifics of the evaluation of the linearity of the calibration and guidance on performing non-linear calibrations. In general, non-linear calibrations also will consider each characteristic Aroclor peak separately. Thus, for the 1016/1260 mixture, non-linear calibration will result in at least five calibration models, one for each selected peak. Each model is then applied to the determination of the concentration of that specific peak in the sample chromatogram, as

describe din Sec. 7.9. When the Aroclor 1016/1260 mixture is used to demonstrate the detector response, the calibration models (see Method 8000) chosen for this mixture must be applied to the other five Aroclors for which only single standards are analyzed. If multi-point calibration is performed for individual Aroclors (see Sec. 7.4.3.3), use the calibration factors from those standards to evaluate linearity.

7.5 Retention time windows

Retention time windows are crucial to the identification of target compounds. Absolute retention times are used for the identification of PCBs as Aroclors. When PCBs are determined as congeners by an internal standard technique, absolute retention times may be used in conjunction with relative retention times (relative to the internal standard). Retention time windows are established to compensate for minor shifts in absolute retention times as a result of sample loadings and normal chromatographic variability. The width of the retention time window should be carefully established to minimize the occurrence of both false positive and false negative results. Tight retention time windows may result in false negatives and/or may cause unnecessary reanalysis of samples when surrogates or spiked compounds are erroneously not identified. Overly wide retention time windows may result in false positive results that cannot be confirmed upon further analysis. Analysts should consult Method 8000 for the details of establishing retention time windows.

7.6 Gas chromatographic analysis of sample extracts

7.6.1 The same GC operating conditions used for the initial calibration must be employed for samples analyses.

7.6.2 Verify calibration each 12-hour shift by injecting calibration verification standards prior to conducting any sample analyses. A calibration standard must also be injected at intervals of not less than once every twenty samples (after every 10 samples is recommended to minimize the number of samples requiring re-injection when QC limits are exceeded) and at the end of the analysis sequence. For Aroclor analyses, the calibration verification standard should be a mixture of Aroclor 1016 and Aroclor 1260. The calibration verification process does not *require* analysis of the other Aroclor standards used for pattern recognition, but the analyst may wish to include a standard for one of these Aroclors after the 1016/1260 mixture used for calibration verification throughout the analytical sequence.

7.6.2.1 The calibration factor for each analyte calculated from the calibration verification standard (CF_v) must not exceed a difference of more than ± 15 percent when compared to the mean calibration factor from the initial calibration curve.

$$\% \text{ Difference} = \frac{CF - CF_v}{\overline{CF}} \times 100$$

7.6.2.2 When internal standard calibration is used for PCB congeners, the response factor calculated from the calibration verification standard (RF_v) must not exceed a ± 15 percent difference when compared to the mean response factor from the initial calibration.

$$\% \text{ Difference} = \frac{RF - RF_v}{\overline{RF}} \times 100$$

7.6.2.3 If this criterion is exceeded for any calibration factor or response factor, inspect the gas chromatographic system to determine the cause and perform whatever maintenance is necessary before verifying calibration and proceeding with sample analysis.

7.6.2.4 If routine maintenance does not return the instrument performance to meet the QC requirements (Sec. 8.2) based on the last initial calibration, then a new initial calibration must be performed.

7.6.3 Inject a 2-µL aliquot of the concentrated sample extract. Record the volume injected to the nearest 0.05 µL and the areas (or heights) of the resulting peaks.

7.6.4 Qualitative identifications of target analytes are made by examination of the sample chromatograms, as described in Sec. 7.7.

7.6.5 Quantitative results are determined for each identified analyte (Aroclors or congeners), using the procedures described in Secs. 7.8 and 7.9 for either the internal or the external calibration procedure (Method 8000). If the responses in the sample chromatogram exceed the calibration range of the system, dilute the extract and reanalyze. Peak height measurements are recommended over peak area when overlapping peaks cause errors in area integration.

7.6.6 Each sample analysis must be bracketed with an acceptable initial calibration, calibration verification standard(s) (each 12-hour shift), or calibration standards interspersed within the samples. When a calibration verification standard fails to meet the QC criteria, all samples that were injected after the last standard that last met the QC criteria must be re-injected.

Multi-level standards (mixtures or multi-component analytes) are highly recommended to ensure that detector response remains stable for all analytes over the calibration range.

7.6.7 Sample injections may continue for as long as the calibration verification standards and standards interspersed with the samples meet instrument QC requirements. It is *recommended* that standards be analyzed after every 10 samples (*required* after every 20 samples and at the end of a set) to minimize the number of samples that must be re-injected when the standards fail the QC limits. The sequence ends when the set of samples has been injected or when qualitative or quantitative QC criteria are exceeded.

7.6.8 If the peak response is less than 2.5 times the baseline noise level, the validity of the quantitative result may be questionable. The analyst should consult with the source of the sample to determine whether further concentration of the sample is warranted.

7.6.9 Use the calibration standards analyzed during the sequence to evaluate retention time stability. If any of the standards fall outside their daily retention time windows, the system is out of control. Determine the cause of the problem and correct it.

7.6.10 If compound identification or quantitation is precluded due to interferences (e.g., broad, rounded peaks or ill-defined baselines are present), corrective action is warranted. Cleanup of the extract or replacement of the capillary column or detector may be necessary. The analyst may begin by rerunning the sample on another instrument to determine if the problem results from analytical hardware or the sample matrix. Refer to Method 3600 for the procedures to be followed in sample cleanup.

7.7 Qualitative identification

The identification of PCBs as either Aroclors or congeners using this method with an electron capture detector is based on agreement between the retention times of peaks in the sample chromatogram with the retention time windows established through the analysis of standards of the target analytes. See Method 8000 for information on the establishment of retention time windows.

Tentative identification of an analyte occurs when a peak from a sample extract falls within the established retention time window for a specific target analyte. Each tentative identification must be confirmed: using a second GC column of dissimilar stationary phase (as in the dual-column analysis), based on a clearly identifiable Aroclor pattern, or using another technique such as GC/MS (see Sec. 7.10).

7.7.1 When simultaneous analyses are performed from a single injection (the dual-column GC configuration described in Sec. 7.3), it is not practical to designate one column as the analytical (primary) column and the other as the confirmation column. Since the calibration standards are analyzed on both columns, the results for both columns must meet the calibration acceptance criteria. If the retention times of the peaks on both columns fall within the retention time windows on the respective columns, then the target analyte identification has been confirmed.

7.7.2 The results of a single column/single injection analysis may be confirmed on a second, dissimilar, GC column. In order to be used for confirmation, retention time windows must have been established for the second GC column. In addition, the analyst must demonstrate the sensitivity of the second column analysis. This demonstration must include the analysis of a standard of the target analyte at a concentration at least as low as the concentration estimated from the primary analysis. That standard may be either the individual congeners, individual Aroclor or the Aroclor 1016/1260 mixture.

7.7.3 When samples are analyzed from a source known to contain specific Aroclors, the results from a single-column analysis may be confirmed on the basis of a clearly recognizable Aroclor pattern. This approach should <u>not</u> be attempted for samples from unknown or unfamiliar sources or for samples that appear to contain mixtures of Aroclors. In order to employ this approach, the analyst must document:

- The peaks that were evaluated when comparing the sample chromatogram and the Aroclor standard.
- The absence of major peaks representing any other Aroclor.
- The source-specific information indicating that Aroclors are anticipated in the sample (e.g., historical data, generator knowledge, etc.).

This information should either be provided to the data user or maintained by the laboratory.

7.7.4 See Sec. 7.10 for information on GC/MS confirmation.

7.8 Quantitation of PCBs as congeners

7.8.1 The quantitation of PCB congeners is accomplished by the comparison of the sample chromatogram to those of the PCB congener standards, using the internal standard technique (see Method 8000). Calculate the concentration of each congener.

7.8.2 Depending on project requirements, the PCB congener results may be reported as congeners, or may be summed and reported as total PCBs. The analyst should use caution when using the congener method for quantitation when regulatory requirements are based on Aroclor concentrations. See Sec. 7.9.3.

7.9 Quantitation of PCBs as Aroclors

The quantitation of PCB residues as Aroclors is accomplished by comparison of the sample chromatogram to that of the most similar Aroclor standard. A choice must be made as to which Aroclor is most similar to that of the residue and whether that standard is truly representative of the PCBs in the sample.

7.9.1 Use the individual Aroclor standards (not the 1016/1260 mixtures) to determine the pattern of peaks on Aroclors 1221, 1232, 1242, 1248, and 1254. The patterns for Aroclors 1016 and 1260 will be evident in the mixed calibration standards.

7.9.2 Once the Aroclor pattern has been identified, compare the responses of 3 to 5 major peaks in the single-point calibration standard for that Aroclor with the peaks observed in the sample extract. The amount of Aroclor is calculated using the individual calibration factor for each of the 3 to 5 characteristic peaks chosen in Sec. 7.4.6.1. and the calibration model (linear or non-linear) established from the multi-point calibration of the 1016/1260 mixture. Non-linear calibration may result in different models for each selected peak. A concentration is determined using each of the characteristic peaks, using the individual calibration factor calculated for that peak in Sec. 7.4.8, and then those 3 to 5 concentrations are averaged to determine the concentration of that Aroclor.

7.9.3 Weathering of PCBs in the environment and changes resulting from waste treatment processes may alter the PCBs to the point that the pattern of a specific Aroclor is no longer recognizable. Samples containing more than one Aroclor present similar problems. If the purpose of the analysis is <u>not</u> regulatory compliance monitoring on the basis of Aroclor concentrations, then it may be more appropriate to perform the analyses using the PCB congener approach described in this method. If results in terms of Aroclors <u>are</u> required, then the quantitation as Aroclors may be performed by measuring the total area of the PCB pattern and quantitating on the basis of the Aroclor standard that is most similar to the sample. Any peaks that are not identifiable as PCBs on the basis of retention times should be subtracted from the total area. When quantitation is performed in this manner, the problems should be fully described for the data user and the specific procedures employed by the analyst should be thoroughly documented.

7.10 GC/MS confirmation may be used in conjunction with either single-or dual-column analysis if the concentration is sufficient for detection by GC/MS.

7.10.1 Full-scan quadrupole GC/MS will normally require a higher concentration of the analyte of interest than full-scan ion trap or selected ion monitoring techniques. The concentrations will be instrument-dependent, but values for full-scan quadrupole GC/MS may

be as high as 10 ng/µL in the final extract, while ion trap or SIM may only require a concentration of 1 ng/µL.

7.10.2 The GC/MS must be calibrated for the target analytes when it is used for quantitative analysis. If GC/MS is used only for confirmation of the identification of the target analytes, then the analyst must demonstrate that those pesticides identified by GC/ECD can be confirmed by GC/MS. This demonstration may be accomplished by analyzing a single-point standard containing the analytes of interest at or below the concentrations reported in the GC/EC analysis. When using SIM techniques, the ions and retention times should be characteristic of the Aroclors to be confirmed.

7.10.3 GC/MS confirmation should be accomplished by analyzing the same extract used for GC/ECD analysis and the extract of the associated blank.

7.11 Chromatographic system maintenance as corrective action

When system performance does not meet the established QC requirements, corrective action is required, and may include one or more of the following.

7.11.1 Splitter connections

For dual columns which are connected using a press-fit Y-shaped glass splitter or a Y-shaped fused-silica connector, clean and deactivate the splitter port insert or replace with a cleaned and deactivated splitter. Break off the first few inches (up to one foot) of the injection port side of the column. Remove the columns and solvent backflush according to the manufacturer's instructions. If these procedures fail to eliminate the degradation problem, it may be necessary to deactivate the metal injector body and/or replace the columns.

7.11.2 Metal injector body

Turn off the oven and remove the analytical columns when the oven has cooled. Remove the glass injection port insert (instruments with on-column injection). Lower the injection port temperature to room temperature. Inspect the injection port and remove any noticeable foreign material.

7.11.2.1 Place a beaker beneath the injector port inside the oven. Using a wash bottle, rinse the entire inside of the injector port with acetone and then rinse it with toluene, catching the rinsate in the beaker.

7.11.2.2 Consult the manufacturer's instructions regarding deactivating the injector port body. Glass injection port liners may require deactivation with a silanizing solution containing dimethyldichlorosilane.

7.11.3 Column rinsing

The column should be rinsed with several column volumes of an appropriate solvent. Both polar and nonpolar solvents are recommended. Depending on the nature of the sample residues expected, the first rinse might be water, followed by methanol and acetone. Methylene chloride is a good final rinse and in some cases may be the only solvent required. The column should then be filled with methylene chloride and allowed to stand flooded overnight to allow materials within the stationary phase to migrate into the solvent. The column

is then flushed with fresh methylene chloride, drained, and dried at room temperature with a stream of ultrapure nitrogen.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Quality control procedures to ensure the proper operation of the various sample preparation techniques can be found in Method 3500. If an extract cleanup procedure was performed, refer to Method 3600 for the appropriate quality control procedures. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures necessary to evaluate the GC system operation are found in Method 8000, Sec. 7.0 and include evaluation of retention time windows, calibration verification and chromatographic analysis of samples.

8.2.1 Include a calibration standard after each group of 20 samples (it is *recommended* that a calibration standard be included after every 10 samples to minimize the number of repeat injections) in the analysis sequence as a calibration check. The response factors for the calibration should be within 15 percent of the initial calibration. When this continuing calibration is out of this acceptance window, the laboratory should stop analyses and take corrective action.

8.2.2 Whenever quantitation is accomplished using an internal standard, internal standard responses must be evaluated for acceptance. The measured area of the internal standard must be no more than 50 percent different from the average area calculated during calibration. When the internal standard peak area is outside the limit, all samples that fall outside the QC criteria must be reanalyzed.

8.3 Initial Demonstration of Proficiency - Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Method 8000, Sec. 8.0, for information on how to accomplish this demonstration.

8.3.1 The QC Reference Sample concentrate (Method 3500) should contain PCBs as Aroclors at 10-50 mg/L for water samples, or PCBs as congeners at the same concentrations. A 1-mL volume of this concentrate spiked into 1 L of organic-free reagent water will result in a sample concentration of 10-50 µg/L. If Aroclors are not expected in samples from a particular source, then prepare the QC reference samples with a mixture of Aroclors 1016 and 1260. However, when specific Aroclors are known to be present or expected in samples, the specific Aroclors should be used for the QC reference sample. See Method 8000, Sec. 8.0, for additional information on how to accomplish this demonstration.

8.3.2 Calculate the average recovery and the standard deviation of the recoveries of the analytes in each of the four QC reference samples. Refer to Sec. 8.0 of Method 8000 for procedures for evaluating method performance.

8.4 Sample Quality Control for Preparation and Analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy,

and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, a matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch and the addition of surrogates to each field sample and QC sample.

8.4.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair, spiked with the Aroclor 1016/1260 mixture. However, when specific Aroclors are known to be present or expected in samples, the specific Aroclors should be used for spiking. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample.

8.4.2 A Laboratory Control Sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

8.4.3 See Method 8000, Sec. 8.0, for the details on carrying out sample quality control procedures for preparation and analysis.

8.5 Surrogate recoveries - The laboratory must evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000, Sec. 8.0, for information on evaluating surrogate data and developing and updating surrogate limits.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 The MDL is defined in Chapter One. The MDLs for Aroclors vary in the range of 0.054 to 0.90 µg/L in water and 57 to 70 µg/kg in soils, with the higher MDLs for the more heavily chlorinated Aroclors. Estimated quantitation limits may be determined using the factors in Table 1.

9.2 Estimated quantitation limits for PCBs as congeners vary by congener, in the range of 5 - 25 ng/L in water and 160 - 800 ng/kg in soils, with the higher values for the more heavily chlorinated congeners.

9.3 The accuracy and precision obtainable with this method depend on the sample matrix, sample preparation technique, optional cleanup techniques, and calibration procedures used. Table 9 provides single laboratory recovery data for Aroclors spiked into clay and soil and extracted with automated Soxhlet. Table 10 provides multiple laboratory data on the precision and accuracy for Aroclors spiked into soil and extracted by automated Soxhlet.

9.4 During method performance studies, the concentrations determined as Aroclors were larger than those obtained using the congener method. In certain soils, interference prevented the measurement of congener 66. Recoveries of congeners from soils spiked with Aroclor 1254 and Aroclor 1260 were between 80% and 90%. Recoveries of congeners from environmental reference materials ranged from 51 - 66% of the certified Aroclor values.

9.5 Tables 11 through 13 contain laboratory performance data for several PCB congeners using supercritical fluid extraction Method 3562 on an HP 7680. Seven replicate extractions were performed on each sample. The method was performed using a variable restrictor and solid trapping material (Florisil). Sample analysis was performed by GC/ECD. The following soil samples were used for this study:

9.5.1 Two field-contaminated certified reference materials were extracted by a single laboratory. One of the materials was a lake sediment from Environment Canada (EC-5). The other material was soil from a dump site and was provided by the National Science and Engineering Research Council of Canada (EC-1). The average recoveries for EC-5 are based on the certified value for that sample. The average recoveries for EC-1 are based on the certified value of the samples or a Soxhlet value, if a certified value was unavailable for a specific analyte.

9.5.2 Four certified reference materials were extracted by two independent laboratories. The materials were: a marine sediment from NIST (SRM 1941), a fish tissue from NIST (SRM 2974), a sewage sludge from BCR European Union (CRM 392), and a soil sample from BCR European Union (CRM 481). The average recoveries are based on the certified value of the samples or a Soxhlet value, if a certified value was unavailable for a specific analyte.

9.5.3 A weathered sediment sample from Michigan (Saginaw Bay) was extracted by a single laboratory. Soxhlet extractions were carried out on this sample and the SFE recovery is relative to that for each congener. The average recoveries are based on the certified value of the samples. Additional data are shown in the tables for some congeners for which no certified values were available.

9.6 Tables 14 through 16 contain single laboratory recovery data for Aroclor 1254 using SPE Method 3535. Recovery data at 2, 10, and 100 ug/L are presented. Results represent three replicate solid-phase extractions of spiked wastewaters. Two different wastewaters from each wastewater type were spiked. All of the extractions were performed using 90-mm C_{18} disks.

10.0 REFERENCES

1. Lopez-Avila, V., Baldin, E., Benedicto, J, Milanes, J., Beckert. W.F., Application of Open-Tubular Columns to SW-846 GC Methods", final report to the U.S. Environmental Protection Agency on Contract 68-03-3511, Mid-Pacific Environmental Laboratory, Mountain View, CA, 1990.

2. Development and Application of Test Procedures for Specific Organic Toxic Substances in Wastewaters. Category 10 - Pesticides and PCB Report for the U.S. Environmental Protection Agency on Contract 68-03-2606.

3. Ahnoff, M., Josefsson, B., "Cleanup Procedures for PCB Analysis on River Water Extracts", Bull. Environ. Contam. Toxicol., 1975, 13, 159.

4. Marsden, P.J., "Performance Data for SW-846 Methods 8270, 8081, and 8141", U.S. Environmental Protection Agency, EMSL-Las Vegas, EPA/600/4-90/015.

5. Marsden, P.J., "Analysis of PCBs", U.S. Environmental Protection Agency, EMSL-Las Vegas, NV, EPA/600/8-90/004.

6. Erickson, M., Analytical Chemistry of PCBs, Butterworth Publishers, Ann Arbor Science Book, (1986).

7. Stewart, J., "EPA Verification Experiment for Validation of the SOXTEC® PCB Extraction Procedure", Oak Ridge National Laboratory, Oak Ridge, TN, 37831-6138, October 1988.

8. Lopez-Avila, V. (Beckert, W., Project Officer), "Development of a Soxtec Extraction Procedure for Extracting Organic Compounds from Soils and Sediments", EPA 600/X-91/140, U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Las Vegas, NV, October 1991.

9. Stewart, J.H., Bayne, C.K., Holmes, R.L., Rogers, W.F., and Maskarinec, M.P., "Evaluation of a Rapid Quantitative Organic Extraction System for Determining the Concentration of PCB in Soils", Proceedings of the U.S. EPA Symposium on Waste Testing and Quality Assurance, Oak Ridge National Laboratory, Oak Ridge, TN, 37831, July 11-15, 1988.

10. Tsang, S.F., Marsden, P.J., and Lesnik, B., "Quantitation of Polychlorinated Biphenyls Using 19 Specific Congeners", Proceedings of the 9th Annual Waste Testing and Quality Assurance Symposium, Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, DC, July 1993.

11. S. Bøwadt, B. Johansson, S. Wunderli, M. Zennegg, L. F. de Alencastro and D. Grandjean, "Independent Comparison of Soxhlet and Supercritical Fluid Extraction for the Determination of PCBs in an Industrial Soil," *Anal. Chem.*, 1995, 67(14) 2424-2430.

12. Markell, C., "3M Data Submission to EPA," letter to B. Lesnik, June 27, 1995.

13. Richter, B, Ezzell, J., and Felix D, "Single Laboratory Method Validation Report - Extraction of Organophosphorus Pesticides, Herbicides and Polychlorinated Biphenyls using Accelerated Solvent Extraction (ASE) with Analytical Validation by GC/NPD and GC/ECD," Dionex, Salt Lake City, UT, Document 101124, December 2, 1994.

TABLE 1

FACTORS FOR DETERMINATION OF ESTIMATED QUANTITATION LIMITS[a] (EQLs) FOR VARIOUS MATRICES

Matrix	Factor
Ground water	10
Low-concentration soil by sonication with GPC cleanup	670
High-concentration soil and sludges by sonication	10,000
Non-water miscible waste	100,000

[a]EQL = [MDL for water (see Sec. 1.8)] times [Factor in this table]

For nonaqueous samples, the factor is on a wet-weight basis. Sample EQLs are highly matrix-dependent. EQLs determined using these factors are provided as guidance and may not always be achievable.

TABLE 2

GC OPERATING CONDITIONS FOR PCBs AS AROCLORS
SINGLE COLUMN ANALYSIS

Narrow-bore columns

Narrow-bore Column 1 - 30 m x 0.25 or 0.32 mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1 µm film thickness.

Carrier gas (He)	16 psi
Injector temperature	225°C
Detector temperature	300°C
Initial temperature	100°C, hold 2 minutes
Temperature program	100°C to 160°C at 15°C/min, followed by 160°C to 270°C at 5°C/min
Final temperature	270°C

Narrow-bore Column 2 - 30 m x 0.25 mm ID fused-silica capillary column chemically bonded with 35 percent phenyl methylpolysiloxane (DB-608, SPB-608, or equivalent) 25 µm coating thickness, 1 µm film thickness

Carrier gas (N_2)	20 psi
Injector temperature	225°C
Detector temperature	300°C
Initial temperature	160°C, hold 2 minutes
Temperature program	160°C to 290°C at 5°C/min
Final temperature	290°C, hold 1 min

Wide-bore columns

Wide-bore Column 1 - 30 m x 0.53 mm ID fused-silica capillary column chemically bonded with 35 percent phenyl methylpolysiloxane (DB-608, SPB-608, RTx-35, or equivalent), 0.5 µm or 0.83 µm film thickness.

Wide-bore Column 2 - 30 m x 0.53 mm ID fused-silica capillary column chemically bonded with 14% cyanopropylmethylpolysiloxane (DB-1701, or equivalent), 1.0 µm film thickness.

Carrier gas (He)	5-7 mL/minute
Makeup gas (argon/methane [P-5 or P-10] or N_2)	30 mL/min
Injector temperature	250°C
Detector temperature	290°C
Initial temperature	150°C, hold 0.5 minute
Temperature program	150°C to 270°C at 5°C/min
Final temperature	270°C, hold 10 min

TABLE 2
(continued)

GC OPERATING CONDITIONS FOR PCBs AS AROCLORS
SINGLE COLUMN ANALYSIS

Wide-bore Columns (continued)

Wide-bore Column 3 - 30 m x 0.53 mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5, SPB-5, RTx-5, or equivalent), 1.5 µm film thickness.

Carrier gas (He)	6 mL/minute
Makeup gas (argon/methane [P-5 or P-10] or N_2)	30 mL/min
Injector temperature	205°C
Detector temperature	290°C
Initial temperature	140°C, hold 2 min
Temperature program	140°C to 240°C at 10°C/min, hold 5 minutes at 240°C, 240°C to 265°C at 5°C/min
Final temperature	265°C, hold 18 min

TABLE 3

GC OPERATING CONDITIONS FOR PCBs AS AROCLORS
FOR THE DUAL COLUMN METHOD OF ANALYSIS HIGH TEMPERATURE, THICK FILM

Column 1 - DB-1701 or equivalent, 30 m x 0.53 mm ID, 1.0 µm film thickness.

Column 2 - DB-5 or equivalent, 30 m x 0.53 mm ID, 1.5 µm film thickness.

Carrier gas (He) flow rate	6 mL/min
Makeup gas (N_2) flow rate	20 mL/min
Temperature program	0.5 min hold 150°C to 190°C, at 12°C/min, 2 min hold 190°C to 275°C, at 4°C/min, 10 min hold
Injector temperature	250°C
Detector temperature	320°C
Injection volume	2 µL
Solvent	Hexane
Type of injector	Flash vaporization
Detector type	Dual ECD
Range	10
Attenuation	64 (DB-1701)/64 (DB-5)
Type of splitter	J&W Scientific press-fit Y-shaped inlet splitter

TABLE 4

SUMMARY OF RETENTION TIMES OF AROCLORS
ON THE DB-5 COLUMN[a], DUAL-COLUMN ANALYSIS

Peak No.[b]	Aroclor 1016	Aroclor 1221	Aroclor 1232	Aroclor 1242	Aroclor 1248	Aroclor 1254	Aroclor 1260
1		5.85	5.85				
2		7.63	7.64	7.57			
3	8.41	8.43	8.43	8.37			
4	8.77	8.77	8.78	8.73			
5	8.98	8.99	9.00	8.94	8.95		
6	9.71			9.66			
7	10.49	10.50	10.50	10.44	10.45		
8	10.58	10.59	10.59	10.53			
9	10.90		10.91	10.86	10.85		
10	11.23	11.24	11.24	11.18	11.18		
11	11.88		11.90	11.84	11.85		
12	11.99		12.00	11.95			
13	12.27	12.29	12.29	12.24	12.24		
14	12.66	12.68	12.69	12.64	12.64		
15	12.98	12.99	13.00	12.95	12.95		
16	13.18		13.19	13.14	13.15		
17	13.61		13.63	13.58	13.58	13.59	13.59
18	13.80		13.82	13.77	13.77	13.78	
19	13.96		13.97	13.93	13.93	13.90	
20	14.48		14.50	14.46	14.45	14.46	
21	14.63		14.64	14.60	14.60		
22	14.99		15.02	14.98	14.97	14.98	
23	15.35		15.36	15.32	15.31	15.32	
24	16.01			15.96			
25			16.14	16.08	16.08	16.10	
26	16.27		16.29	16.26	16.24	16.25	16.26
27						16.53	
28			17.04		16.99	16.96	16.97
29			17.22	17.19	17.19	17.19	17.21
30			17.46	17.43	17.43	17.44	
31					17.69	17.69	
32				17.92	17.91	17.91	
33				18.16	18.14	18.14	
34			18.41	18.37	18.36	18.36	18.37
35			18.58	18.56	18.55	18.55	
36							18.68

[a] GC operating conditions are given in Table 3. All retention times in minutes.
[b] The peaks listed in this table are sequentially numbered in elution order for illustrative purposes only and are not isomer numbers.

TABLE 4
(continued)

Peak No.[b]	Aroclor 1016	Aroclor 1221	Aroclor 1232	Aroclor 1242	Aroclor 1248	Aroclor 1254	Aroclor 1260
37			18.83	18.80	18.78	18.78	18.79
38			19.33	19.30	19.29	19.29	19.29
39						19.48	19.48
40						19.81	19.80
41			20.03	19.97	19.92	19.92	
42						20.28	20.28
43					20.46	20.45	
44						20.57	20.57
45				20.85	20.83	20.83	20.83
46			21.18	21.14	21.12	20.98	
47					21.36	21.38	21.38
48						21.78	21.78
49				22.08	22.05	22.04	22.03
50						22.38	22.37
51						22.74	22.73
52						22.96	22.95
53						23.23	23.23
54							23.42
55						23.75	23.73
56						23.99	23.97
57							24.16
58						24.27	
59							24.45
60						24.61	24.62
61						24.93	24.91
62							25.44
63						26.22	26.19
64							26.52
65							26.75
66							27.41
67							28.07
68							28.35
69							29.00

[a] GC operating conditions are given in Table 3. All retention times in minutes.
[b] The peaks listed in this table are sequentially numbered in elution order for illustrative purposes only and are not isomer numbers.

TABLE 5

SUMMARY OF RETENTION TIMES OF AROCLORS
ON THE DB-1701 COLUMN[a], DUAL COLUMN ANALYSIS

Peak No.[b]	Aroclor 1016	Aroclor 1221	Aroclor 1232	Aroclor 1242	Aroclor 1248	Aroclor 1254	Aroclor 1260
1		4.45	4.45				
2		5.38					
3		5.78					
4		5.86	5.86				
5	6.33	6.34	6.34	6.28			
6	6.78	6.78	6.79	6.72			
7	6.96	6.96	6.96	6.90	6.91		
8	7.64			7.59			
9	8.23	8.23	8.23	8.15	8.16		
10	8.62	8.63	8.63	8.57			
11	8.88		8.89	8.83	8.83		
12	9.05	9.06	9.06	8.99	8.99		
13	9.46		9.47	9.40	9.41		
14	9.77	9.79	9.78	9.71	9.71		
15	10.27	10.29	10.29	10.21	10.21		
16	10.64	10.65	10.66	10.59	10.59		
17				10.96	10.95	10.95	
18	11.01		11.02	11.02	11.03		
19	11.09		11.10				
20	11.98		11.99	11.94	11.93	11.93	
21	12.39		12.39	12.33	12.33	12.33	
22			12.77	12.71	12.69		
23	12.92			12.94	12.93		
24	12.99		13.00	13.09	13.09	13.10	
25	13.14		13.16				
26						13.24	
27	13.49		13.49	13.44	13.44		
28	13.58		13.61	13.54	13.54	13.51	13.52
29				13.67		13.68	
30			14.08	14.03	14.03	14.03	14.02
31			14.30	14.26	14.24	14.24	14.25
32					14.39	14.36	
33			14.49	14.46	14.46		
34						14.56	14.56
35					15.10	15.10	
36			15.38	15.33	15.32	15.32	

[a]GC operating conditions are given in Table 3. All retention times in minutes.
[b]The peaks listed in this table are sequentially numbered in elution order for illustrative purposes only and are not isomer numbers.

TABLE 5
(continued)

Peak No.[b]	Aroclor 1016	Aroclor 1221	Aroclor 1232	Aroclor 1242	Aroclor 1248	Aroclor 1254	Aroclor 1260
37			15.65	15.62	15.62	15.61	16.61
38			15.78	15.74	15.74	15.74	15.79
39			16.13	16.10	16.10	16.08	
40							16.19
41						16.34	16.34
42						16.44	16.45
43						16.55	
44			16.77	16.73	16.74	16.77	16.77
45			17.13	17.09	17.07	17.07	17.08
46						17.29	17.31
47				17.46	17.44	17.43	17.43
48				17.69	17.69	17.68	17.68
49					18.19	18.17	18.18
50				18.48	18.49	18.42	18.40
51						18.59	
52						18.86	18.86
53				19.13	19.13	19.10	19.09
54						19.42	19.43
55						19.55	19.59
56						20.20	20.21
57						20.34	
58							20.43
59					20.57	20.55	
60						20.62	20.66
61						20.88	20.87
62							21.03
63						21.53	21.53
64						21.83	21.81
65						23.31	23.27
66							23.85
67							24.11
68							24.46
69							24.59
70							24.87
71							25.85
72							27.05
73							27.72

[a] GC operating conditions are given in Table 3. All retention times in minutes.
[b] The peaks listed in this table are sequentially numbered in elution order for illustrative purposes only and are not isomer numbers.

TABLE 6

PEAKS DIAGNOSTIC OF PCBs OBSERVED ON 0.53 mm ID COLUMN
DURING SINGLE COLUMN ANALYSIS

Peak No.[a]	RT on DB-608[b]	RT on DB-1701[b]	Aroclor[c]
I	4.90	4.66	1221
II	7.15	6.96	1221, 1232, 1248
III	7.89	7.65	1061, 1221, 1232, 1242
IV	9.38	9.00	1016, 1232, 1242, 1248
V	10.69	10.54	1016, 1232, 1242
VI	14.24	14.12	1248, 1254
VII	14.81	14.77	1254
VIII	16.71	16.38	1254
IX	19.27	18.95	1254, 1260
X	21.22	21.23	1260
XI	22.89	22.46	1260

[a] Peaks are sequentially numbered in elution order and are not isomer numbers

[b] Temperature program: T_i = 150°C, hold 30 seconds; 5°C/minute to 275°C.

[c] Underline indicates largest peak in the pattern for that Aroclor

TABLE 7

SPECIFIC PCB CONGENERS IN AROCLORS

Congener	IUPAC number	Aroclor 1016	1221	1232	1242	1248	1254	1260
Biphenyl	--		X					
2-CB	1	X	X	X	X			
23-DCB	5	X	X	X	X	X		
34-DCB	12	X		X	X	X		
244'-TCB	28*	X		X	X	X	X	
22'35'-TCB	44			X	X	X	X	X
23'44'-TCB	66*					X	X	X
233'4'6-PCB	110						X	
23'44'5-PCB	118*						X	X
22'44'55'-HCB	153							X
22'344'5'-HCB	138							X
22'344'55'-HpCB	180							X
22'33'44'5-HpCB	170							X

*Apparent co-elution of: 28 with 31 (2,4',5-trichlorobiphenyl)
66 with 95 (2,2',3,5',6-pentachlorobiphenyl)
118 with 149 (2,2',3,4',5',6-hexachlorobiphenyl)

TABLE 8

RETENTION TIMES OF PCB CONGENERS
ON THE DB-5 WIDE-BORE COLUMN

IUPAC #	Retention Time (min)	
1	6.52	
5	10.07	
18	11.62	
31	13.43	
52	14.75	
44	15.51	
66	17.20	
101	18.08	
87	19.11	
110	19.45	
151	19.87	
153	21.30	
138	21.79	
141	22.34	
187	22.89	
183	23.09	
180	24.87	
170	25.93	
206	30.70	
209	32.63	(internal standard)

TABLE 9

SINGLE LABORATORY RECOVERY DATA FOR EXTRACTION OF PCBs FROM CLAY AND SOIL BY METHOD 3541[a] (AUTOMATED SOXHLET)

Matrix	Aroclor	Spike Level (ppm)	Trial	Percent Recovery[b]
Clay	1254	5	1	87.0
			2	92.7
			3	93.8
			4	98.6
			5	79.4
			6	28.3
Clay	1254	50	1	65.3
			2	72.6
			3	97.2
			4	79.6
			5	49.8
			6	59.1
Clay	1260	5	1	87.3
			2	74.6
			3	60.8
			4	93.8
			5	96.9
			6	113.1
Clay	1260	50	1	73.5
			2	70.1
			3	92.4
			4	88.9
			5	90.2
			6	67.3

TABLE 9
(continued)

Matrix	Aroclor	Spike Level (ppm)	Trial	Percent Recovery[b]
Soil	1254	5	1	69.7
			2	89.1
			3	91.8
			4	83.2
			5	62.5
Soil	1254	50	1	84.0
			2	77.5
			3	91.8
			4	66.5
			5	82.3
			6	61.6
Soil	1260	5	1	83.9
			2	82.8
			3	81.6
			4	96.2
			5	93.7
			6	93.8
			7	97.5
Soil	1260	50	1	76.9
			2	69.4
			3	92.6
			4	81.6
			5	83.1
			6	76.0

[a] The operating conditions for the automated Soxhlet

Immersion time: 60 min
Reflux time: 60 min

[b] Multiple results from two different extractors

Data from Reference 9

TABLE 10

MULTIPLE LABORATORY PRECISION AND ACCURACY DATA FOR THE EXTRACTION OF PCBs FROM SPIKED SOIL BY METHOD 3541 (AUTOMATED SOXHLET)

		Percent Recovery at Aroclor 1254 Spike Concentration (µg/kg)			Percent Recovery at Aroclor 1260 Spike Concentration (µg/kg)			Mean Recovery
		5	50	500	5	50	500	All Levels
Lab 1	n	3	3		3	3		12
	Mean	101.2	74.0		83.9	78.5		84.4
	S. D.	34.9	41.8		7.4	7.4		26.0
Lab 2	n		6	6		6	6	24
	Mean		56.5	66.9		70.1	74.5	67.0
	S. D.		7.0	15.4		14.5	10.3	13.3
Lab 3	n	3	3		3	3		12
	Mean	72.8	63.3		70.6	57.2		66.0
	S. D.	10.8	8.3		2.5	5.6		9.1
Lab 4	n	6	6		6	6		24
	Mean	112.6	144.3		100.3	84.8		110.5
	S. D.	18.2	30.4		13.3	3.8		28.5
Lab 5	n		3	3		3	3	12
	Mean		97.1	80.1		79.5	77.0	83.5
	S. D.		8.7	5.1		3.1	9.4	10.3
Lab 6	n	2	3		3	4		12
	Mean	140.9	127.7		138.7	105.9		125.4
	S. D.	4.3	15.5		15.5	7.9		18.4
Lab 7	n	3	3		3	3		12
	Mean	100.1	123.4		82.1	94.1		99.9
	S. D.	17.9	14.6		7.9	5.2		19.0
Lab 8	n	3	3		3	3		12
	Mean	65.0	38.3		92.8	51.9		62.0
	S. D.	16.0	21.9		36.5	12.8		29.1
All Labs	n	20	30	9	21	31	9	120
	Mean	98.8	92.5	71.3	95.5	78.6	75.3	87.6
	S. D.	28.7	42.9	14.1	25.3	18.0	9.5	29.7

Data from Reference 7

TABLE 11

PERCENT RECOVERY (BIAS) OF PCBs IN VARIOUS SOILS USING SFE METHOD 3562

PCB No. [a]	EC-1[c] Dump Site Soil Low #1	SRM 1941 Marine Sediment Low #2	EC-5[c] Lake Sediment Low #3	CRM 481[b] European Soil High #1	Mich Bay Saginaw Sediment High #2	CRM 392 Sewage Sludge High #3	SRM 2974 Fish Tissue Mussel Low #4	Congener Mean
28	148.4	63.3	147.7	67.3	114.7	89.2	101.7	104.6
52	88.5	106.6	115.8	84.5	111.1	96.2	131.4	104.9
101	93.3	91.2	100.2	84.5	111.5	93.9	133.2	101.1
149	92.6	105.1	101.5	73.2	111.2		69.4	92.2
118	89.9	66.1	108.9	82.1	110.8	73.5	82.7	87.7
153	90.8	65.1	95.1	82.8	118.6	97.3	107.5	94.0
105 [c]	89.1	72.6	96.6	83.4	111.8		79.4	88.8
138	90.1	57.4	97.9	76.9	126.9		73.1	87.1
128	90.8	69.9	101.2	65.9	87.6		62.5	79.7
156 [c]	90.6	88.9	94.3	85.2	101.1		59.3	86.6
180	92.4	142.4	93.3	82.2	109.2	100.5	65.7	98.0
170	91.3	101.1	95.2	80.5			33.0	81.8
Matrix Mean	95.7	85.8	104.0	79.0	108.7	91.8	83.2	92.2

[a] Congeners which are either certified or have had Soxhlet confirmation

[b] Parts per million (µg/g)

[c] Congener 105 was not resolved from congener 132 and congener 156 was not resolved from congener 171 by the GC method used for samples EC-1 and EC-5

TABLE 12

% RELATIVE STANDARD DEVIATION (PRECISION) OF PCBs USING SFE METHOD 3562

PCB No. [a]	EC-1[c] Dump Site Soil Low #1	SRM 1941 Marine Sediment Low #2	EC-5[c] Lake Sediment Low #3	CRM 481[b] European Soil High #1	Mich Bay Saginaw Sediment High #2	CRM 392 Sewage Sludge High #3	SRM 2974 Fish Tissue Mussel Low #4	Congener Mean
28	11.5	1.5	3.8	5.6	2.4	1.9	2.7	4.2
52	9.1	3.3	3.9	5.4	2.2	2.9	3.1	4.3
101	9.1	2.9	2.8	4.9	1.4	5.2	2.9	4.2
149	7.1	0.7	3.8	3.9	3.4		2.2	3.0
118	9.8	1.9	4.5	5.4	2.0	3.3	2.4	4.2
153	8.4	1.5	3.0	4.3	4.3	9.5	3.0	4.9
105 [c]	6.6	3.7	2.7	4.3	2.7		2.5	3.2
138	9.2	1.8	3.1	4.7	2.3		2.9	3.4
128	6.0	5.3	3.3	4.9	2.8		3.3	3.7
156 [c]	8.3	0.0	5.1	4.5	1.9		3.8	3.4
180	8.0	1.3	3.6	4.3	3.1	9.6	2.7	4.7
170	5.7	2.3	3.6	3.9	2.3		4.0	3.1
Matrix Mean	8.2	2.2	3.6	4.7	2.6	2.7	3.0	3.8

[a] Congeners which are either certified or have had Soxhlet confirmation.
[b] Parts per million (mg/kg)
[c] Congener 105 was not resolved from congener 132 and congener 156 was not resolved from congener 171 by the GC method used for samples EC-1 and EC-5.

TABLE 13

METHOD DETECTION LIMITS (MDLs)[a] OF PCBs USING SFE METHOD 3562

PCB No. [a]	EC-1[c] Dump Site Soil Low #1	SRM 1941 Marine Sediment Low #2	EC-5[c] Lake Sediment Low #3	CRM 481[b] European Soil High #1	Mich Bay Saginaw Sediment High #2	CRM 392 Sewage Sludge High #3	SRM 2974 Fish Tissue Mussel Low #4	Congener Mean
28	13.2	0.5	0.6	n/a	15.4	5.3	5.0	6.6
52	22.3	0.6	1.9	n/a	29.9	6.9	9.1	11.8
101	23.9	0.9	1.9	n/a	3.3	20.6	9.7	10.1
149	7.1	0.7	3.8	n/a	3.7		4.1	3.2
118	9.8	1.9	4.5	n/a	3.3	7.5	6.9	5.7
153	8.4	1.5	3.0	n/a	3.5	97.3	9.4	20.5
105 [d]	6.6	3.7	2.7	n/a	2.6		3.1	3.1
138	9.2	1.8	3.1	n/a	1.7		7.2	3.8
128	6.0	5.3	3.3	n/a	0.5		0.6	2.6
156 [d]	8.3	0.0	5.1	n/a	0.2		0.6	2.4
180	8.0	1.3	3.6	n/a	1.9	94.5	0.9	18.4
170	5.7	2.3	3.6	n/a	0.6		3.1	2.6
Matrix Mean	10.7	1.7	3.1	n/a	5.6	19.3	5.0	7.6

[a] MDLs are highly matrix-dependant. MDLs provided in SW-846 are for guidance purposes and may not always be achievable. Labs should establish their own in-house MDLs to document method performance.

[b] Congeners which are either certified or have had Soxhlet confirmation.

[c] Parts per million (mg/kg), THEREFORE LOW MDL IS NOT APPROPRIATE - Use mean µg/kg value.

[d] Congener 105 was not resolved from congener 132 and congener 156 was not resolved from congener 171 by the GC method used for samples EC-1 and EC-5.

TABLE 14

SINGLE LABORATORY RECOVERY DATA FOR SPE (METHOD 3535)
OF AROCLOR 1254 FROM WASTEWATER MATRICES SPIKED AT 2 µg/L

Wastewater Type	Mean Conc. (µg/L)	Percent Recovery	Std. Dev. (µg/L)	RSD (%)
Chemical Industry	2.39	120	0.41	17.2
Chemical Industry	0.56	28	0.03	5.4
Paper Industry	3.00	150	0.56	18.5
Paper Industry	2.30	115	0.08	3.7
Pharmaceutical Industry	1.52	76	0.03	1.7
Pharmaceutical Industry	1.02	51	0.03	2.9
Refuse	0.54	27	0.04	6.7
Refuse	0.63	31	0.10	16.0
POTW	1.92	96	0.15	7.8
POTW	2.10	105	0.04	1.8

Results represent three replicate solid-phase extractions of spiked wastewaters. Two different wastewaters from each wastewater type were spiked. All extractions were performed using 90-mm C_{18} extraction disks.

TABLE 15

SINGLE LABORATORY RECOVERY DATA FOR SPE (METHOD 3535)
OF AROCLOR 1254 FROM WASTEWATER MATRICES SPIKED AT 10 µg/L

Wastewater Type	Mean Conc. (µg/L)	Percent Recovery	Std. Dev. (µg/L)	RSD (%)
Chemical Industry	8.75	88	1.07	12.2
Chemical Industry	8.08	81	0.06	0.7
Paper Industry	8.88	889	0.71	7.9
Paper Industry	10.14	101	0.15	1.4
Pharmaceutical Industry	9.19	92	0.24	2.6
Pharmaceutical Industry	8.42	84	0.17	2.0
Refuse	8.80	88	0.49	5.6
Refuse	8.00	80	1.44	18.0
POTW	9.52	82	0.17	2.1
POTW	8.18	82	0.17	2.1

Results represent three replicate solid-phase extractions of spiked wastewaters. Two different wastewaters from each wastewater type were spiked. All extractions were performed using 90-mm C_{18} extraction disks.

TABLE 16

SINGLE LABORATORY RECOVERY DATA SPE (METHOD 3535)
OF AROCLOR 1254 FROM WASTEWATER MATRICES SPIKED AT 100 µg/L

Wastewater Type	Mean Conc. (µg/L)	Percent Recovery	Std. Dev. (µg/L)	RSD (%)
Chemical Industry	81.72	82	1.46	1.8
Chemical Industry	89.71	90	0.66	0.7
Paper Industry	73.73	74	3.94	5.3
Paper Industry	95.26	95	1.89	2.0
Pharmaceutical Industry	86.41	86	1.95	2.3
Pharmaceutical Industry	79.16	79	3.92	4.9
Refuse	85.70	86	1.59	1.9
Refuse	71.50	72	1.61	2.2
POTW	87.76	88	1.76	2.0
POTW	80.59	81	0.40	0.5

Results represent three replicate solid-phase extractions of spiked wastewaters. Two different wastewaters from each wastewater type were spiked. All extractions were performed using 90-mm C_{18} extraction disks.

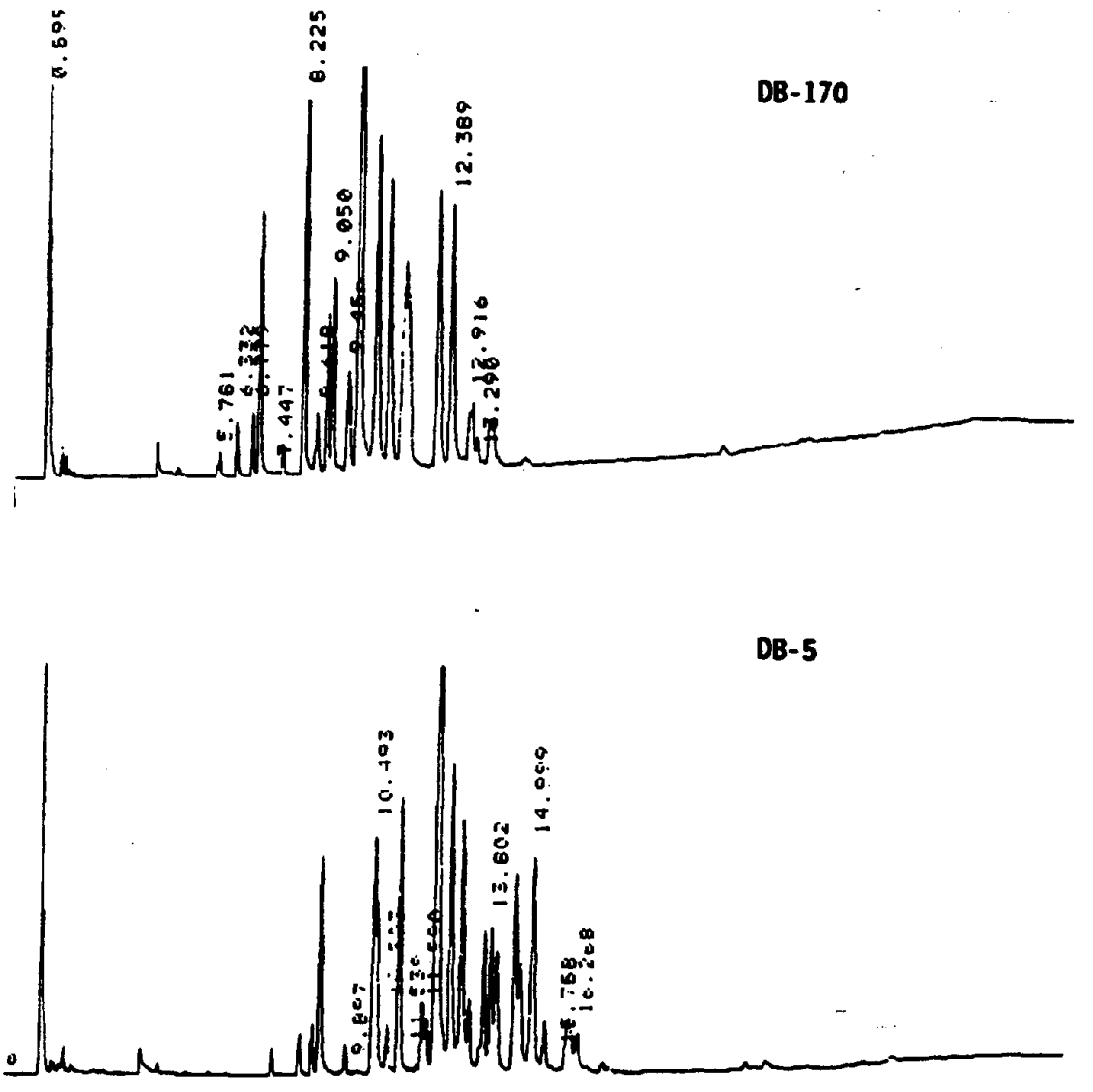


FIGURE 1. GC/ECD chromatogram of Aroclor 1016 analyzed on a DB-5/DB-1701 fused-silica open-tubular column pair. The GC operating conditions were as follows: 30 m x 0.53 mm ID DB-5 (1.5-µm film thickness) and 30 m x 0.53 mm ID DB-1701 (1.0-µm film thickness) connected to a J&W Scientific press-fit Y-shaped inlet splitter. Temperature program: 150°C (0.5 min hold) to 190°C (2 min hold) at 12°C/min then to 275°C (10 min hold) at 4°C/min.

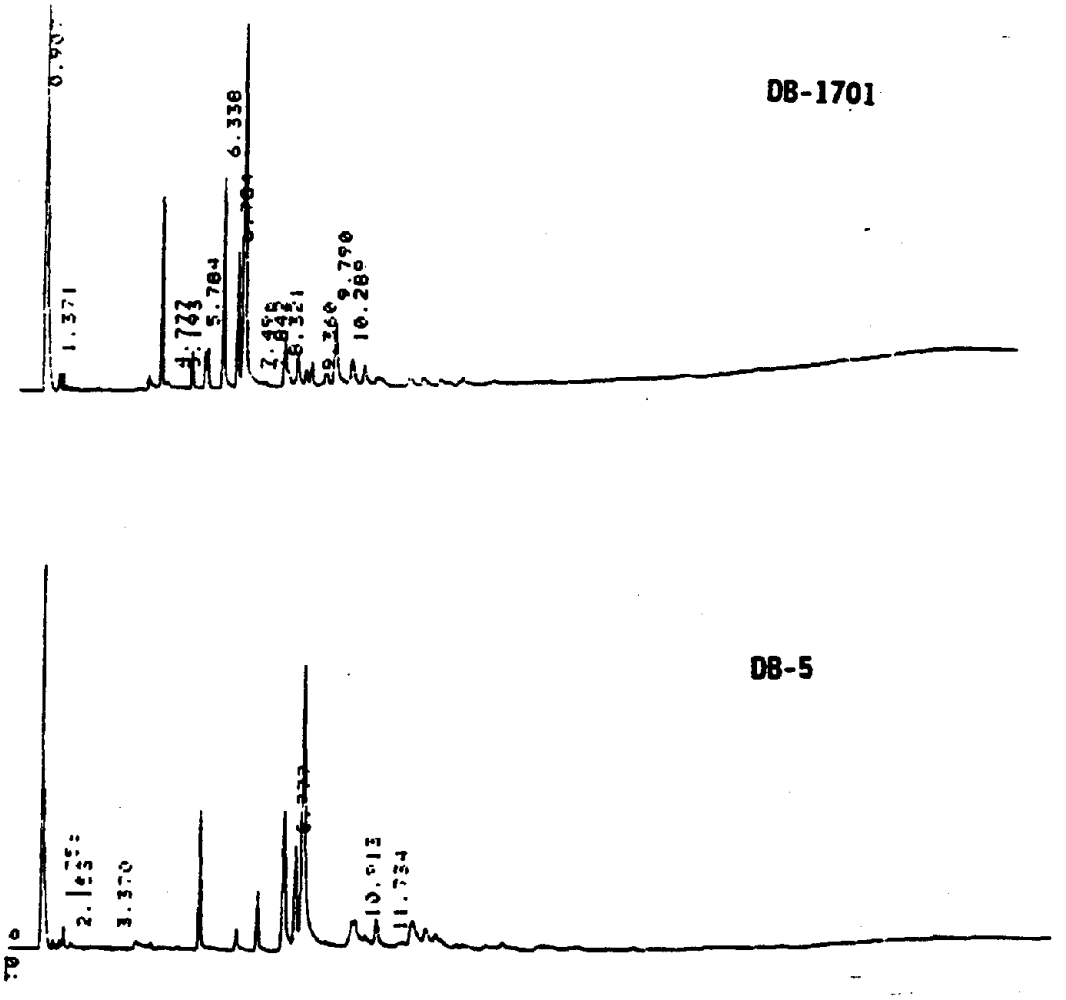


FIGURE 2. GC/ECD chromatogram of Aroclor 1221 analyzed on a DB-5/DB-1701 fused-silica open-tubular column pair. The GC operating conditions were as follows: 30 m x 0.53 mm ID DB-5 (1.5-µm film thickness) and 30 m x 0.53 mm ID DB-1701 (1.0-µm film thickness) connected to a J&W Scientific press-fit Y-shaped inlet splitter. Temperature program: 150°C (0.5 min hold) to 190°C (2 min hold) at 12°C/min then to 275°C (10 min hold) at 4°C/min.

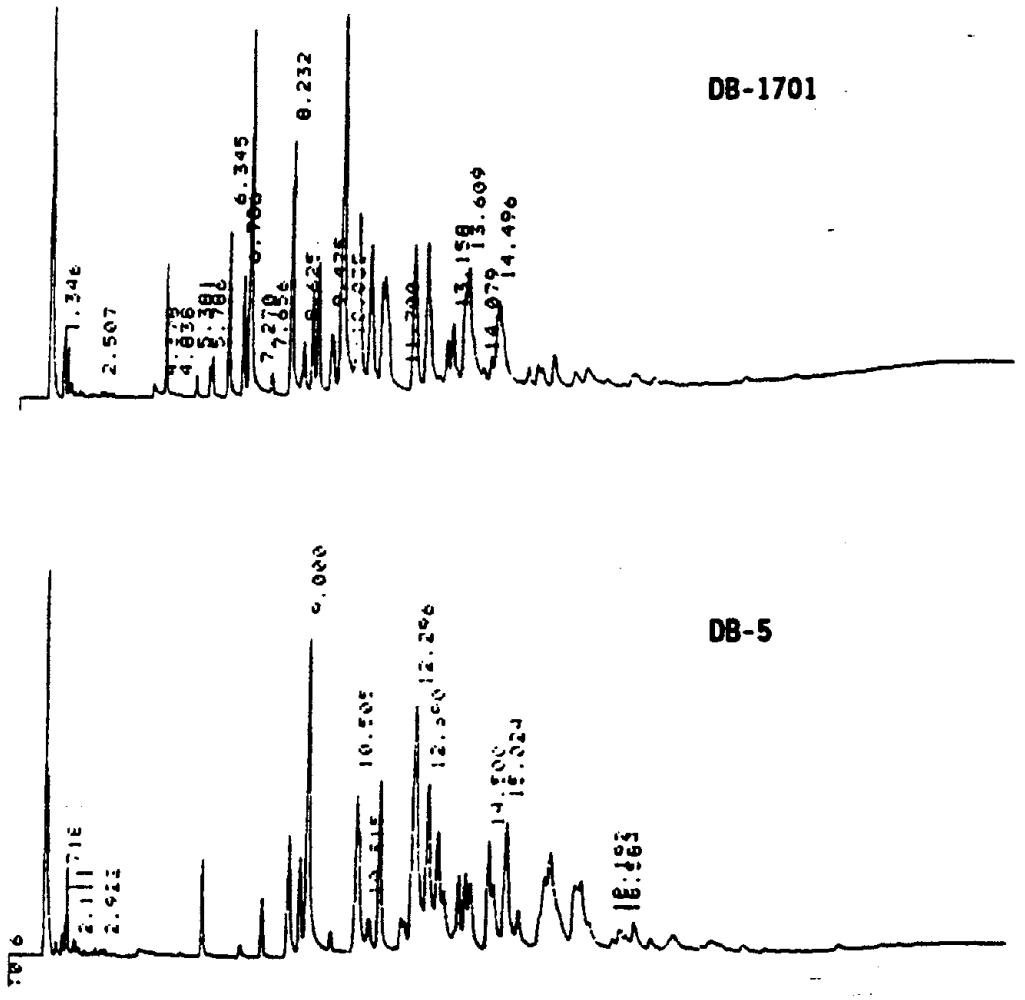


FIGURE 3. GC/ECD chromatogram of Aroclor 1232 analyzed on a DB-5/DB-1701 fused-silica open-tubular column pair. The GC operating conditions were as follows: 30 m x 0.53 mm ID DB-5 (1.5-µm film thickness) and 30 m x 0.53 mm ID DB-1701 (1.0-µm film thickness) connected to a J&W Scientific press-fit Y-shaped inlet splitter. Temperature program: 150°C (0.5 min hold) to 190°C (2 min hold) at 12°C/min then to 275°C (10 min hold) at 4°C/min.

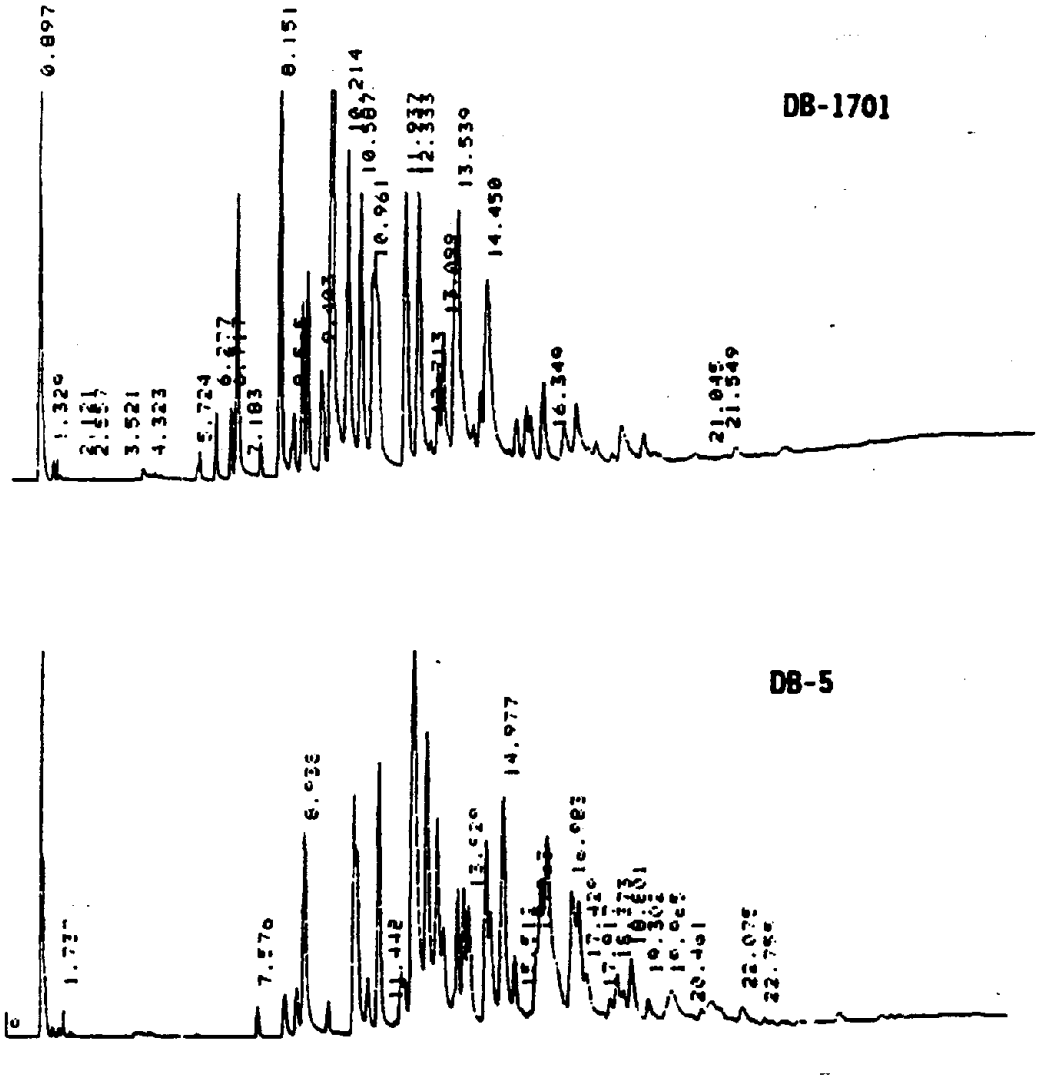


FIGURE 4. GC/ECD chromatogram of Aroclor 1242 analyzed on a DB-5/DB-1701 fused-silica open-tubular column pair. The GC operating conditions were as follows: 30 m x 0.53 mm ID DB-5 (1.5-µm film thickness) and 30 m x 0.53 mm ID DB-1701 (1.0-µm film thickness) connected to a J&W Scientific press-fit Y-shaped inlet splitter. Temperature program: 150°C (0.5 min hold) to 190°C (2 min hold) at 12°C/min then to 275°C (10 min hold) at 4°C/min.

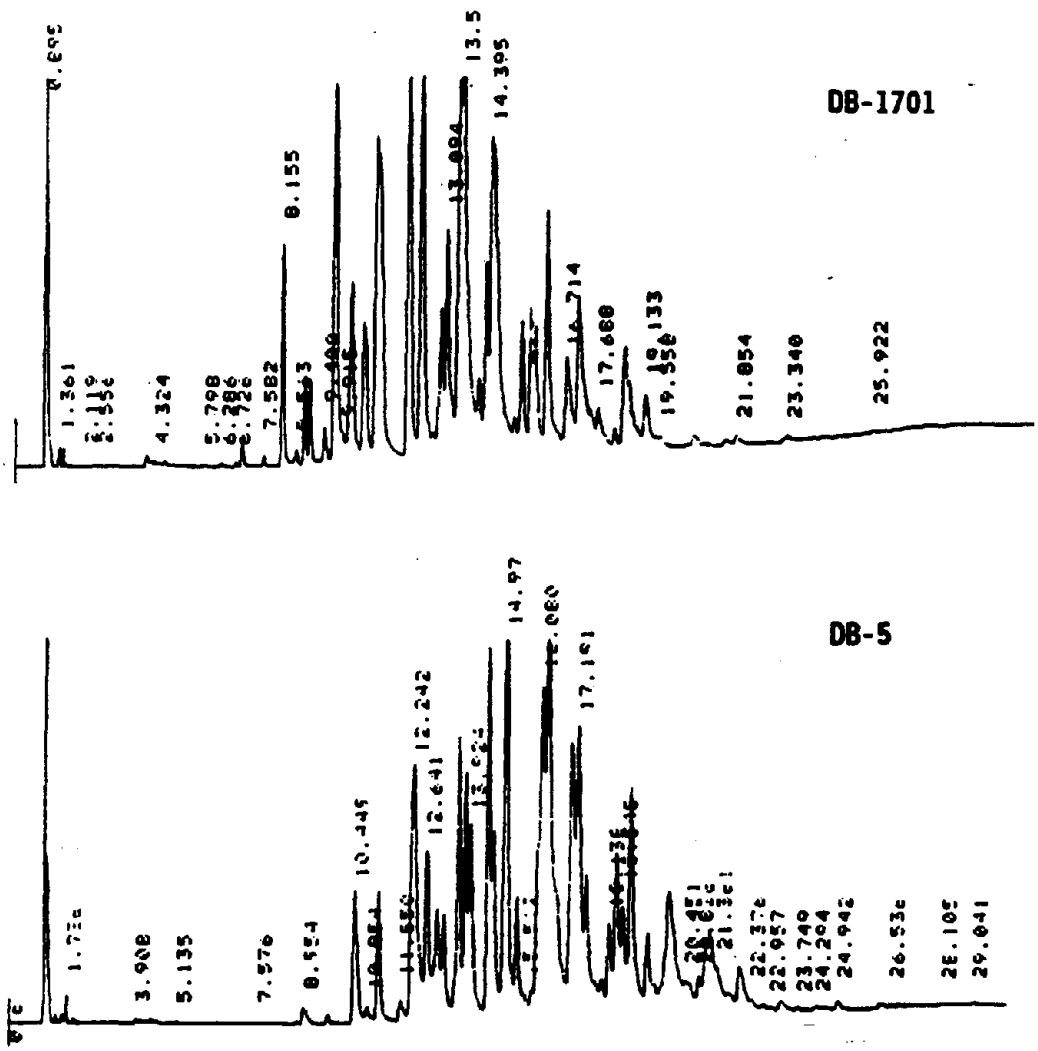


FIGURE 5. GC/ECD chromatogram of Aroclor 1248 analyzed on a DB-5/DB-1701 fused-silica open-tubular column pair. The GC operating conditions were as follows: 30 m x 0.53 mm ID DB-5 (1.5-µm film thickness) and 30 m x 0.53 mm ID DB-1701 (1.0-µm film thickness) connected to a J&W Scientific press-fit Y-shaped inlet splitter. Temperature program: 150°C (0.5 min hold) to 190°C (2 min hold) at 12°C/min then to 275°C (10 min hold) at 4°C/min.

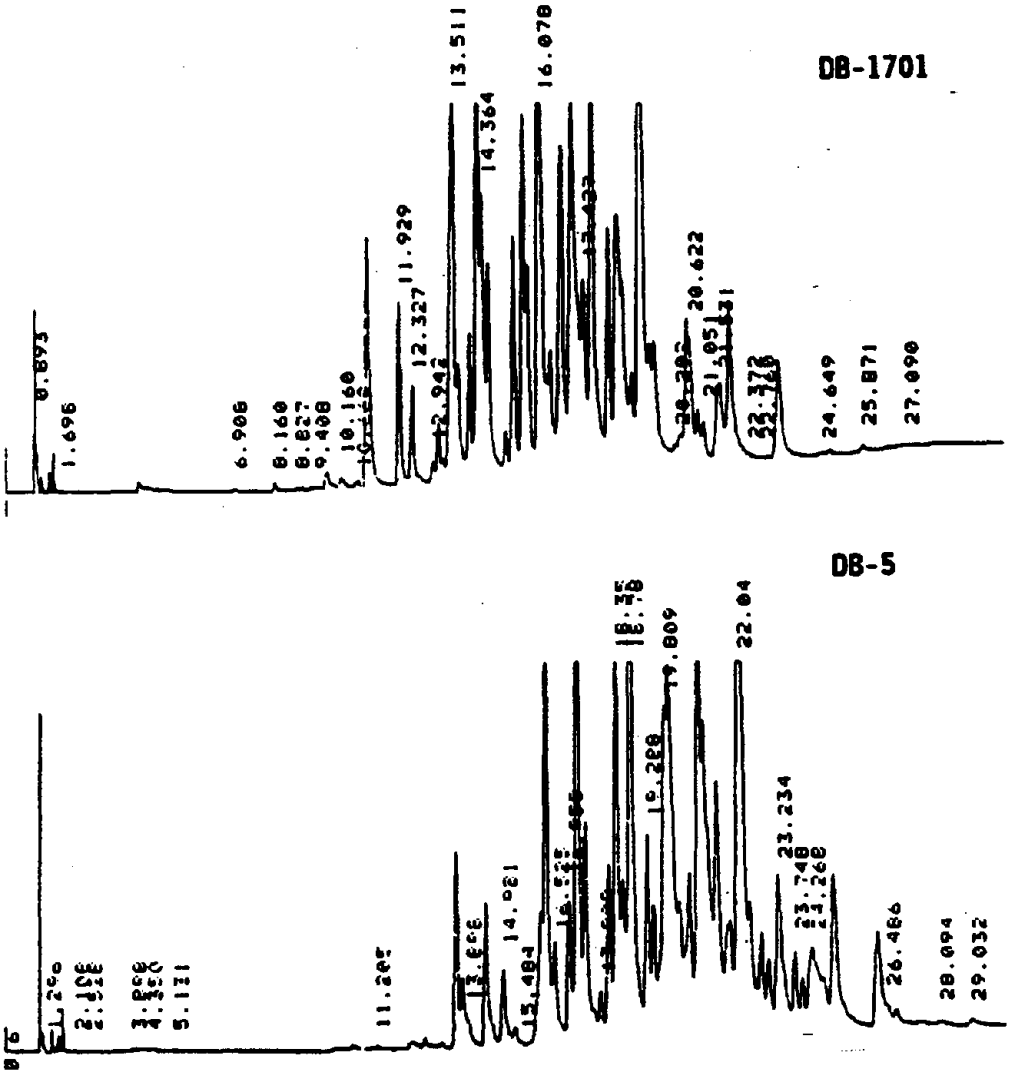


FIGURE 6. GC/ECD chromatogram of Aroclor 1254 analyzed on a DB-5/DB-1701 fused-silica open-tubular column pair. The GC operating conditions were as follows: 30 m x 0.53 mm ID DB-5 (1.5-µm film thickness) and 30 m x 0.53 mm ID DB-1701 (1.0-µm film thickness) connected to a J&W Scientific press-fit Y-shaped inlet splitter. Temperature program: 150°C (0.5 min hold) to 190°C (2 min hold) at 12°C/min then to 275°C (10 min hold) at 4°C/min.

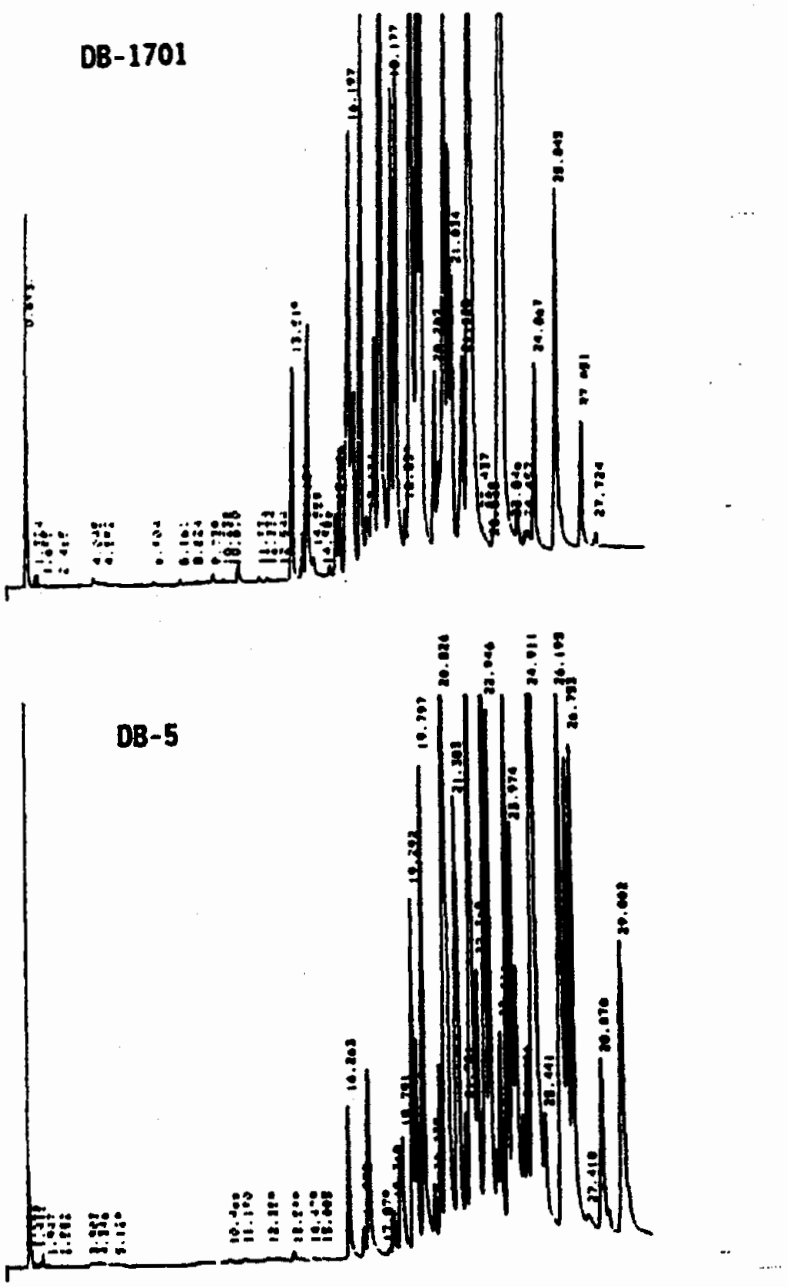
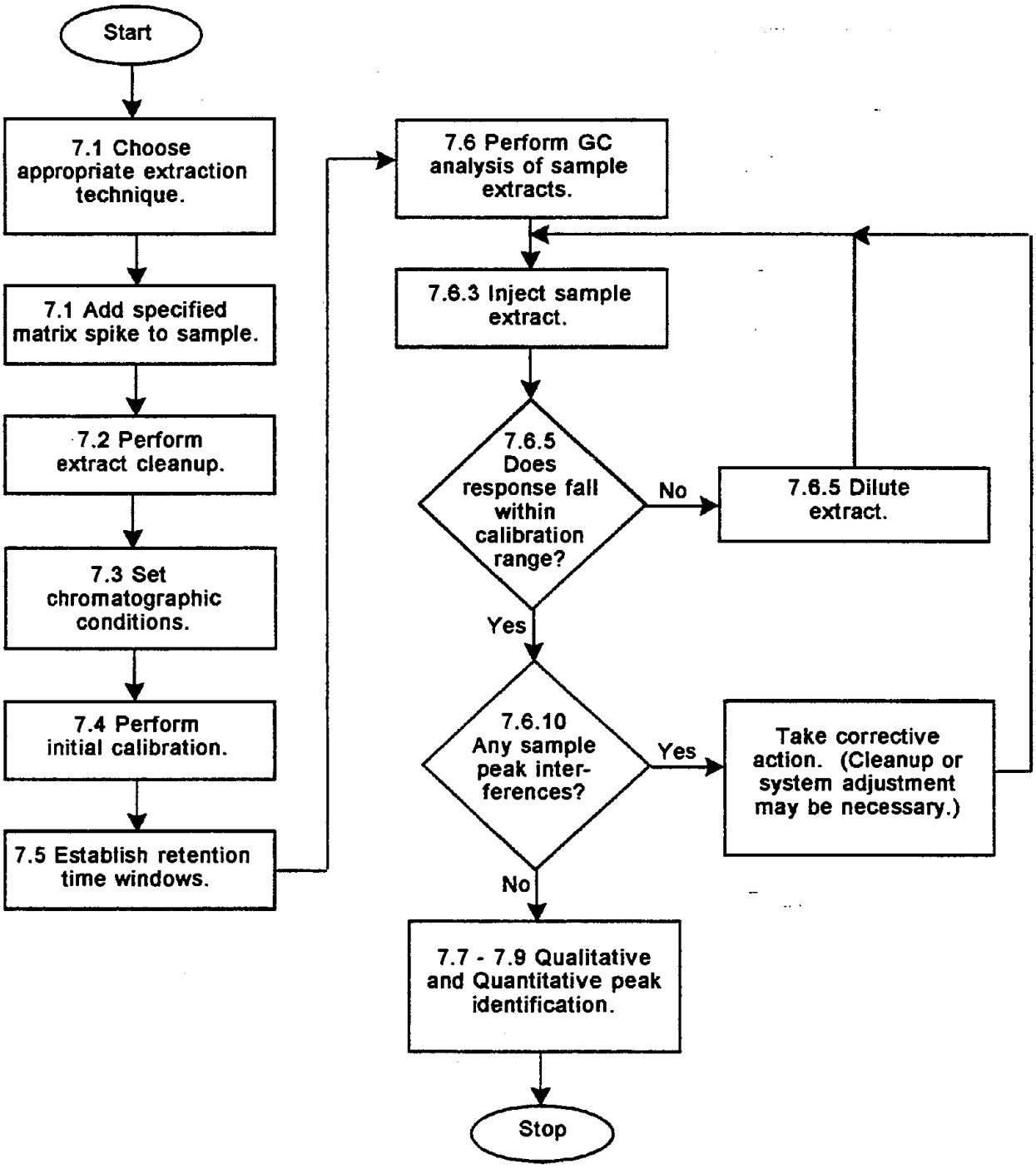


FIGURE 7. GC/ECD chromatogram of Aroclor 1260 analyzed on a DB-5/DB-1701 fused-silica open-tubular column pair. The GC operating conditions were as follows: 30 m x 0.53 mm ID DB-5 (1.5-µm film thickness) and 30 m x 0.53 mm ID DB-1701 (1.0-µm film thickness) connected to a J&W Scientific press-fit Y-shaped inlet splitter. Temperature program: 150°C (0.5 min hold) to 190°C (2 min hold) at 12°C/min then to 275°C (10 min hold) at 4°C/min.


Start
7.1 Choose appropriate extraction technique.
7.1 Add specified matrix spike to sample.
7.2 Perform extract cleanup.
7.3 Set chromatographic conditions.
7.4 Perform initial calibration.
7.5 Establish retention time windows.
7.6 Perform GC analysis of sample extracts.
7.6.3 Inject sample extract.
7.6.5 Does response fall within calibration range?
No
7.6.5 Dilute extract.
Yes
7.6.10 Any sample peak interferences?
Yes
Take corrective action. (Cleanup or system adjustment may be necessary.)
No
7.7 - 7.9 Qualitative and Quantitative peak identification.
Stop

METHOD 8141B

ORGANOPHOSPHORUS COMPOUNDS BY GAS CHROMATOGRAPHY

1.0 SCOPE AND APPLICATION

1.1 This method provides procedures for the gas chromatographic (GC) determination of organophosphorus (OP) compounds. The compounds listed in the table below can be determined by GC using capillary columns with a flame photometric detector (FPD) or a nitrogen-phosphorus detector (NPD). Triazine herbicides can also be determined with this method when the NPD is used. Although performance data are presented for each of the listed chemicals, it is unlikely that all of them could be determined in a single analysis. This limitation results because the chemical and chromatographic behavior of many of these chemicals can result in co-elution. The analyst must select columns, detectors, and calibration procedures for the specific analytes of interest. Any listed chemical is a potential method interference when it is not a target analyte.

Analyte	CAS Registry No.
Organophosphorus Pesticides	
Aspon[b]	3244-90-4
Azinphos-methyl	86-50-0
Azinphos-ethyl[a]	2642-71-9
Bolstar (Sulprofos)	35400-43-2
Carbophenothion[a]	786-19-6
Chlorfenvinphos[a]	470-90-6
Chlorpyrifos	2921-88-2
Chlorpyrifos methyl[a]	5598-13-0
Coumaphos	56-72-4
Crotoxyphos[a]	7700-17-6
Demeton-O[c]	8065-48-3
Demeton-S[c]	8065-48-3
Diazinon	333-41-5
Dichlorofenthion[a]	97-17-6
Dichlorvos (DDVP)	62-73-7
Dicrotophos[a]	141-66-2
Dimethoate	60-51-5
Dioxathion[a,c]	78-34-2
Disulfoton	298-04-4
EPN	2104-64-5
Ethion[a]	563-12-2
Ethoprop	13194-48-4
Famphur[a]	52-85-7

Analyte	CAS Registry No.
Fenitrothion[a]	122-14-5
Fensulfothion	115-90-2
Fenthion	55-38-9
Fonophos[a]	944-22-9
Leptophos[a,d]	21609-90-5
Malathion	121-75-5
Merphos[c]	150-50-5
Mevinphos[e]	7786-34-7
Monocrotophos	6923-22-4
Naled	300-76-5
Parathion, ethyl	56-38-2
Parathion, methyl	298-00-0
Phorate	298-02-2
Phosmet[a]	732-11-6
Phosphamidon[a]	13171-21-6
Ronnel	299-84-3
Stirophos (Tetrachlorvinphos)	22248-79-9
Sulfotepp	3689-24-5
Tetraethyl pyrophosphate (TEPP)[d]	107-49-3
Terbufos[a]	13071-79-9
Thionazin[a,b] (Zinophos)	297-97-2
Tokuthion[b] (Prothiofos)	34643-46-4
Trichlorfon[a]	52-68-6
Trichloronate[b]	327-98-0
Industrial Chemicals	
Hexamethyl phosphoramide[a] (HMPA)	680-31-9
Tri-o-cresyl phosphate[a,d] (TOCP)	78-30-8
Triazine Herbicides (NPD only)	
Atrazine[a]	1912-24-9
Simazine[a]	122-34-9

[a] This analyte has been evaluated using a 30-m column only (see Sec. 1.5).
[b] Production discontinued in the U.S., standard not readily available.
[c] Standards may have multiple components because of oxidation.
[d] Compound is extremely toxic or neurotoxic.
[e] Adjacent major/minor peaks can be observed due to cis/trans isomers.

1.2 A dual-column/dual-detector approach may be used for the analysis of relatively clean extracts. Two 15- or 30-m x 0.53-mm ID fused-silica, open-tubular columns of different polarities are connected to an injection tee and each is connected to a detector. Analysts are cautioned regarding the use of a dual column configuration when their instrument is subject to mechanical stress, when many samples are analyzed over a short time, or when extracts of contaminated samples are analyzed.

1.3 Two detectors can be used for the listed organophosphorus chemicals. The FPD works by measuring the emission of phosphorus- or sulfur-containing species. Detector performance is optimized by selecting the proper optical filter and adjusting the hydrogen and air flows to the flame. The NPD is a flame ionization detector with a rubidium ceramic flame tip which enhances the response of phosphorus- and nitrogen-containing analytes. The FPD is more sensitive and more selective, but is a less common detector in environmental laboratories.

1.4 Table 1 lists method detection limits (MDLs) for the target analytes, using 15-m columns and FPD, for water and soil matrices. Table 2 lists the estimated quantitation limits (EQLs) for other matrices. MDLs and EQLs using 30-m columns will be very similar to those obtained from 15-m columns, however, laboratories should determine in-house MDLs for the analytes of interest using the specific instrumentation employed for sample analysis.

1.5 The use of a 15-m column system has not been fully validated for the determination of all of the compounds listed in Sec. 1.1. The analyst must demonstrate chromatographic resolution of all analytes, recoveries of greater than 70 percent, with precision of no more than 15 percent RSD, before data generated on the 15-m column system can be reported for the following analytes, or any additional analytes:

Azinphos-ethyl	Phosphamidon	Dioxathion
Ethion	Chlorfenvinphos	Leptophos
Carbophenothion	HMPA	TOCP
Famphur	Terbufos	Phosmet

1.6 When Method 8141 is used to analyze unfamiliar samples, compound identifications should be supported by confirmatory analysis. Sec. 8.0 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

1.7 This method is restricted to use by, or under the supervision of, analysts experienced in the use of capillary gas chromatography and in the interpretation of chromatograms.

2.0 SUMMARY OF METHOD

2.1 Method 8141 provides gas chromatographic conditions for the determination of part per billion concentrations of organophosphorus compounds. Prior to the use of this method, appropriate sample preparation techniques must be used. Water samples are extracted at a neutral pH with methylene chloride by using a separatory funnel (Method 3510), a continuous liquid-liquid extractor (Method 3520), solid-phase extraction (Method 3535), or other appropriate technique. Solid samples are extracted using Soxhlet extraction (Method 3540) or automated Soxhlet extraction (Method 3541), using methylene chloride/acetone (1:1), pressurized fluid extraction (Method 3545), or other

appropriate technique. Both neat and diluted organic liquids (Method 3580, Waste Dilution) may be analyzed by direct injection.

2.2 Analysis is performed on a gas chromatograph with a flame photometric or nitrogen-phosphorus detector.

2.3 Organophosphorus esters and thioesters can hydrolyze under both acid and base conditions. Therefore, sample preparation procedures employing acid and base partitioning procedures are not appropriate for extracts to be analyzed by Method 8141.

2.4 Ultrasonic Extraction (Method 3550) is not an appropriate sample preparation method for Method 8141 because of the potential for destruction of target analytes during the ultrasonic extraction process and should not be used.

3.0 INTERFERENCES

3.1 Refer to Methods 3500, 3600, and 8000, as well as to Sec. 1.1.

3.2 The use of Florisil Cleanup (Method 3620) for some of the compounds in this method has been demonstrated to yield recoveries less than 85 percent and is therefore not recommended for all compounds. Refer to Table 2 of Method 3620 for recoveries of organophosphorus compounds. Use of an FPD often eliminates the need for sample cleanup. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each analyte is not less than 85 percent.

3.3 The use of gel permeation cleanup (GPC) (Method 3640) for extract cleanup has been demonstrated to yield recoveries of less than 85 percent for many method analytes because they elute before bis-(2-ethylhexyl) phthalate. Therefore Method 3640 is not recommended for use with this method, unless analytes of interest are listed in Method 3640 or are demonstrated to give greater than 85 percent recovery.

3.4 Use of a flame photometric detector in the phosphorus mode will minimize interferences from materials that do not contain phosphorus or sulfur. Elemental sulfur will interfere with the determination of certain organophosphorus compounds by flame photometric gas chromatography. If Method 3660 is used for sulfur cleanup, only the tetrabutylammonium (TBA)-sulfite option should be employed, since copper may destroy OP pesticides. The stability of each analyte must be tested to ensure that the recovery from the TBA-sulfite sulfur cleanup step is not less than 85 percent.

3.5 A halogen-specific detector (i.e., electrolytic conductivity or microcoulometry) is very selective for the halogen-containing compounds and may be used for the determination of Chlorpyrifos, Ronnel, Coumaphos, Tokuthion, Trichloronate, Dichlorvos, EPN, Naled, and Stirophos only. Many of the OP pesticides may also be detected by the electron capture detector (ECD), however, the ECD is not as specific as the NPD or FPD. The ECD should only be used when previous analyses have demonstrated that interferences will not adversely effect quantitation, and that the detector sensitivity is sufficient to meet project requirements..

3.6 Certain analytes will coelute, particularly on 15-m columns (Table 3). If coelution is observed, analysts should (1) select a second column of different polarity for confirmation, (2) use 30-m x 0.53-mm columns, or (3) use 0.25- or 0.32-mm ID columns. See Figures 1 through 4 for combinations of compounds that do not coelute on 15-m columns.

3.7 The following pairs coeluted on the DB-5/DB-210 30-m column pair:

GC Column	Coeluting pair
DB-5	Terbufos/tri-o-cresyl phosphate
	Naled/Simazine/Atrazine
	Dichlorofenthion/Demeton-O
	Trichloronate/Aspon
	Bolstar/Stirophos/Carbophenothion
	Phosphamidon/Crotoxyphos
	Fensulfothion/EPN
DB-210	Terbufos/tri-o-cresyl phosphate
	Dichlorofenthion/Phosphamidon
	Chlorpyrifos, methyl/Parathion, methyl
	Chlorpyrifos/Parathion, ethyl
	Aspon/Fenthion
	Demeton-O/Dimethoate
	Leptophos/Azinphos-methyl
	EPN/Phosmet
	Famphur/Carbophenothion

See Table 4 for the retention times of these compounds on 30-m columns.

3.8 Analytical difficulties encountered for target analytes

3.8.1 Tetraethyl pyrophosphate (TEPP) is an unstable diphosphate which is readily hydrolyzed in water and is thermally labile (decomposes at 170°C). Care must be taken to minimize loss during GC analysis and during sample preparation. Identification of bad standard lots is difficult since the electron impact (EI) mass spectrum of TEPP is nearly identical to its major breakdown product, triethyl phosphate.

3.8.2 The water solubility of Dichlorvos (DDVP) is 10 g/L at 20°C, and recovery is poor from aqueous solution.

3.8.3 Naled is converted to Dichlorvos (DDVP) on column by debromination. This reaction may also occur during sample preparation. The extent of debromination will depend on the nature of the matrix being analyzed. The analyst must consider the potential for debromination when Naled is to be determined.

3.8.4 Trichlorfon rearranges and is dehydrochlorinated in acidic, neutral, or basic media to form Dichlorvos (DDVP) and hydrochloric acid. If this method is to be used for the

determination of organophosphates in the presence of Trichlorfon, the analyst should be aware of the possibility of its rearrangement to Dichlorvos and the possibility of misidentification.

3.8.5 Demeton (Systox) is a mixture of two compounds; O,O-diethyl O-[2-(ethylthio)ethyl]phosphorothioate (Demeton-O) and O,O-diethyl S-[2-(ethylthio)ethyl]phosphorothioate (Demeton-S). Two peaks are observed in all the chromatograms corresponding to these two isomers. It is recommended that the early eluting compound (Demeton-S) be used for quantitation.

3.8.6 Dioxathion is a single-component pesticide. However, several extra peaks are observed in the chromatograms of standards. These peaks appear to be the result of spontaneous oxygen-sulfur isomerization. Because of this, Dioxathion is not included in composite standard mixtures.

3.8.7 Merphos (tributyl phosphorotrithioite) is a single-component pesticide that is readily oxidized to its phosphorotrithioate (Merphos oxone). Chromatographic analysis of Merphos almost always results two peaks (unoxidized Merphos elutes first). As the relative amounts of oxidation of the sample and the standard are probably different, quantitation based on the sum of both peaks may be most appropriate.

3.8.8 Retention times of some analytes, particularly Monocrotophos, may increase with increasing concentrations in the injector. Analysts should check for retention time shifts in highly-contaminated samples.

3.8.9 Many analytes will degrade on reactive sites in the chromatographic system. Analysts must ensure that injectors and splitters are free from contamination and are silanized. Columns should be installed and maintained properly.

3.8.10 Performance of chromatographic systems will degrade with time. Column resolution, analyte breakdown and baselines may be improved by column washing (Sec. 7). Oxidation of columns is not reversible.

3.9 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts or elevated baselines in gas chromatograms. All these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing reagent blanks (Sec. 8.0).

3.10 NP Detector interferences - Triazine herbicides, such as atrazine and simazine, and other nitrogen-containing compounds may interfere.

4.0 APPARATUS AND MATERIALS

4.1 Gas chromatograph

An analytical system complete with a gas chromatograph suitable for on-column or split/splitless injection, and all required accessories, including syringes, analytical columns, gases, suitable detector(s), and a recording device. The analyst should select the detector for the specific measurement application, either the flame photometric detector or the nitrogen-phosphorus detector. A data system for measuring peak areas and dual display of chromatograms is highly recommended.

4.2 GC columns

This method employs capillary columns (0.53-mm, 0.32-mm, or 0.25-mm ID and 15-m or 30-m length, depending on the resolution required). Columns of 0.53-mm ID are recommended for most environmental and waste analysis applications. Dual-column, single-injector analysis requires columns of equal length and bore. See Sec. 3.0 and Figures 1 through 4 for guidance on selecting the proper length and diameter for the column(s). Four columns are recommended.

4.2.1 Column 1 - 15-m or 30-m x 0.53-mm wide-bore capillary column, 1.0-µm film thickness, chemically bonded with 50% trifluoropropyl polysiloxane, 50% methyl polysiloxane (DB-210), or equivalent.

4.2.2 Column 2 - 15-m or 30-m x 0.53-mm wide-bore capillary column, 0.83-µm film thickness, chemically bonded with 35% phenyl methyl polysiloxane (DB-608, SPB-608, RTx-35), or equivalent.

4.2.3 Column 3 - 15-m or 30-m x 0.53-mm wide-bore capillary column, 1.0 µm film thickness, chemically bonded with 5% phenyl polysiloxane, 95% methyl polysiloxane (DB-5, SPB-5, RTx-5), or equivalent.

4.2.4 Column 4 - 15- or 30-m x 0.53-mm ID wide-bore capillary column, chemically bonded with methyl polysiloxane (DB-1, SPB-1, or equivalent), 1.0-µm or 1.5-µm film thickness.

4.2.5 Column rinsing kit (optional) - Bonded-phase column rinse kit (J&W Scientific, catalog no. 430-3000, or equivalent).

4.3 Splitter - If a dual-column, single-injector configuration is used, the open tubular columns should be connected to one of the following splitters, or equivalent:

4.3.1 Splitter 1 - J&W Scientific press-fit Y-shaped glass 3-way union splitter (J&W Scientific, catalog no. 705-0733).

4.3.2 Splitter 2 - Supelco 8-in glass injection tee, deactivated (Supelco, catalog no. 2-3665M).

4.3.3 Splitter 3 - Restek Y-shaped fused-silica connector (Restek, catalog no. 20405).

4.4 Injectors

4.4.1 Packed column, 1/4-in injector port with hourglass liner are recommended for 0.53-mm column. These injector ports can be fitted with splitters (Sec. 4.3) for dual-column analysis.

4.4.2 Split/splitless capillary injectors operated in the split mode are required for 0.25-mm and 0.32-mm columns.

4.5 Detectors

4.5.1 Flame Photometric Detector (FPD) operated in the phosphorus-specific mode is recommended.

4.5.2 Nitrogen-Phosphorus Detector (NPD) operated in the phosphorus-specific mode is less selective but can detect triazine herbicides.

4.5.3 Halogen-Specific Detectors (electrolytic conductivity or microcoulometry) may be used only for a limited number of halogenated or sulfur-containing analytes (Sec. 3.5).

4.5.4 Electron-capture detectors may be used for a limited number of analytes (Sec. 3.5).

4.6 Data system

4.6.1 A data system capable of presenting chromatograms, retention time, and peak integration data is strongly recommended.

4.6.2 Use of a data system that allows storage of raw chromatographic data is strongly recommended.

5.0 REAGENTS

5.1 Solvents - All solvents must be pesticide quality or equivalent.

5.1.1 Isooctane, $(CH_3)_3CCH_2CH(CH_3)_2$

5.1.2 Hexane, C_6H_{14}

5.1.3 Acetone, CH_3COCH_3

5.1.4 Tetrahydrofuran (THF), C_4H_8O - for triazine standards only.

5.1.5 Methyl *tert*-butyl-ether (MTBE), $CH_3Ot\text{-}C_4H_9$ -for triazine standards only.

5.2 Stock standard solutions (1000 mg/L) - May be prepared from pure standard materials or can be purchased as certified solutions.

5.2.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure compounds. Dissolve the compounds in suitable mixtures of acetone and hexane and dilute to volume in a 10-mL volumetric flask. If compound purity is 96 percent or greater, the weight may be used without correction to calculate the concentration of the stock standard solution. Commercially-prepared stock standard solutions may be used at any concentration if they are certified by the manufacturer or by an independent source.

5.2.2 Both Simazine and Atrazine have low solubilities in hexane. If standards of these compounds are required, Atrazine should be dissolved in MTBE, and Simazine should be dissolved in acetone/MTBE/THF (1:3:1).

5.2.3 Composite stock standard - This standard may be prepared from individual stock solutions. The analyst must demonstrate that the individual analytes and common oxidation products are resolved by the chromatographic system. For composite stock standards containing less than 25 components, take exactly 1 mL of each individual stock solution at 1000 mg/L, add solvent, and mix the solutions in a 25-mL volumetric flask. For example, for a composite containing 20 individual standards, the resulting concentration of

each component in the mixture, after the volume is adjusted to 25 mL, will be 40 mg/L. This composite solution can be further diluted to obtain the desired concentrations. Composite stock standards containing more than 25 components are not recommended.

5.2.4 Store the standard solutions (stock, composite, calibration, internal, and surrogate) at 4°C in PTFE-sealed containers in the dark. All standard solutions should be replaced after two months, or sooner if routine QC (Sec. 8.0) indicates a problem. Standards for easily hydrolyzed chemicals including TEPP, Methyl Parathion, and Merphos should be checked every 30 days.

5.2.5 It is recommended that lots of standards be subdivided and stored in small vials. Individual vials should be used as working standards to minimize the potential for contamination or hydrolysis of the entire lot.

5.3 Calibration standards should be prepared at a minimum of five concentrations by dilution of the composite stock standard with isooctane or hexane. The concentrations should correspond to the expected range of concentrations found in real samples and should bracket the linear range of the detector. Organophosphorus calibration standards should be replaced after one or two months, or sooner if comparison with check samples or historical data indicates that there is a problem. Laboratories may wish to prepare separate calibration solutions for the easily hydrolyzed standards identified above.

5.4 Internal standard

Internal standards should only be used on well-characterized samples by analysts experienced in the technique. Use of internal standards is complicated by coelution of some OP pesticides and by the differences in detector response to dissimilar chemicals. If internal standards are to be used, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences.

5.4.1 FPD response for organophosphorus compounds is enhanced by the presence of sulfur atoms bonded to the phosphorus atom. It has not been established that a thiophosphate can be used as an internal standard for an OP with a different numbers of sulfur atoms (e.g., phosphorothioates [P=S] as an internal standard for phosphates [PO_4]) or phosphorodithioates [$P=S_2$]).

5.4.2 When 15-m columns are used, it may be difficult to fully resolve internal standards from target analytes and interferences. The analyst must demonstrate that the measurement of the internal standard is not affected by method or matrix interferences.

5.4.3 1-bromo-2-nitrobenzene has been used as an NPD internal standard for a 30-m column pair. Prepare a solution of 1000 mg/L of 1-bromo-2-nitrobenzene. For spiking, dilute this solution to 5 mg/L. Use a spiking volume of 10 µL/mL of extract. The spiking concentration of the internal standards should be kept constant for all samples and calibration standards. Since its FPD response is small, 1-bromo-2-nitrobenzene is not an appropriate internal standard for that detector. No FPD internal standard is suggested.

5.5 Surrogates

The analyst should monitor the performance of the extraction, cleanup (when used), and analytical system, and the effectiveness of the method in dealing with each sample matrix, by spiking

each sample, standard, and blank with one or two surrogates (e.g., organophosphorus compounds not expected to be present in the sample). If multiple analytes are to be measured, two surrogates (an early and a late eluter) are recommended. Deuterated analogs of analytes are not appropriate surrogates for gas chromatographic/FPD/NPD analysis.

5.5.1 If surrogates are to be used, the analyst must select one or more compounds that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of a surrogate is not affected by method or matrix interferences. General guidance on the selection and use of surrogates is provided in Sec. 5.0 of Method 3500.

5.5.2 Tributyl phosphate and triphenyl phosphate are recommended as surrogates for either FPD and NPD analyses. A volume of 1.0 mL of a 1-µg/L spiking solution (containing 1 ng of surrogate) is added to each sample. If there is a co-elution problem with either of these compounds, 4-chloro-3-nitrobenzo-trifluoride has also been used as a surrogate for NPD analysis.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to Chapter Four, "Organic Analytes," Sec. 4.0.

6.2 Even with storage at 4°C and use of mercuric chloride as a preservative, most organophosphorus pesticides in groundwater samples collected for a national pesticide survey degraded within a 14-day period. Therefore, begin sample extraction within 7 days of collection.

6.3 Store extracts at 4°C and perform analyses within 40 days of extraction.

6.4 Organophosphorus esters will hydrolyze under acidic or basic conditions. Adjust samples to a pH of 5 to 8 using sodium hydroxide or sulfuric acid solution as soon as possible after sample collection. Record the volume used.

7.0 PROCEDURE

7.1 Extraction and cleanup

Refer to Chapter Two and Method 3500 for guidance on choosing the appropriate extraction procedure. In general, water samples are extracted at a neutral pH with methylene chloride, using Method 3510, 3520, 3535, or other appropriate technique. Solid samples are extracted using either Method 3540 or 3541 with methylene chloride/acetone (1:1 v/v) or hexane/acetone (1:1 v/v) as the extraction solvent, Method 3545, or other appropriate technique.

Method 3550 is not an appropriate extraction technique for the target analytes of this method (See Sec. 2.4).

Extraction and cleanup procedures that use solutions below pH 4 or above pH 8 are not appropriate for this method.

7.1.1 If required, the sample extracts may be cleaned up using Florisil column cleanup (Method 3620) and sulfur cleanup (Method 3660, TBA-sulfite option), which may have

particular application for organophosphorus pesticides. Gel permeation cleanup (Method 3640) should not generally be used for organophosphorus pesticides.

7.1.2 If sulfur cleanup by Method 3660 is required, do not use the copper technique, as the target analytes may be degraded in the presence of copper.

7.1.3 GPC may only be employed if all the target organophosphorus pesticides of interest are listed as analytes of Method 3640, or if the laboratory has demonstrated a recovery of greater than 85 percent for target organophosphorus pesticides at a concentration not greater than 5 times the levels of interest (e.g., the regulatory limit). Laboratories must retain data demonstrating acceptable recovery.

7.1.4 Prior to gas chromatographic analysis, the extract solvent may be exchanged to hexane. The analyst must ensure quantitative transfer of the extract concentrate. Single-laboratory data indicate that samples should not be transferred with 100-percent hexane during sample workup, as the more polar organophosphorus compounds may be lost. Transfer of organophosphorus esters is best accomplished using methylene chloride or a hexane/acetone solvent mixture.

7.1.5 Methylene chloride may be used as an injection solvent with both the FPD and the NPD.

NOTE: Follow manufacturer's instructions as to suitability of using methylene chloride with any specific detector.

7.2 Gas chromatographic conditions

Four different 0.53-mm ID capillary columns are suggested for the determination of organophosphates by this method. Column 1 (DB-210,or equivalent) and Column 2 (SPB-608,or equivalent) of 30-m lengths are recommended if a large number of organophosphorus analytes are to be determined. If superior chromatographic resolution is *not* required, 15-m columns may be appropriate.

7.2.1 Suggested operating conditions for 15-m columns are listed in Table 8. Suggested operating conditions for 30-m columns are listed in Table 9.

7.2.2 Retention times for analytes on each set of columns are presented in Tables 3 and 4. These data were developed using the operating conditions in Tables 8 and 9.

7.2.3 Establish the GC operating conditions appropriate for the column employed, using Tables 8 and 9 as guidance. Optimize the instrumental conditions for resolution of the target analytes and sensitivity.

NOTE: Once established, the same operating conditions must be used for both calibrations and sample analyses.

7.3 Calibration

Refer to Method 8000 for proper calibration techniques. Use Table 8 and Table 9 for establishing the proper operating parameters for the set of columns being employed in the analyses.

7.4 Gas chromatographic analysis

Method 8000 provides instructions on the analysis sequence, appropriate dilutions, establishing daily retention time windows and identification criteria.

7.4.1 Automated 1-µL injections are recommended. Manual injections of no more than 2 µL may be used if the analyst demonstrates quantitation precision of ≤ 10 percent relative standard deviation. The solvent flush technique may be used if the amount of solvent is kept at a minimum. If the internal standard calibration technique is used, add 10 µL of internal standard to each 1 mL of sample, prior to injection. Chromatograms of the target organophosphorus compounds are shown in Figures 1 through 4.

7.4.2 Figures 5 and 6 show chromatograms with and without Simazine, Atrazine, and Carbophenothion on 30-m columns.

7.5 Record the sample volume injected to the nearest 0.05 µL and the resulting peak sizes (in area units or peak heights). Using either the internal or external calibration procedure (Method 8000), determine the identity and quantity of each component peak in the sample chromatogram which corresponds to the compounds used for calibration purposes. See Method 8000 for calculations.

7.5.1 If peak detection and identification are prevented by the presence of interferences, the use of an FPD or further sample cleanup is required. Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to establish elution patterns and to determine recovery of target compounds.

7.5.2 If the responses exceed the linear range of the system, dilute the extract and reanalyze. It is recommended that extracts be diluted so that all peaks are on scale. Overlapping peaks are not always evident when peaks are off-scale. Computer reproduction of chromatograms, manipulated to ensure all peaks are on scale over a 100-fold range, are acceptable if linearity is demonstrated. Peak height measurements are recommended over peak area integration when overlapping peaks cause errors in area integration.

7.5.3 If the peak response is less than 2.5 times the baseline noise level, the validity of the quantitative result may be questionable. The analyst should consult with the source of the sample to determine whether further concentration of the sample extract is warranted.

7.5.4 If partially overlapping or coeluting peaks are found, change columns or try a GC/MS technique. Refer to Sec. 8.0 and Method 8270.

7.6 Suggested chromatograph maintenance

Corrective measures may require any one or more of the following remedial actions. Refer to Method 8000 for general information on the maintenance of capillary columns and injectors.

7.6.1 Splitter connections - For dual columns which are connected using a press-fit Y-shaped glass splitter or a Y-shaped fused-silica connector, clean and deactivate the splitter. Reattach the columns after cleanly cutting off at least one foot from the injection port side of the column using a capillary cutting tool or scribe. The accumulation of high boiling residues can change split ratios between dual columns and thereby change calibration factors.

7.6.2 Columns will be damaged permanently and irreversibly by contact with oxygen at elevated temperature. Oxygen can enter the column during a septum change, when oxygen traps are exhausted, through neoprene diaphragms of regulators, and through leaks in the gas manifold. Polar columns including the DB-210 and DB-608 are more prone to oxidation. Oxidized columns will exhibit baselines that rise rapidly during temperature programming.

7.6.3 Peak tailing for all components will be exacerbated by dirty injectors, pre-columns, and glass "Y"s. Additionally, cleaning of this equipment (or replacement/clipping, as appropriate) will greatly reduce the peak tailing. Components such as Fensulfothion, Naled, Azinphos-methyl, and Dimethoate are very good indicators of system performance.

7.7 Detector maintenance

7.7.1 Older FPDs may be susceptible to stray light in the photomultiplier tube compartment. This stray light will decrease the sensitivity and the linearity of the detector. Analysts can check for leaks by initiating an analysis in a dark room and turning on the lights. A shift in the baseline indicates that light may be leaking into the photomultiplier tube compartment. Additional shielding should be applied to eliminate light leaks and minimize stray light interference.

7.7.2 The bead of the NPD will become exhausted with time, which will decrease the sensitivity and the selectivity of the detector. The collector may become contaminated which decreased detector sensitivity.

7.7.3 Both types of detectors use a flame to generate a response. Flow rates of air and hydrogen should be optimized to give the most sensitive, linear detector response for target analytes.

7.8 GC/MS confirmation

7.8.1 GC/MS techniques should be judiciously employed to support qualitative identifications made with this method. Follow the GC/MS operating requirements described in Method 8270. GC/MS confirmation may be used in conjunction with either single-column or dual-column analysis if the concentration is sufficient for detection by GC/MS.

7.8.2 The GC/MS must be calibrated for the specific target pesticides when it is used for quantitative analysis. If GC/MS is used only for confirmation of the identification of the target analytes, then the analyst must demonstrate that those pesticides identified by GC/ECD can be confirmed by GC/MS. This demonstration may be accomplished by analyzing a single-point standard containing the analytes of interest at or below the concentrations reported in the GC/EC analysis.

7.8.3 GC/MS confirmation should be accomplished by analyzing the same extract that is used for GC analysis and the extract of the associated method blank.

7.8.4 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process because of the extensive fragmentation of organophosphorus pesticides during electron impact MS processes.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Quality control procedures to ensure the proper performance of the various sample preparation techniques can be found in Method 3500. If an extract cleanup procedure was performed, refer to Method 3600 for the appropriate quality control procedures. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures necessary to evaluate the GC system operation are found in Method 8000, Sec. 7.0 and include evaluation of retention time windows, calibration verification, and chromatographic analysis of samples.

8.3 Initial Demonstration of Proficiency

8.3.1 Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made.

8.3.2 It is suggested that the quality control (QC) reference sample concentrate (as discussed in Section 8.0 of Methods 8000 and 3500) contain each analyte of interest at 10 mg/L. See Method 8000, Sec. 8.0 for additional information on how to accomplish this demonstration.

8.3.3 Calculate the average recovery and the standard deviation of the recoveries of the analytes in each of the four QC reference samples. Refer to Sec. 8.0 of Method 8000 for procedures for evaluating method performance.

8.4 Sample Quality Control for Preparation and Analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, a matrix spike, a duplicate, a laboratory control sample (LCS), and the addition of surrogates to each field sample and QC sample.

8.4.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, the laboratories should use a matrix spike and matrix spike duplicate pair.

8.4.2 In-house method performance criteria should be developed using the guidance found in Sec. 8.0 of Method 8000 for procedures for evaluating method performance.

8.4.3 A Laboratory Control Sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicates a

potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

8.4.4 Include a calibration standard after each group of 20 samples (it is *recommended* that a calibration standard be included after every 10 samples to minimize the number of repeat injections) in the analysis sequence as a calibration check. Thus, injections of method blank extracts, matrix spike samples, and other non-standards are counted in the total. Solvent blanks, injected as a check on cross-contamination, need not be counted in the total. The calibration factors for the calibration should be within ±15% of the initial calibration. When this calibration verification standard falls out of this acceptance window, the laboratory should stop analyses and take corrective action.

8.4.5 Whenever quantitation is accomplished using an internal standard, internal standards must be evaluated for acceptance. The measured area of the internal standard must be no more than 50 percent different from the average area calculated during calibration. When the internal standard peak area is outside the limit, all samples that fall outside the QC criteria must be reanalyzed.

8.4.6 See Method 8000, Sec. 8.0 for the details on carrying out sample quality control procedures for preparation and analysis.

8.5 Surrogate recoveries

The laboratory must evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000, Sec. 8.0 for information on evaluating surrogate data and developing and updating surrogate limits.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 Estimated MDLs and associated chromatographic conditions for water and clean soil (uncontaminated with synthetic organics) are listed in Table 1. As detection limits will vary with the particular matrix to be analyzed, guidance for determining EQLs is given in Table 2.

9.2 Recoveries for some method analytes are provided in Tables 5, 6, and 7.

9.3 Tables 11 and 12 present data for solid-phase extraction of ground water and waste water samples. Forty four organophosphorus compounds were divided into three sets of analytes. Each set was spiked into seven 250-mL replicate samples of ground water and a waste water at 10 ppb and at 250 ppb. Ground water was obtained from the Stroh Brewery in St. Paul, MN, while the waste water was obtained from a chemical manufacturing plant. The water samples were extracted using a 47-mm Empore™ Extraction Disk with SDB-RPS, a reverse-phase, sulfonated, poly(styrenedivinylbenzene) copolymer adsorbent. The samples were analyzed using gas chromatography with a nitrogen-phosphorous detector.

9.4 Single-laboratory accuracy data were obtained for organophosphorus pesticides at two different spiking concentrations in three different soil types. Spiking concentrations ranged from 250

to 2500 µg/kg for the OPPs. Spiked samples were extracted both by the Dionex Accelerated Solvent Extractor and by Soxhlet. Table 13 contains the data for the recoveries of the analytes from pressurized fluid extraction as a percentage of the amount recovered by Soxhlet for all three soils. Table 14 contains the bias, calculated as a percentage of the spiked concentration and the precision of those results, calculated as the relative standard deviation (RSD). All data are taken from Reference 15.

10.0 REFERENCES

1. Taylor, V.; Hickey, D.M.; Marsden, P.J. "Single Laboratory Validation of EPA Method 8140," U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Office of Research and Development, Las Vegas, NV, 1987; EPA-600/4-87-009.

2. Pressley, T.A; Longbottom, J.E. "The Determination of Organophosphorus Pesticides in Industrial and Municipal Wastewater: Method 614," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH, 1982; EPA-600/4-82-004.

3. "Analysis of Volatile Hazardous Substances by GC/MS: Pesticide Methods Evaluation," Letter Reports 6, 12A, and 14 to the U.S. Environmental Protection Agency on Contract 68-03-2697, 1982.

4. "Method 622, Organophosphorus Pesticides," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

5. Lopez-Avila, V.; Baldin, E.; Benedicto, J; Milanes, J.; Beckert, W. F. "Application of Open-Tubular Columns to SW-846 GC Methods," final report to the U.S. Environmental Protection Agency on Contract 68-03-3511, Mid-Pacific Environmental Laboratory, Mountain View, CA, 1990.

6. Hatcher, M.D.; Hickey, D.M.; Marsden, P.J.; and Betowski, L.D.; "Development of a GC/MS Module for RCRA Method 8141," final report to the U.S. EPA Environmental Protection Agency on Contract 68-03-1958, S-Cubed, San Diego, CA, 1988.

7. Chau, A.S.Y.; Afghan, B.K. *Analysis of Pesticides in Water*; "Chlorine and Phosphorus-Containing Pesticides," CRC Press, Boca Raton, FL, 1982, Vol. 2, pp 91-113, 238.

8. Hild, J.; Schulte, E; Thier, H.P. "Separation of Organophosphorus Pesticides and Their Metabolites on Glass-Capillary Columns," *Chromatographia*, 1978, 11-17.

9. Luke, M.A.; Froberg, J.E.; Doose, G.M.; Masumoto, H.T. "Improved Multiresidue Gas Chromatographic Determination of Organophosphorus, Organonitrogen, and Organohalogen Pesticides in Produce, Using Flame Photometric and Electrolytic Conductivity Detectors," *J. Assoc. Off. Anal. Chem.* 1981, 1187, 64.

10. Sherma, J.; Berzoa, M. "Analysis of Pesticide Residues in Human and Environmental Samples," U.S. Environmental Protection Agency, Research Triangle Park, NC, EPA-600/8-80-038.

11. Desmarchelier, J.M.; Wustner, D.A.; Fukuto, T.R. "Mass Spectra of Organophosphorus Esters and Their Alteration Products," *Residue Reviews*, 1974, pp 63, 77.

12. Munch, D.J. and Frebis, C.P., "Analyte Stability Studies Conducted during the National Pesticide Survey," *ES & T*, 1992, vol 26, 921-925.

13. T.L. Jones, "Organophosphorus Pesticide Standards: Stability Study," EMSL-LV Research Report, EPA 600/X-92/040, April, 1992

14. Kotronarou, A., et al., "Decomposition of Parathion in Aqueous Solution by Ultrasonic Irradiation," *ES&T*, 1992, Vol. 26, 1460-1462.

15. Richter, B, Ezzell, J., and Felix D, "Single Laboratory Method Validation Report - Extraction of Organophosphorus Pesticides, Herbicides and Polychlorinated Biphenyls using Accelerated Solvent Extraction (ASE) with Analytical Validation by GC/NPD and GC/ECD," Dionex, Salt Lake City, UT, Document 101124, December 2, 1994.

TABLE 1
METHOD DETECTION LIMITS IN A WATER AND A SOIL MATRIX
USING 15-m COLUMNS AND A FLAME PHOTOMETRIC DETECTOR

Compound	Reagent Water[a] (µg/L)	Soil[b] (µg/kg)
Azinphos-methyl	0.10	5.0
Bolstar (Sulprofos)	0.07	3.5
Chlorpyrifos	0.07	5.0
Coumaphos	0.20	10.0
Demeton, -O, -S	0.12	6.0
Diazinon	0.20	10.0
Dichlorvos (DDVP)	0.80	40.0
Dimethoate	0.26	13.0
Disulfoton	0.07	3.5
EPN	0.04	2.0
Ethoprop	0.20	10.0
Fensulfothion	0.08	4.0
Fenthion	0.08	5.0
Malathion	0.11	5.5
Merphos	0.20	10.0
Mevinphos	0.50	25.0
Naled	0.50	25.0
Parathion, ethyl	0.06	3.0
Parathion, methyl	0.12	6.0
Phorate	0.04	2.0
Ronnel	0.07	3.5
Sulfotepp	0.07	3.5
TEPP[c]	0.80	40.0
Tetrachlorovinphos	0.80	40.0
Tokuthion (Protothiofos)[c]	0.07	5.5
Trichloronate[c]	0.80	40.0

[a]Sample extracted using Method 3510, Separatory Funnel Liquid-Liquid Extraction.
[b]Sample extracted using Method 3540, Soxhlet Extraction.
[c]Purity of these standards not established by the EPA Pesticides and Industrial Chemicals Repository, Research Triangle Park, NC.

TABLE 2

DETERMINATION OF ESTIMATED QUANTITATION LIMITS (EQLs) FOR VARIOUS MATRICES[a]

Matrix	Factor
Ground water (Methods 3510 or 3520)	10[b]
Low-concentration soil by Soxhlet and no cleanup	10[c]
Non-water miscible waste (Method 3580)	1000[c]

[a] EQL = [Method detection limit (see Table 1)] X [Factor found in this table]. For non-aqueous samples, the factor is on a wet-weight basis. Sample EQLs are highly matrix dependent. The EQLs to be determined herein are for guidance and may not always be achievable.

[b] Multiply this factor times the reagent water MDL in Table 1.

[c] Multiply this factor times the soil MDL in Table 1.

TABLE 3

RETENTION TIMES ON 15-m COLUMNS

Analyte	Retention Time (min)			
	DB-5	SPB-608	DB-210	DB-1
TEPP		6.44	5.12	10.66
Dichlorvos (DDVP)	9.63	7.91	12.79	
Mevinphos	14.18	12.88	18.44	
Demeton, -O and -S	18.31	15.90	17.24	
Ethoprop	18.62	16.48	18.67	
Naled		19.01	17.40	19.35
Phorate	19.94	17.52	18.19	
Monochrotophos	20.04	20.11	31.42	
Sulfotepp	20.11	18.02	19.58	
Dimethoate	20.64	20.18	27.96	
Disulfoton	23.71	19.96	20.66	
Diazinon	24.27	20.02	19.68	
Merphos	26.82	21.73	32.44	
Ronnel	29.23	22.98	23.19	
Chlorpyrifos	31.17	26.88	25.18	
Malathion	31.72	28.78	32.58	
Parathion, methyl	31.84	23.71	32.17	
Parathion, ethyl	31.85	27.62	33.39	
Trichloronate	32.19	28.41	29.95	
Tetrachlorovinphos	34.65	32.99	33.68	
Tokuthion (Protothiofos)	34.67	24.58	39.91	
Fensulfothion	35.85	35.20	36.80	
Bolstar (Sulprofos)	36.34	35.08	37.55	
Famphur[b]	36.40	36.93	37.86	
EPN		37.80	36.71	36.74
Azinphos-methyl	38.34	38.04	37.24	
Fenthion	38.83	29.45	28.86	
Coumaphos	39.83	38.87	39.47	

[a]GC operating conditions are shown in Table 8.
[b]Method 8141 has not been fully validated for Famphur.

TABLE 4

RETENTION TIMES ON 30-m COLUMNS[a]

Analyte	Retention Time (min)			
	DB-5	DB-210	DB-608	DB-1
Trimethylphosphate	b	2.36		
Dichlorvos (DDVP)	7.45	6.99	6.56	10.43
Hexamethylphosphoramide	b	7.97		
Trichlorfon	11.22	11.63	12.69	
TEPP	b	13.82		
Thionazin	12.32	24.71		
Mevinphos	12.20	10.82	11.85	14.45
Ethoprop	12.57	15.29	18.69	18.52
Diazinon	13.23	18.60	24.03	21.87
Sulfotepp	13.39	16.32	20.04	19.60
Terbufos	13.69	18.23	22.97	
Tri-o-cresyl phosphate	13.69	18.23		
Naled	14.18	15.85	18.92	18.78
Phorate	12.27	16.57	20.12	19.65
Fonophos	14.44	18.38		
Disulfoton	14.74	18.84	23.89	21.73
Merphos	14.89	23.22		26.23
Oxidized Merphos	20.25	24.87	35.16	
Dichlorofenthion	15.55	20.09	26.11	
Chlorpyrifos, methyl	15.94	20.45	26.29	
Ronnel	16.30	21.01	27.33	23.67
Chlorpyrifos	17.06	22.22	29.48	24.85
Trichloronate	17.29	22.73	30.44	
Aspon	17.29	21.98		
Fenthion	17.87	22.11	29.14	24.63
Demeton-S	11.10	14.86	21.40	20.18
Demeton-O	15.57	17.21	17.70	
Monocrotophos[c]	19.08	15.98	19.62	19.3
Dimethoate	18.11	17.21	20.59	19.87
Tokuthion	19.29	24.77	33.30	27.63
Malathion	19.83	21.75	28.87	24.57
Parathion, methyl	20.15	20.45	25.98	22.97

TABLE 4
(continued)

Analyte	Retention Time (min)			
	DB-5	DB-210	DB-608	DB-1
Fenithrothion	20.63	21.42		
Chlorfenvinphos	21.07	23.66	32.05	
Parathion, ethyl	21.38	22.22	29.29	24.82
Bolstar	22.09	27.57	38.10	29.53
Stirophos	22.06	24.63	33.40	26.90
Ethion	22.55	27.12	37.61	
Phosphamidon	22.77	20.09	25.88	
Crotoxyphos	22.77	23.85	32.65	
Leptophos	24.62	31.32	44.32	
Fensulfothion	27.54	26.76	36.58	28.58
EPN	27.58	29.99	41.94	31.60
Phosmet	27.89	29.89	41.24	
Azinphos-methyl	28.70	31.25	43.33	32.33
Azinphos-ethyl	29.27	32.36	45.55	
Famphur	29.41	27.79	38.24	
Coumaphos	33.22	33.64	48.02	34.82
Atrazine	13.98	17.63		
Simazine	13.85	17.41		
Carbophenothion	22.14	27.92		
Dioxathion	d	d	22.24	
Trithion methyl			36.62	
Dicrotophos			19.33	
Internal Standard				
1-Bromo-2-nitrobenzene	8.11	9.07		
Surrogates				
Tributyl phosphate			11.1	
Triphenyl phosphate			33.4	
4-Chloro-3-nitrobenzotrifluoride	5.73	5.40		

[a]GC operating conditions are shown in Table 8.
[b] Not detected at 20 ng per injection.
[c] Retention times may shift to longer times with larger amounts injected (shifts of over 30 seconds have been observed, see Reference 6).
[d] Shows multiple peaks; therefore, not included in the composite.

TABLE 5

RECOVERY OF 27 ORGANOPHOSPHATES BY SEPARATORY FUNNEL EXTRACTION

	Percent Recovery at Three Spiking Levels		
Analyte	Low	Medium	High
Azinphos methyl	126	143 ± 8	101
Bolstar	134	141 ± 8	101
Chlorpyrifos	7	89 ± 6	86
Coumaphos	103	90 ± 6	96
Demeton	33	67 ± 11	74
Diazinon	136	121 ± 9.5	82
Dichlorvos	80	79 ± 11	72
Dimethoate	NR	47 ± 3	101
Disulfoton	48	92 ± 7	84
EPN	113	125 ± 9	97
Ethoprop	82	90 ± 6	80
Fensulfonthion	84	82 ± 12	96
Fenthion	NR	48 ± 10	89
Malathion	127	92 ± 6	86
Merphos	NR	79	81
Mevinphos	NR	NR	55
Monocrotophos	NR	18 ± 4	NR
Naled	NR	NR	NR
Parathion, ethyl	101	94 ± 5	86
Parathion, methyl	NR	46 ± 4	44
Phorate	94	77 ± 6	73
Ronnel	67	97 ± 5	87
Sulfotep	87	85 ± 4	83
TEPP	96	55 ± 72	63
Tetrachlorvinphos	79	90 ± 7	80
Tokuthion	NR	45 ± 3	90
Trichloroate	NR	35	4

NR = Not recovered

TABLE 6

RECOVERY OF 27 ORGANOPHOSPHATES BY CONTINUOUS LIQUID-LIQUID EXTRACTION

	Percent Recovery at Three Spiking Levels		
Analyte	Low	Medium	High
Azinphos methyl	NR	129	122
Bolstar	NR	126	128
Chlorpyrifos	13	82 ± 4	88
Coumaphos	94	79 ± 1	89
Demeton	38	23 ± 3	41
Diazinon	NR	128 ± 37	118
Dichlorvos	81	32 ± 1	74
Dimethoate	NR	10 ± 8	102
Disulfoton	94	69 ± 5	81
EPN	NR	104 ± 18	119
Ethoprop	39	76 ± 2	83
Famphur	–	63 ± 15	–
Fensulfonthion	90	67 ± 26	90
Fenthion	8	32 ± 2	86
Malathion	105	87 ± 4	86
Merphos	NR	80	79
Mevinphos	NR	87	49
Monocrotophos	NR	30	1
Naled	NR	NR	74
Parathion, ethyl	106	81 ± 1	87
Parathion, methyl	NR	50 ± 30	43
Phorate	84	63 ± 3	74
Ronnel	82	83 ± 7	89
Sulfotep	40	77 ± 1	85
TEPP	39	18 ± 7	70
Tetrachlorvinphos	56	70 ± 14	83
Tokuthion	132	32 ± 14	90
Trichloroate	NR	NR	21

NR = Not recovered

TABLE 7

RECOVERY OF 27 ORGANOPHOSPHATES BY SOXHLET EXTRACTION

	Percent Recovery at Three Spiking Levels		
Analyte	Low	Medium	High
Azinphos methyl	156	110 ± 6	87
Bolstar	102	103 ± 15	79
Chlorpyrifos	NR	66 ± 17	79
Coumaphos	93	89 ± 11	90
Demeton	169	64 ± 6	75
Diazinon	87	96 ± 3	75
Dichlorvos	84	39 ± 21	71
Dimethoate	NR	48 ± 7	98
Disulfoton	78	78 ± 6	76
EPN	114	93 ± 8	82
Ethoprop	65	70 ± 7	75
Fensulfonthion	72	81 ± 18	111
Fenthion	NR	43 ± 7	89
Malathion	100	81 ± 8	81
Merphos	62	53	60
Mevinphos	NR	71	63
Monocrotophos	NR	NR	NR
Naled	NR	48	NR
Parathion, ethyl	75	80 ± 8	80
Parathion, methyl	NR	41 ± 3	28
Phorate	75	77 ± 6	78
Ronnel	NR	83 ± 12	79
Sulfotep	67	72 ± 8	78
TEPP	36	34 ± 33	63
Tetrachlorvinphos	50	81 ± 7	83
Tokuthion	NR	40 ± 6	89
Trichloroate	56	53	53

NR = Not recovered

TABLE 8

SUGGESTED OPERATING CONDITIONS FOR 15-m COLUMNS

Columns 1 and 2 (DB-210 and SPB-608 or their equivalents)	
Carrier gas (He) flow rate	5mL/min
Initial temperature	50°C, hold for 1 minute
Temperature program	50°C to 140°C at 5°C/min, hold for 10 minutes, followed by 140°C to 240°C at 10°C/min, hold for 10 minutes (or a sufficient amount of time for last compound to elute).
Column 3 (DB-5 or equivalent)	
Carrier gas (He) flow rate	5mL/min
Initial temperature	130°C, hold for 3 minutes
Temperature program	130°C, to 180°C at 5°C/min, hold for 10 minutes, followed by 180°C to 250°C at 2°C/min, hold for 15 minutes (or a sufficient amount of time for last compound to elute).

TABLE 9

SUGGESTED OPERATING CONDITIONS FOR 30-m COLUMNS

Column 1	DB-210
	Dimensions: 30-m x 0.53-mm ID
	Film Thickness (µm): 1.0
Column 2	DB-5
	Dimensions: 30-m x 0.53-mm ID
	Film Thickness (µm): 1.5
Carrier gas flow rate	6 (mL/min) Helium
Makeup gas flow rate	20 (mL/min) Helium
Temperature program	120°C (3-min hold) to 270°C (10-min hold) at 5°C/min
Injector temperature	250°C
Detector temperature	300°C
Injection volume	2 µL
Solvent	Hexane
Type of injector	Flash vaporization
Detector type	Dual NPD
Range	1
Attenuation	64
Type of splitter	Y-shaped or Tee
Data system	Integrator
Hydrogen gas pressure	20 psi
Bead temperature	400°C
Bias voltage	4

TABLE 10

QUANTITATION AND CHARACTERISTIC IONS FOR GC/MS ANALYSIS OF ORGANOPHOSPHORUS PESTICIDES

Analyte	Quantitation ion	Characteristic ions
Azinphos-methyl	160	77,132
Bolstar (Sulprofos)	156	140,143,113,33
Chlorpyrifos	197	97,199,125.314
Coumaphos	109	97,226,362,21
Demeton-S	88	60,114,170
Diazinon	137	179,152,93,199,304
Dichlorvos (DDVP)	109	79,185,145
Dimethoate	87	93,125,58,143
Disulfoton	88	89,60,61,97,142
EPN	157	169,141,63,185
Ethoprop	158	43,97,41,126
Fensulfothion	293	97,125,141,109,308
Fenthion	278	125,109,93,169
Malathion	173	125,127,93,158
Merphos	209	57,153,41,298
Mevinphos	127	109,67,192
Monocrotophos	127	67,97,192,109
Naled	109	145,147,79
Parathion, ethyl	291	97,109,139,155
Parathion, methyl	109	125,263,79
Phorate	75	121,97,47,260
Ronnel	285	125,287,79,109
Stirophos	109	329,331,79
Sulfotepp	322	97,65,93,121,202
TEPP	99	155,127,81,109
Tokuthion	113	43,162,267,309

TABLE 11

PERFORMANCE DATA FOR ORGANOPHOSPHORUS PESTICIDES
IN GROUND WATER USING METHOD 3535

	Ground Water spiked at 250 ppb		Ground Water spiked at 10 ppb		
Analyte	% Recovery	RSD	% Recovery	RSD	MDL[a]
Aspon	85.6	11.5	77.7	6.8	1.7
Azinphos-methyl	83.0	13.4	109.7	7.0	2.4
Azinphos-ethyl	88.3	10.8	92.8	8.1	2.4
Bolstar	96.1	4.2	78.2	4.3	1.1
Carbophenothion	85.6	11.0	81.7	7.2	1.9
Chlorfenvinphos	87.8	10.2	90.1	6.0	1.7
Chlorpyrifos	98.8	5.7	77.5	4.2	1.0
Chlorpyrifos methyl	82.5	12.0	59.4	7.5	1.4
Coumaphos	84.3	8.7	100.8	13.5	4.3
Crotoxyphos	86.3	10.5	89.4	5.9	1.7
Demeton	93.6	4.5	73.8	5.1	1.2
Diazinon	91.7	4.7	70.0	5.0	1.1
Dichlorofenthion	85.2	10.9	75.6	6.0	1.4
Dichlorvos (DDVP)	88.1	6.7	90.1	7.9	2.2
Dicrotophos	88.6	10.8	75.7	5.7	1.3
Dimethoate	99.3	1.8	76.7	9.5	2.3
Dioxathion	81.6	14.1	92.7	11.0	3.2
Disulfoton	93.2	7.6	79.5	6.1	1.5
EPN	73.8	10.6	67.9	7.9	1.7
Ethion	85.5	10.6	79.2	6.5	1.6
Ethoprop	95.6	4.1	81.4	3.7	0.9
Famphur	85.2	10.2	75.6	8.3	2.0
Fenitrothion	91.2	8.8	85.0	5.0	1.3
Fensulfothion	86.2	6.4	97.2	6.0	1.8

TABLE II (cont.)

Analyte	Ground Water spiked at 250 ppb		Ground Water spiked at 10 ppb		
	% Recovery	RSD	% Recovery	RSD	MDL[a]
Fenthion	91.2	5.4	79.5	4.3	1.7
Fonophos	91.0	8.0	81.6	3.6	0.9
Leptophos	81.3	12.2	73.6	8.8	2.0
Malathion	79.5	6.9	78.0	8.7	2.1
Merphos	113.1	9.3	84.6	4.5	1.2
Mevinphos	57.9	6.9	96.8	6.7	2.0
Naled	90.1	6.7	88.1	7.9	2.2
Parathion, ethyl	76.7	9.6	69.6	8.1	1.8
Parathion, methyl	93.9	5.8	83.6	4.7	1.2
Phorate	92.3	7.1	70.8	6.7	1.5
Phosmet	66.1	17.7	90.3	10.7	3.0
Phosphamidon	86.2	11.2	80.6	5.7	1.4
Ronnel	94.7	5.2	77.8	4.7	1.2
Stirophos	78.6	13.1	106.3	5.9	2.0
Sulfotepp	75.3	9.3	68.9	8.6	1.9
Terbufos	87.1	10.5	78.0	3.7	0.9
Thionazin	95.1	8.0	88.6	3.4	1.0
Tokuthion	94.4	4.1	77.8	5.6	1.4
Trichlorfon	72.7	13.5	45.6	6.9	1.0
Trichloronate	95.3	4.5	75.7	3.9	0.9

[a] All MDL values are in µg/L, and are highly matrix dependant. MDLs provided in SW-846 are for guidance purposes and may not always be achievable. Laboratories should establish their own in-house MDLs to document method performance.

TABLE 12

PERFORMANCE DATA FOR ORGANOPHOSPHORUS PESTICIDES
IN WASTEWATER USING METHOD 3535

	Wastewater spiked at 250 ppb		Wastewater spiked at 10 ppb		
Analyte	% Recovery	RSD	% Recovery	RSD	MDL[a]
Aspon	83.7	1.8	76.3	6.7	1.6
Azinphos-methyl	102.6	18.0	129.9	12.4	5.1
Azinphos-ethyl	79.8	6.8	96.0	6.7	2.0
Bolstar	94.4	8.3	84.9	1.4	0.4
Carbophenothion	82.4	2.9	82.1	6.7	1.7
Chlorfenvinphos	81.7	6.5	88.0	7.2	2.0
Chlorpyrifos	91.0	8.3	86.5	1.7	0.5
Chlorpyrifos methyl	77.6	2.2	56.7	7.1	1.3
Coumaphos	100.2	17.2	111.0	8.5	3.0
Crotoxyphos	81.3	5.7	87.5	7.0	1.9
Demeton	95.8	5.3	88.5	5.0	1.4
Diazinon	91.8	6.5	82.4	3.2	0.8
Dichlorfenthion	82.5	1.4	76.2	5.5	1.3
Dichlorvos (DDVP)	60.6	11.1	99.7	6.1	1.9
Dicrotophos	82.0	1.6	73.4	6.1	1.4
Dimethoate	93.5	4.1	115.7	6.7	2.4
Dioxathion	84.6	5.6	100.4	9.4	3.0
Disulfoton	92.5	5.3	90.4	2.6	0.7
EPN	78.1	9.6	80.1	8.6	2.2
Ethion	83.5	2.0	78.4	6.4	1.6
Ethoprop	96.3	4.7	92.9	3.1	0.9
Famphur	85.9	2.5	78.6	7.9	1.9
Fenitrothion	83.5	4.8	82.3	5.9	1.5
Fensulfothion	101.7	11.4	110.5	6.5	2.3

TABLE 12

PERFORMANCE DATA FOR ORGANOPHOSPHORUS PESTICIDES
IN WASTEWATER USING METHOD 3535
(continued)

	Wastewater spiked at 250 ppb		Wastewater spiked at 10 ppb		
Analyte	% Recovery	RSD	% Recovery	RSD	MDL[a]
Fenthion	91.7	7.3	88.2	2.7	0.7
Fonophos	83.4	2.6	81.3	5.0	1.3
Leptophos	81.9	3.3	73.2	7.5	1.7
Malathion	94.8	6.7	94.7	5.5	1.6
Merphos	94.5	12.7	90.7	1.4	0.4
Mevinphos	62.6	11.2	109.0	4.8	1.6
Naled	60.6	11.1	99.7	6.1	1.9
Parathion ethyl	80.2	8.1	83.6	8.6	2.3
Parathion methyl	92.9	6.5	93.8	4.4	1.3
Phorate	92.4	6.4	85.6	2.4	0.6
Phosmet	63.5	8.2	101.3	9.1	2.9
Phosphamidon	81.1	3.1	78.0	5.7	1.4
Ronnel	91.4	8.4	88.3	2.2	0.6
Stirophos	101.4	14.3	126.5	6.5	2.6
Sulfotepp	78.7	10.7	87.9	8.8	2.4
Terbufos	83.0	1.5	80.1	6.4	1.6
Thionazin	85.1	5.8	84.8	4.9	1.3
Tokuthion	91.8	8.4	83.6	1.8	0.5
Trichlorfon	66.8	4.6	52.2	8.7	1.4
Trichloronate	91.3	8.1	84.3	1.6	0.4

[a] All MDL values are in µg/L, and are highly matrix dependant. MDLs provided in SW-846 are for guidance purposes and may not always be achievable. Labs should establish their own in-house MDLs to document method performance.

TABLE 13

RECOVERIES OF ANALYTES FROM SPIKED SOIL SAMPLES
USING PRESSURIZED FLUID EXTRACTION (METHOD 3545)

	Clay		Loam		Sand	
Analyte	Low	High	Low	High	Low	High
Dichlorvos	100.0	280.0	135.1	158.5	103.0	230.2
Mevinphos	100.6	98.0	104.0	99.8	91.5	107.8
Demeton O&S	103.7	106.2	124.3	108.4	103.4	106.0
Ethoprop	97.4	95.8	101.2	97.2	90.0	98.4
TEPP	100.0	100.0	100.0	100.0	100.0	100.0
Phorate	98.8	96.6	104.6	98.5	92.6	100.0
Sulfotep	102.6	99.3	113.2	119.1	129.4	104.2
Naled	100.0	100.0	100.0	100.0	100.0	100.0
Diazinon	97.6	96.2	104.2	101.7	89.3	100.4
Disulfoton	121.8	86.8	112.0	92.5	76.9	90.7
Monocrotophos	100.0	100.0	100.0	100.0	100.0	100.0
Dimethoate	92.5	90.7	94.3	89.0	88.5	101.8
Ronnel	96.4	95.3	102.4	85.0	94.1	98.7
Chlorpyrifos	98.3	96.3	98.0	97.0	90.2	100.2
Parathion methyl	94.9	97.7	98.9	98.5	91.3	98.3
Parathion ethyl	95.4	97.4	99.1	99.5	87.9	98.2
Fenthion	95.9	96.5	104.0	100.4	83.1	99.2
Tokuthion	97.1	95.8	102.4	96.5	94.2	98.2
Tetrachlorvinphos	93.8	93.8	144.0	92.3	95.2	101.9
Bolstar	99.1	98.4	105.1	97.5	96.6	102.5
Fensulfothion	100.0	89.9	81.2	76.8	90.1	102.1
EPN	85.8	97.3	88.8	97.1	92.8	104.8
Azinphos-methyl	100.0	92.4	85.0	81.4	96.3	103.4
Coumaphos	100.0	94.0	85.1	90.6	102.6	109.8

Results are expressed as the percentage of amount determined by a Soxhlet extraction. Data from Reference 15.

TABLE 14

BIAS AND PRECISION OF PRESSURIZED FLUID EXTRACTION
(METHOD 3545) OF THREE SPIKED SOIL SAMPLES

	Clay				Loam				Sand			
	Low		High		Low		High		Low		High	
Analyte	Bias	Pre	Bias	Pre	Bias	Pre	Bias	Pre	Bias	Pre	Bias	Pre
Dichlorvos	0.0	NA	5.6	19.0	10.4	11.4	6.5	22.2	13.9	13.4	9.9	22.2
Mevinphos	66.1	3.8	67.2	4.8	57.3	11.2	63.1	6.5	61.6	14.3	64.7	12.1
Demeton O&S	79.0	3.4	80.2	4.2	73.7	10.0	77.6	6.4	60.0	12.5	77.6	12.7
Ethoprop	83.0	4.7	84.8	4.8	76.1	10.7	77.0	4.9	75.5	12.8	79.0	10.6
TEPP	0.0	NA	0.0	NA	0.0	NA	0.0	NA	0.0	NA	0.0	NA
Phorate	67.5	3.2	79.4	5.1	63.4	11.8	73.5	5.4	62.9	13.6	76.2	10.8
Sulfotep	66.6	3.7	69.4	4.7	62.6	11.0	66.8	7.3	62.1	13.8	67.7	13.2
Naled	0.0	NA	0.0	NA	0.0	NA	0.0	NA	0.0	NA	0.0	NA
Diazinon	80.2	4.7	80.3	4.8	74.4	12.0	75.9	6.0	73.9	14.0	77.4	11.2
Disulfoton	55.9	3.6	93.9	4.7	58.9	11.8	89.4	6.2	52.2	15.3	88.5	12.3
Monocrotophos	0.0	NA	0.0	NA	0.0	NA	0.0	NA	0.0	NA	0.0	NA
Dimethoate	87.0	5.0	86.7	5.3	70.7	12.1	71.7	18.8	75.0	13.1	80.6	12.5
Ronnel	81.3	3.7	81.1	5.0	73.1	11.1	64.7	6.5	69.0	13.6	73.8	11.6
Chlorpyrifos	99.5	3.1	99.0	5.1	81.7	14.1	87.7	16.8	84.1	13.1	91.6	12.7
Parathion methyl	82.5	3.9	84.5	5.2	74.4	11.5	79.6	5.8	74.9	13.2	80.3	11.3
Parathion ethyl	85.0	3.8	83.5	5.2	77.3	11.9	79.6	6.1	78.0	12.7	80.3	11.5
Fenthion	56.4	3.8	71.4	5.0	44.1	10.8	50.9	6.6	44.3	12.5	51.9	12.6
Tokuthion	96.1	4.7	97.0	5.7	93.2	12.2	93.8	6.1	81.2	12.5	85.4	11.9
Tetrachlorvinphos	72.1	3.3	69.7	5.6	101.4	12.6	64.7	6.5	69.3	11.9	69.6	13.0
Bolstar	89.0	3.4	109.5	6.8	82.2	9.9	89.2	6.2	77.3	11.7	94.2	12.8
Fensulfothion	0.0	NA	69.7	4.3	70.4	9.3	52.2	7.1	63.0	9.2	62.0	13.1
EPN	72.6	44.3	76.9	8.0	92.9	10.1	70.4	7.1	68.6	11.2	71.9	11.6
Azinphos-methyl	0.0	NA	90.6	5.3	69.7	13.9	70.5	8.7	94.5	12.5	82.5	11.4
Coumaphos	0.0	NA	79.6	4.8	62.8	13.4	6.5	10.2	74.8	16.1	72.9	9.2

NA = not applicable
Bias was calculated as the percent recovery of the certified spiking value. Precision was calculated as the relative standard deviation (RSD). Total number of analyses of each sample was 7.
Data from Reference 15.

FIGURE 1

Chromatogram of target organophosphorus compounds from a 15-m DB-210 column with FPD detector. More compounds are shown in Figure 2. See Table 3 for retention times.

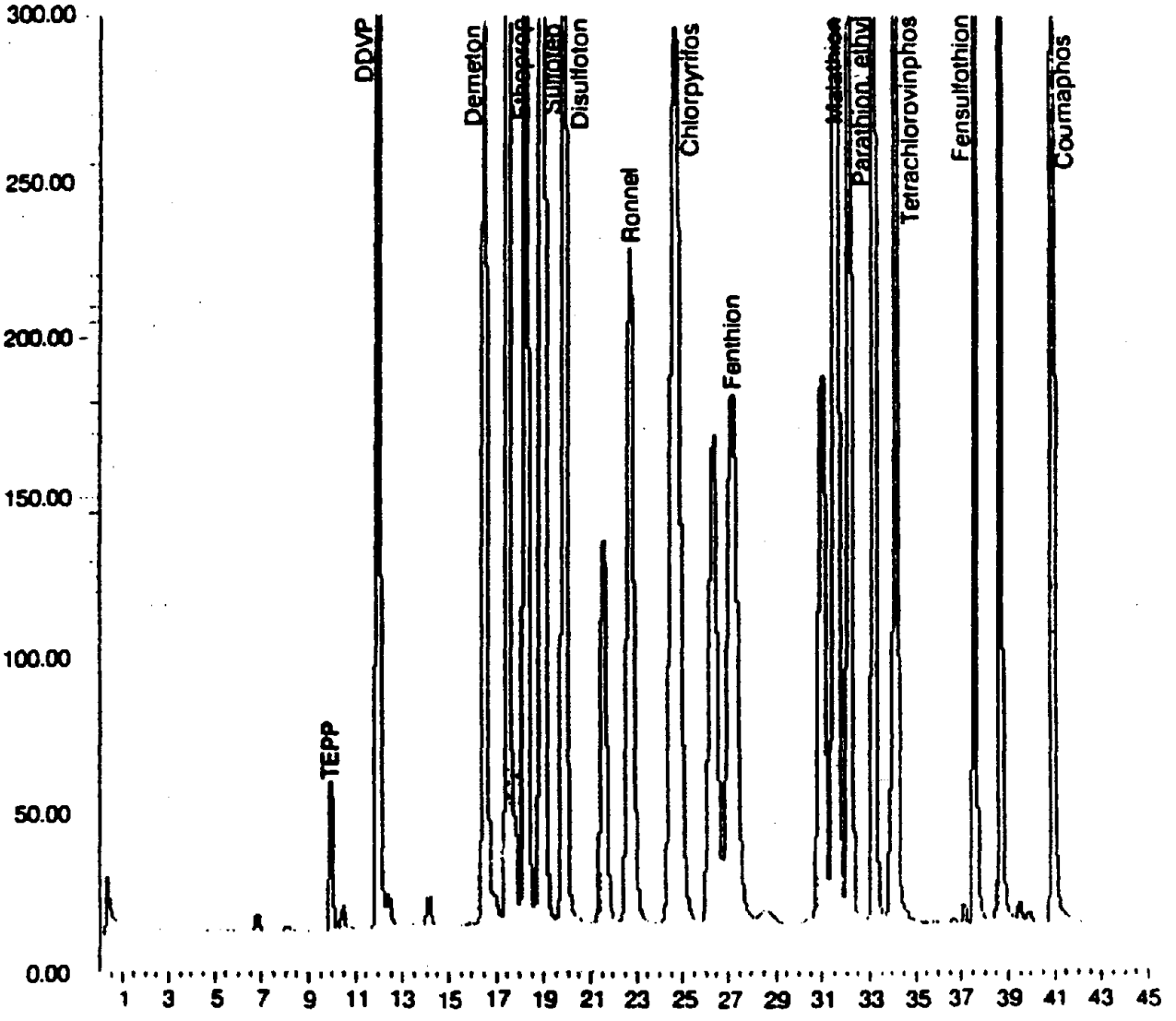


FIGURE 2

Chromatogram of target organophosphorus compounds from a 15-m DB-210 column with FPD detector. More compounds are shown in Figure 1. See Table 3 for retention times.

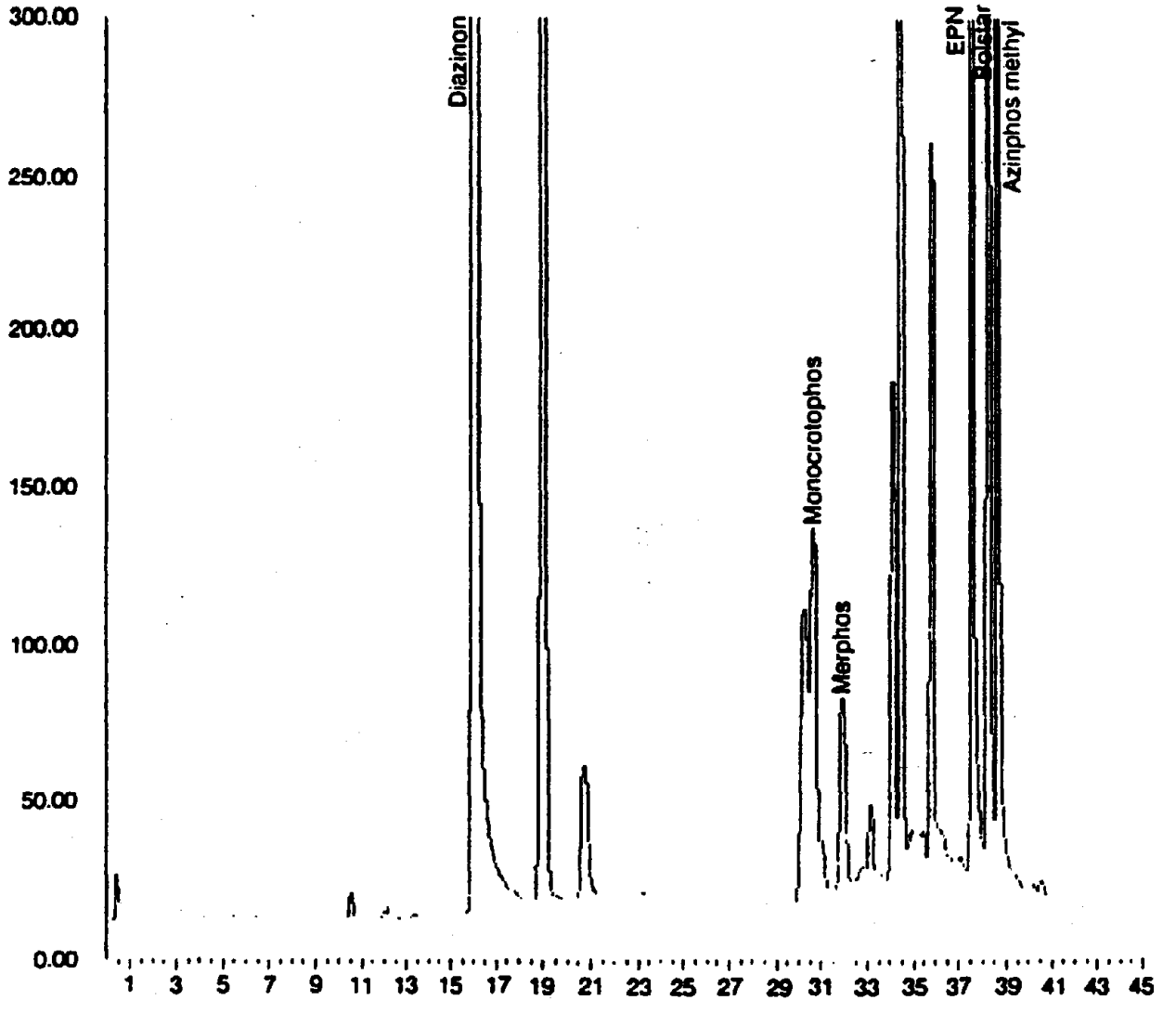


FIGURE 3

Chromatogram of target organophosphorus compounds from a 15-m DB-210 column with NPD detector. More compounds are shown in Figure 4. See Table 3 for retention times.

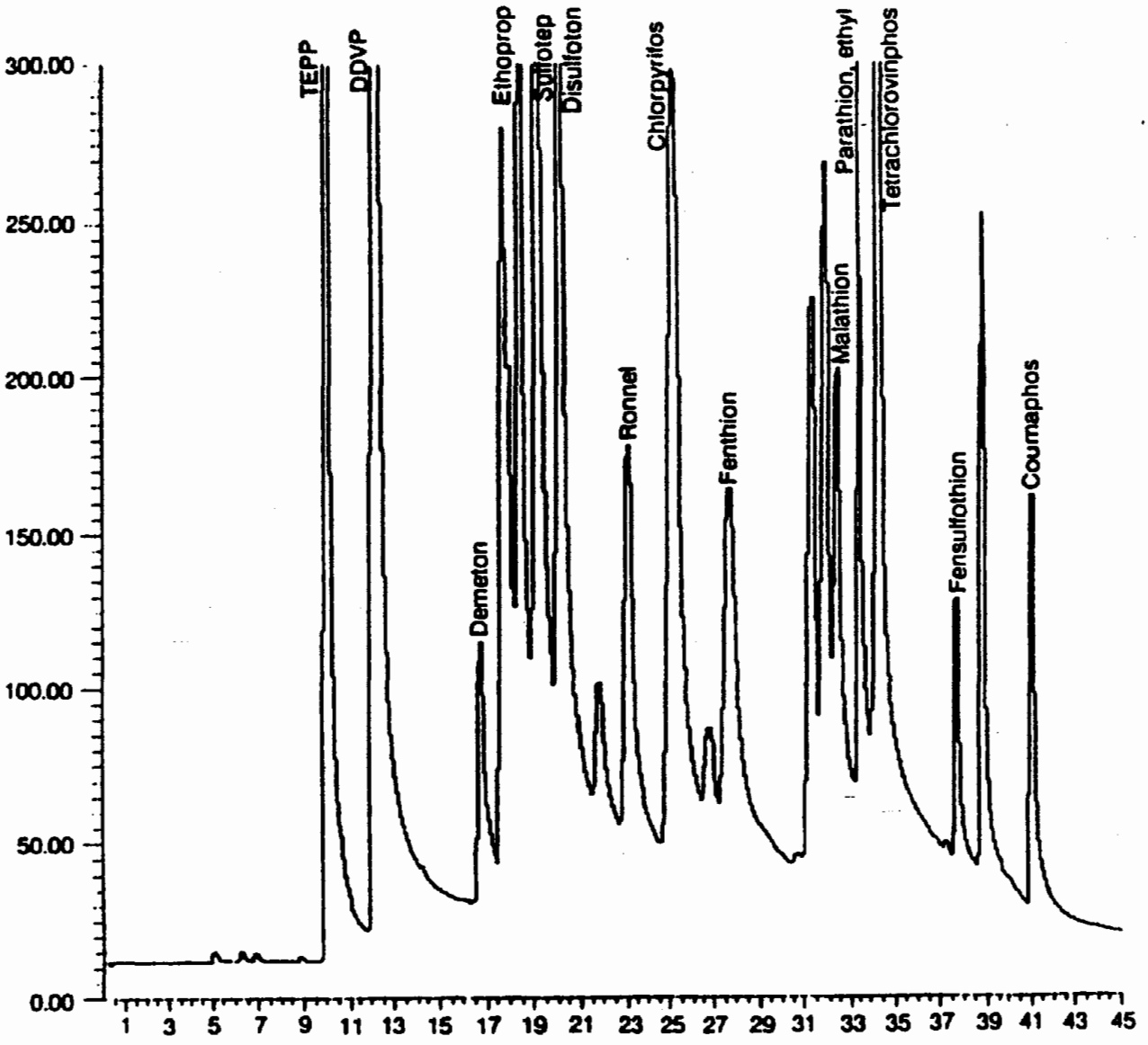


FIGURE 4

Chromatogram of target organophosphorus compounds from a 15-m DB-210 column with NPD detector. More compounds are shown in Figure 3. See Table 3 for retention times.

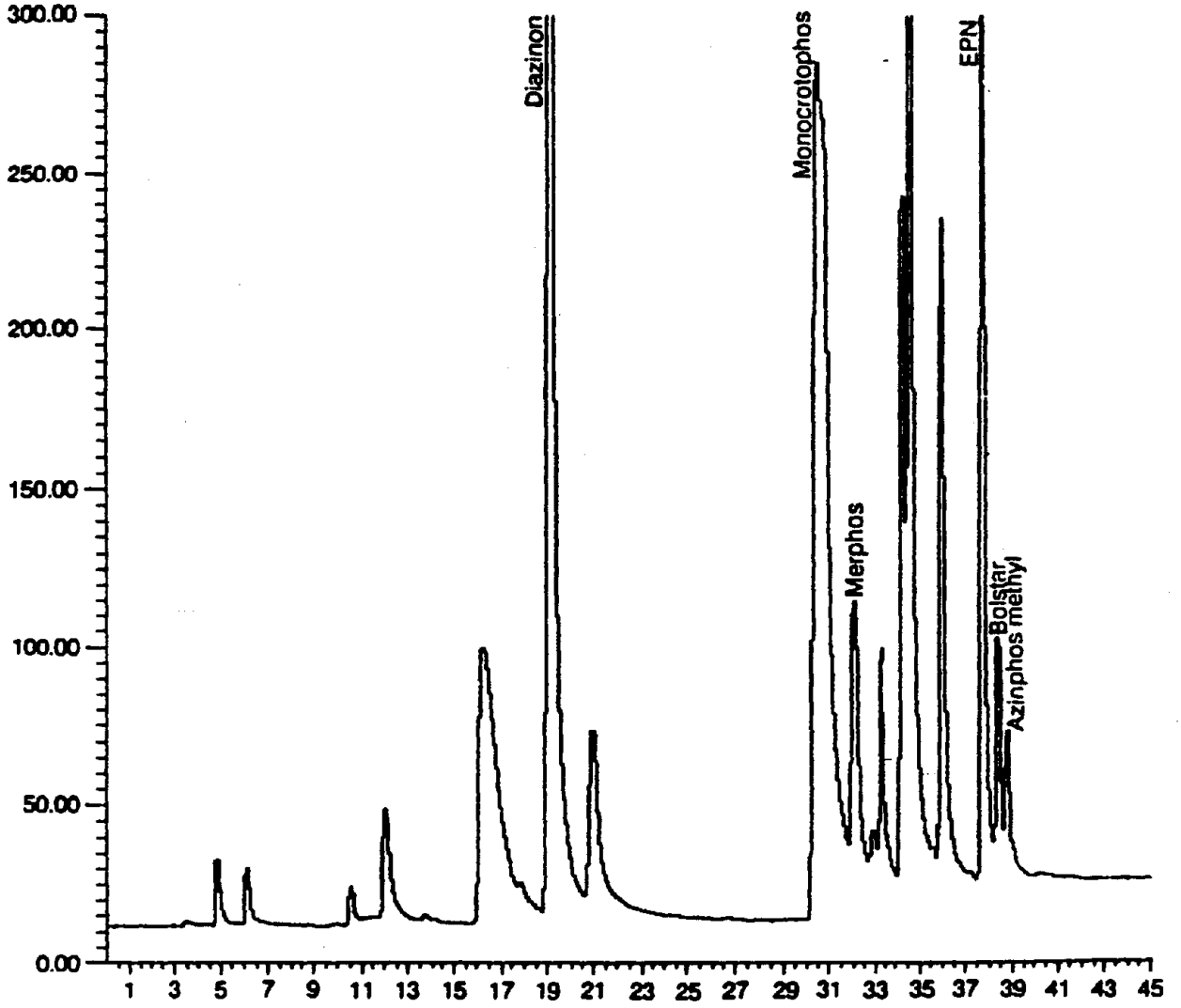


FIGURE 5

Chromatogram of target organophosphorus compounds on a 30-m DB-5/DB-210 column pair with NPD detector, without Simazine, Atrazine and Carbophenothion. See Table 4 for retention times and for GC operating conditions.

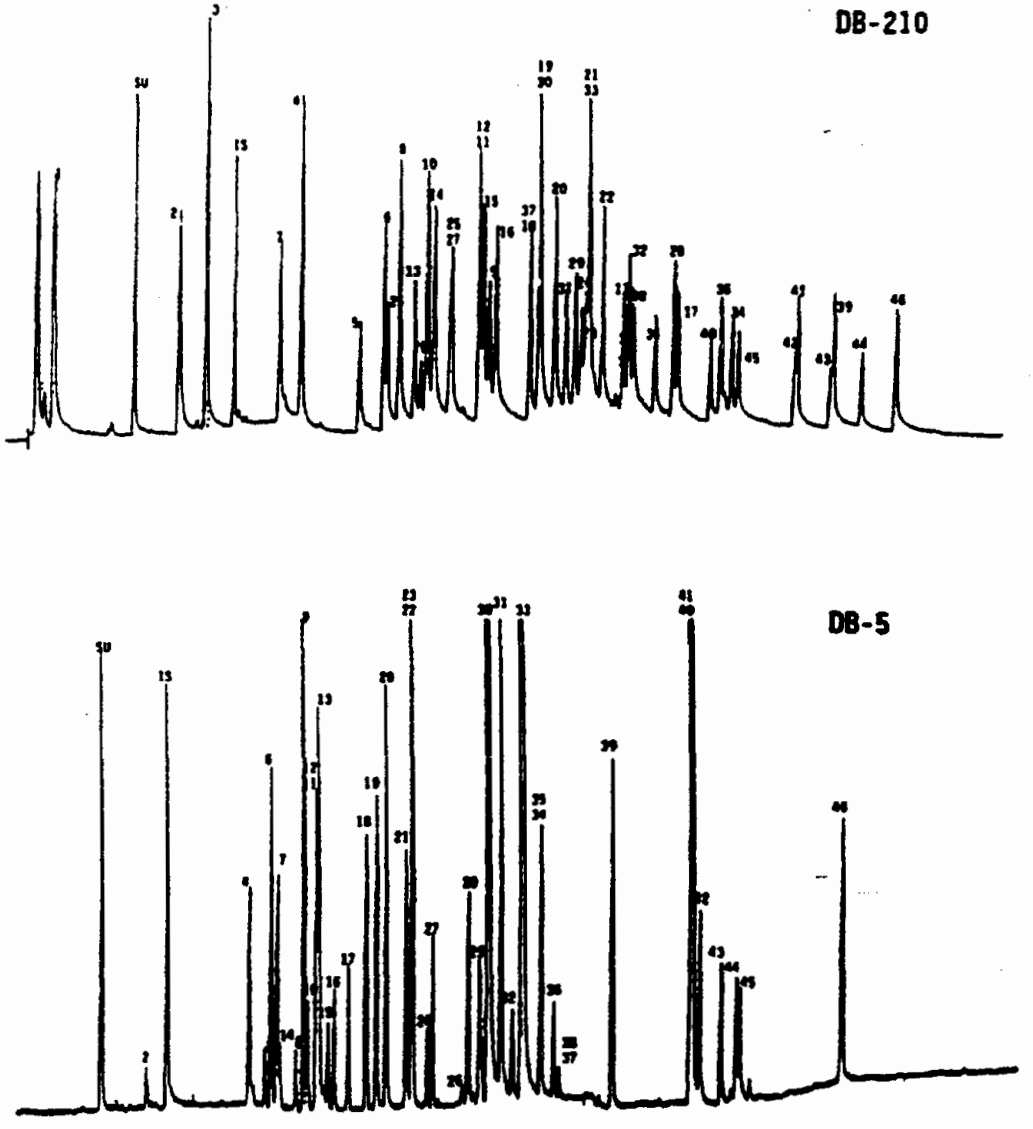
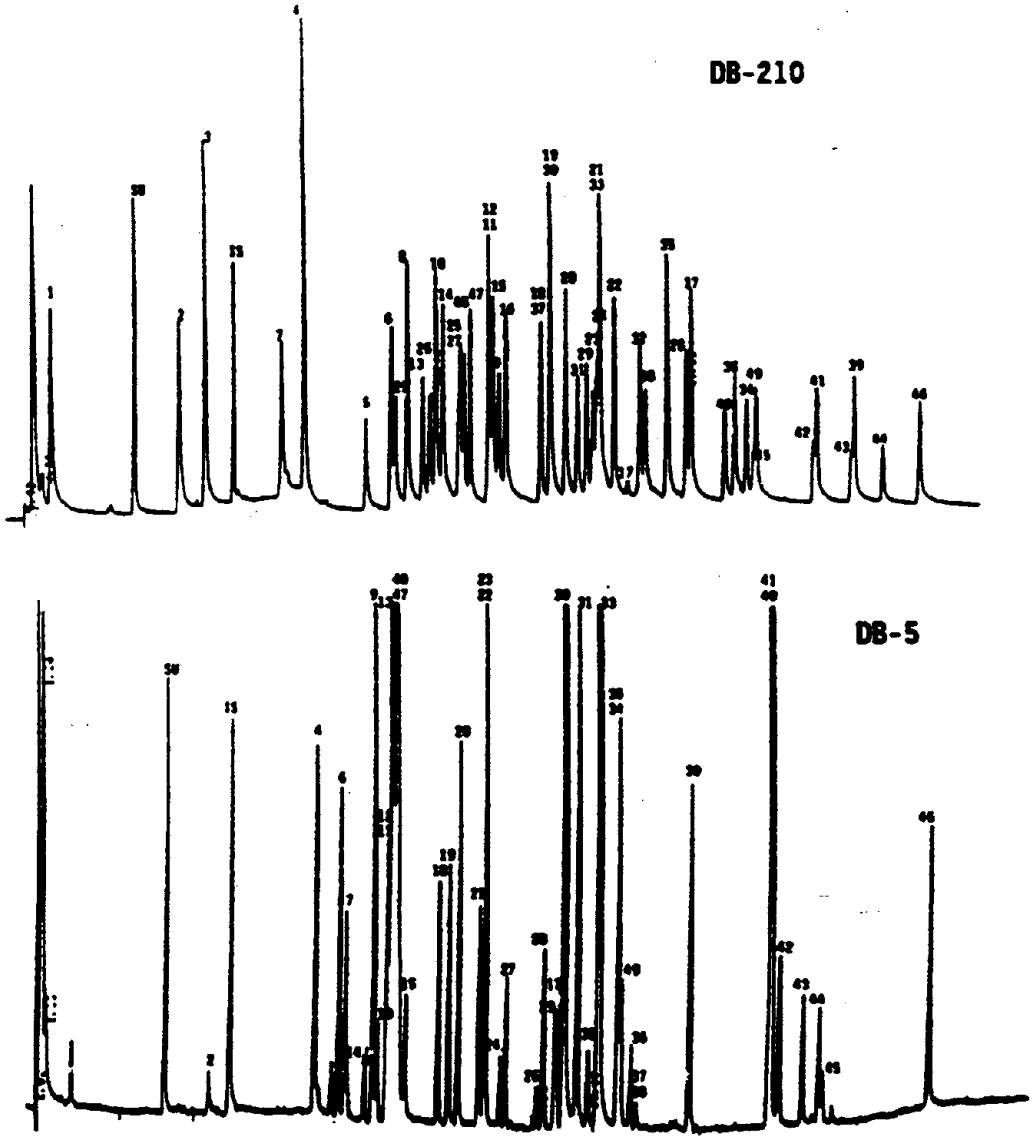
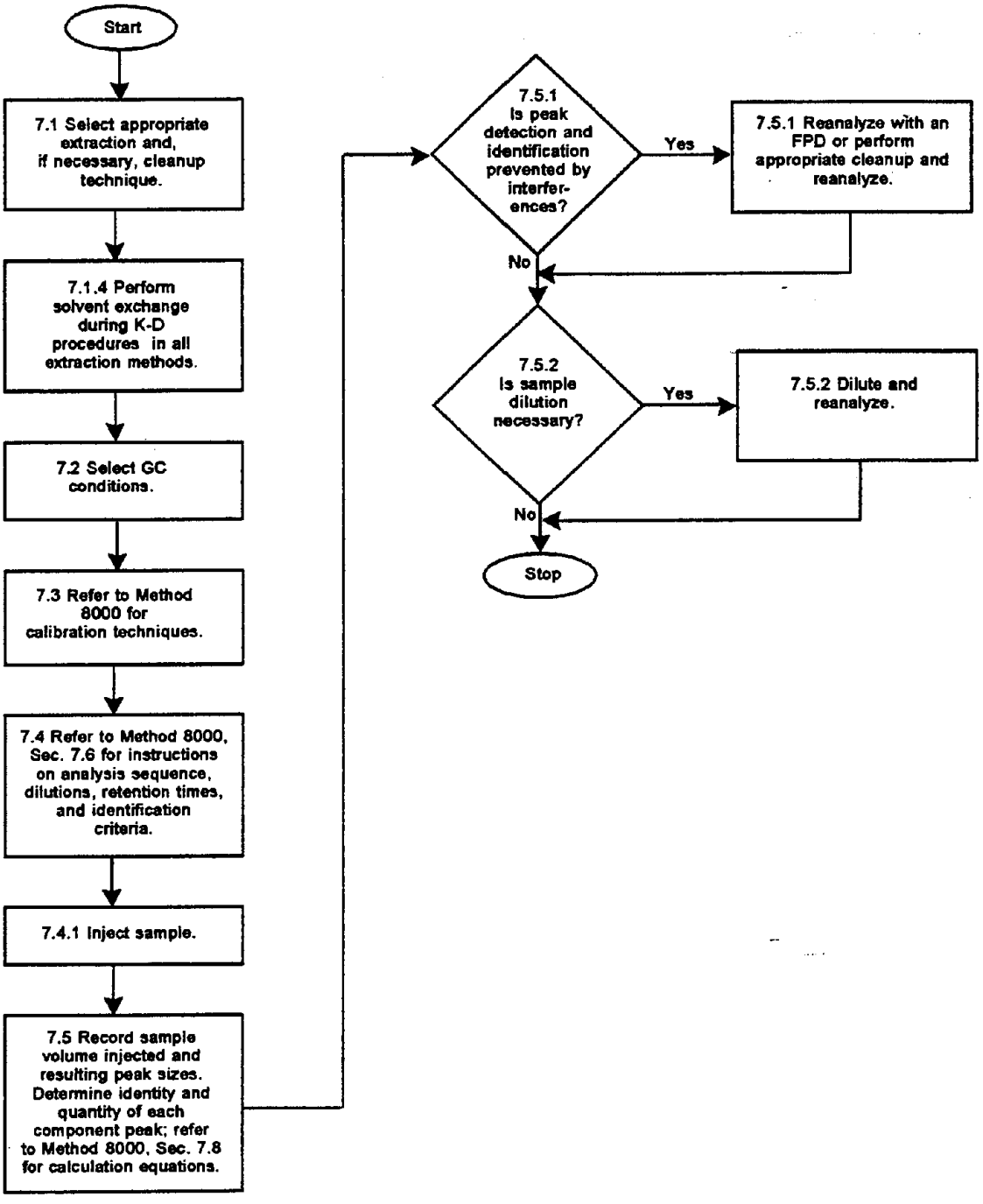


FIGURE 6

Chromatogram of target organophosphorus compounds on a 30-m DB-5/DB-210 column pair with NPD detector, with Simazine, Atrazine and Carbophenothion. See Table 4 for retention times and for GC operating conditions.




Start
7.1 Select appropriate extraction and, if necessary, cleanup technique.
7.1.4 Perform solvent exchange during K-D procedures in all extraction methods.
7.2 Select GC conditions.
7.3 Refer to Method 8000 for calibration techniques.
7.4 Refer to Method 8000, Sec. 7.6 for instructions on analysis sequence, dilutions, retention times, and identification criteria.
7.4.1 Inject sample.
7.5 Record sample volume injected and resulting peak sizes. Determine identity and quantity of each component peak; refer to Method 8000, Sec. 7.8 for calculation equations.
7.5.1 Is peak detection and identification prevented by interfer-ences?
Yes
7.5.1 Reanalyze with an FPD or perform appropriate cleanup and reanalyze.
No
7.5.2 Is sample dilution necessary?
Yes
7.5.2 Dilute and reanalyze.
No
Stop

4.3 DETERMINATION OF ORGANIC ANALYTES

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgements necessary to meet the data quality objectives or needs for the intended use of the data.

4.3.2 GAS CHROMATOGRAPHIC/MASS SPECTROMETRIC METHODS

The following methods are included in this section:

Method 8260B: Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)

Method 8270D: Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)

Method 8275A: Semivolatile Organic Compounds (PAHs and PCBs) in Soils/Sludges and Solid Wastes Using Thermal Extraction/Gas Chromatography/Mass Spectrometry (TE/GC/MS)

Method 8280B: Polychlorinated Dibenzo-*p*-Dioxins and Polychlorinated Dibenzofurans by High Resolution Gas Chromatography/Low Resolution Mass Spectrometry (HRGC/LRMS)

Method 8290A: Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry (HRGC/HRMS)

Appendix A: Procedures for the Collection, Handling, Analysis, and Reporting of Wipe Tests Performed within the Laboratory

METHOD 8270D

SEMIVOLATILE ORGANIC COMPOUNDS
BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

1.0 SCOPE AND APPLICATION

1.1 Method 8270 is used to determine the concentration of semivolatile organic compounds in extracts prepared from many types of solid waste matrices, soils, air sampling media and water samples. Direct injection of a sample may be used in limited applications. The following compounds can be determined by this method:

		Appropriate Preparation Techniques[b]				
Compounds	CAS No[a]	3510	3520	3540/ 3541	3550	3580
Acenaphthene	83-32-9	X	X	X	X	X
Acenaphthylene	208-96-8	X	X	X	X	X
Acetophenone	98-86-2	X	ND	ND	ND	X
2-Acetylaminofluorene	53-96-3	X	ND	ND	ND	X
1-Acetyl-2-thiourea	591-08-2	LR	ND	ND	ND	LR
Aldrin	309-00-2	X	X	X	X	X
2-Aminoanthraquinone	117-79-3	X	ND	ND	ND	X
Aminoazobenzene	60-09-3	X	ND	ND	ND	X
4-Aminobiphenyl	92-67-1	X	ND	ND	ND	X
3-Amino-9-ethylcarbazole	132-32-1	X	X	ND	ND	ND
Anilazine	101-05-3	X	ND	ND	ND	X
Aniline	62-53-3	X	X	ND	X	X
o-Anisidine	90-04-0	X	ND	ND	ND	X
Anthracene	120-12-7	X	X	X	X	X
Aramite	140-57-8	HS(43)	ND	ND	ND	X
Aroclor 1016	12674-11-2	X	X	X	X	X
Aroclor 1221	11104-28-2	X	X	X	X	X
Aroclor 1232	11141-16-5	X	X	X	X	X
Aroclor 1242	53469-21-9	X	X	X	X	X
Aroclor 1248	12672-29-6	X	X	X	X	X
Aroclor 1254	11097-69-1	X	X	X	X	X
Aroclor 1260	11096-82-5	X	X	X	X	X
Azinphos-methyl	86-50-0	HS(62)	ND	ND	ND	X
Barban	101-27-9	LR	ND	ND	ND	LR
Benzidine	92-87-5	CP	CP	CP	CP	CP
Benzoic acid	65-85-0	X	X	ND	X	X
Benz(a)anthracene	56-55-3	X	X	X	X	X
Benzo(b)fluoranthene	205-99-2	X	X	X	X	X

Compounds	CAS No[a]	Appropriate Preparation Techniques[b]				
		3510	3520	3540/ 3541	3550	3580
Benzo(k)fluoranthene	207-08-9	X	X	X	X	X
Benzo(g,h,i)perylene	191-24-2	X	X	X	X	X
Benzo(a)pyrene	50-32-8	X	X	X	X	X
p-Benzoquinone	106-51-4	OE	ND	ND	ND	X
Benzyl alcohol	100-51-6	X	X	ND	X	X
α-BHC	319-84-6	X	X	X	X	X
β-BHC	319-85-7	X	X	X	X	X
δ-BHC	319-86-8	X	X	X	X	X
γ-BHC (Lindane)	58-89-9	X	X	X	X	X
Bis(2-chloroethoxy)methane	111-91-1	X	X	X	X	X
Bis(2-chloroethyl) ether	111-44-4	X	X	X	X	X
Bis(2-chloroisopropyl) ether	108-60-1	X	X	X	X	X
Bis(2-ethylhexyl) phthalate	117-81-7	X	X	X	X	X
4-Bromophenyl phenyl ether	101-55-3	X	X	X	X	X
Bromoxynil	1689-84-5	X	ND	ND	ND	X
Butyl benzyl phthalate	85-68-7	X	X	X	X	X
Captafol	2425-06-1	HS(55)	ND	ND	ND	X
Captan	133-06-2	HS(40)	ND	ND	ND	X
Carbaryl	63-25-2	X	ND	ND	ND	X
Carbofuran	1563-66-2	X	ND	ND	ND	X
Carbophenothion	786-19-6	X	ND	ND	ND	X
Chlordane (NOS)	57-74-9	X	X	X	X	X
Chlorfenvinphos	470-90-6	X	ND	ND	ND	X
4-Chloroaniline	106-47-8	X	ND	ND	ND	X
Chlorobenzilate	510-15-6	X	ND	ND	ND	X
5-Chloro-2-methylaniline	95-79-4	X	ND	ND	ND	X
4-Chloro-3-methylphenol	59-50-7	X	X	X	X	X
3-(Chloromethyl)pyridine hydrochloride	6959-48-4	X	ND	ND	ND	X
1-Chloronaphthalene	90-13-1	X	X	X	X	X
2-Chloronaphthalene	91-58-7	X	X	X	X	X
2-Chlorophenol	95-57-8	X	X	X	X	X
4-Chloro-1,2-phenylenediamine	95-83-0	X	X	ND	ND	ND
4-Chloro-1,3-phenylenediamine	5131-60-2	X	X	ND	ND	ND
4-Chlorophenyl phenyl ether	7005-72-3	X	X	X	X	X
Chrysene	218-01-9	X	X	X	X	X
Coumaphos	56-72-4	X	ND	ND	ND	X
p-Cresidine	120-71-8	X	ND	ND	ND	X
Crotoxyphos	7700-17-6	X	ND	ND	ND	X

Compounds	CAS No[a]	Appropriate Preparation Techniques[b]				
		3510	3520	3540/ 3541	3550	3580
2-Cyclohexyl-4,6-dinitro-phenol	131-89-5	X	ND	ND	ND	LR
4,4'-DDD	72-54-8	X	X	X	X	X
4,4'-DDE	72-55-9	X	X	X	X	X
4,4'-DDT	50-29-3	X	X	X	X	X
Demeton-O	298-03-3	HS(68)	ND	ND	ND	X
Demeton-S	126-75-0	X	ND	ND	ND	X
Diallate (cis or trans)	2303-16-4	X	ND	ND	ND	X
2,4-Diaminotoluene	95-80-7	DC,0E(42)	ND	ND	ND	X
Dibenz(a,j)acridine	224-42-0	X	ND	ND	ND	X
Dibenz(a,h)anthracene	53-70-3	X	X	X	X	X
Dibenzofuran	132-64-9	X	X	ND	X	X
Dibenzo(a,e)pyrene	192-65-4	ND	ND	ND	ND	X
1,2-Dibromo-3-chloropropane	96-12-8	X	X	ND	ND	ND
Di-n-butyl phthalate	84-74-2	X	X	X	X	X
Dichlone	117-80-6	OE	ND	ND	ND	X
1,2-Dichlorobenzene	95-50-1	X	X	X	X	X
1,3-Dichlorobenzene	541-73-1	X	X	X	X	X
1,4-Dichlorobenzene	106-46-7	X	X	X	X	X
3,3'-Dichlorobenzidine	91-94-1	X	X	X	X	X
2,4-Dichlorophenol	120-83-2	X	X	X	X	X
2,6-Dichlorophenol	87-65-0	X	ND	ND	ND	X
Dichlorovos	62-73-7	X	ND	ND	ND	X
Dicrotophos	141-66-2	X	ND	ND	ND	X
Dieldrin	60-57-1	X	X	X	X	X
Diethyl phthalate	84-66-2	X	X	X	X	X
Diethylstilbestrol	56-53-1	AW,0S(67)	ND	ND	ND	X
Diethyl sulfate	64-67-5	LR	ND	ND	ND	LR
Dimethoate	60-51-5	HE,HS(31)	ND	ND	ND	X
3,3'-Dimethoxybenzidine	119-90-4	X	ND	ND	ND	LR
Dimethylaminoazobenzene	60-11-7	X	ND	ND	ND	X
7,12-Dimethylbenz(a)-anthracene	57-97-6	CP(45)	ND	ND	ND	CP
3,3'-Dimethylbenzidine	119-93-7	X	ND	ND	ND	X
α,α-Dimethylphenethylamine	122-09-8	ND	ND	ND	ND	X
2,4-Dimethylphenol	105-67-9	X	X	X	X	X
Dimethyl phthalate	131-11-3	X	X	X	X	X
1,2-Dinitrobenzene	528-29-0	X	ND	ND	ND	X
1,3-Dinitrobenzene	99-65-0	X	ND	ND	ND	X
1,4-Dinitrobenzene	100-25-4	HE(14)	ND	ND	ND	X
4,6-Dinitro-2-methylphenol	534-52-1	X	X	X	X	X

Compounds	CAS No[a]	Appropriate Preparation Techniques[b]				
		3510	3520	3540/ 3541	3550	3580
2,4-Dinitrophenol	51-28-5	X	X	X	X	X
2,4-Dinitrotoluene	121-14-2	X	X	X	X	X
2,6-Dinitrotoluene	606-20-2	X	X	X	X	X
Dinocap	39300-45-3	CP,HS(28)	ND	ND	ND	CP
Dinoseb	88-85-7	X	ND	ND	ND	X
Diphenylamine	122-39-4	X	X	X	X	X
5,5-Diphenylhydantoin	57-41-0	X	ND	ND	ND	X
1,2-Diphenylhydrazine	122-66-7	X	X	X	X	X
Di-n-octyl phthalate	117-84-0	X	X	X	X	X
Disulfoton	298-04-4	X	ND	ND	ND	X
Endosulfan I	959-98-8	X	X	X	X	X
Endosulfan II	33213-65-9	X	X	X	X	X
Endosulfan sulfate	1031-07-8	X	X	X	X	X
Endrin	72-20-8	X	X	X	X	X
Endrin aldehyde	7421-93-4	X	X	X	X	X
Endrin ketone	53494-70-5	X	X	ND	X	X
EPN	2104-64-5	X	ND	ND	ND	X
Ethion	563-12-2	X	ND	ND	ND	X
Ethyl carbamate	51-79-6	DC(28)	ND	ND	ND	X
Ethyl methanesulfonate	62-50-0	X	ND	ND	ND	X
Famphur	52-85-7	X	ND	ND	ND	X
Fensulfothion	115-90-2	X	ND	ND	ND	X
Fenthion	55-38-9	X	ND	ND	ND	X
Fluchloralin	33245-39-5	X	ND	ND	ND	X
Fluoranthene	206-44-0	X	X	X	X	X
Fluorene	86-73-7	X	X	X	X	X
2-Fluorobiphenyl (surr)	321-60-8	X	X	X	X	X
2-Fluorophenol (surr)	367-12-4	X	X	X	X	X
Heptachlor	76-44-8	X	X	X	X	X
Heptachlor epoxide	1024-57-3	X	X	X	X	X
Hexachlorobenzene	118-74-1	X	X	X	X	X
Hexachlorobutadiene	87-68-3	X	X	X	X	X
Hexachlorocyclopentadiene	77-47-4	X	X	X	X	X
Hexachloroethane	67-72-1	X	X	X	X	X
Hexachlorophene	70-30-4	AW,CP(62)	ND	ND	ND	CP
Hexachloropropene	1888-71-7	X	ND	ND	ND	X
Hexamethylphosphoramide	680-31-9	X	ND	ND	ND	X
Hydroquinone	123-31-9	ND	ND	ND	ND	X
Indeno(1,2,3-cd)pyrene	193-39-5	X	X	X	X	X

Compounds	CAS No[a]	Appropriate Preparation Techniques[b] 3510	3520	3540/ 3541	3550	3580
Isodrin	465-73-6	X	ND	ND	ND	X
Isophorone	78-59-1	X	X	X	X	X
Isosafrole	120-58-1	DC(46)	ND	ND	ND	X
Kepone	143-50-0	X	ND	ND	ND	X
Leptophos	21609-90-5	X	ND	ND	ND	X
Malathion	121-75-5	HS(5)	ND	ND	ND	X
Maleic anhydride	108-31-6	HE	ND	ND	ND	X
Mestranol	72-33-3	X	ND	ND	ND	X
Methapyrilene	91-80-5	X	ND	ND	ND	X
Methoxychlor	72-43-5	X	ND	ND	ND	X
3-Methylcholanthrene	56-49-5	X	ND	ND	ND	X
4,4'-Methylenebis (2-chloroaniline)	101-14-4	OE,OS(0)	ND	ND	ND	LR
4,4'-Methylenebis(N,N-dimethyl-aniline)	101-61-1	X	X	ND	ND	ND
Methyl methanesulfonate	66-27-3	X	ND	ND	ND	X
2-Methylnaphthalene	91-57-6	X	X	ND	X	X
Methyl parathion	298-00-0	X	ND	ND	ND	X
2-Methylphenol	95-48-7	X	ND	ND	ND	X
3-Methylphenol	108-39-4	X	ND	ND	ND	X
4-Methylphenol	106-44-5	X	ND	ND	ND	X
Mevinphos	7786-34-7	X	ND	ND	ND	X
Mexacarbate	315-18-4	HE,HS(68)	ND	ND	ND	X
Mirex	2385-85-5	X	ND	ND	ND	X
Monocrotophos	6923-22-4	HE	ND	ND	ND	X
Naled	300-76-5	X	ND	ND	ND	X
Naphthalene	91-20-3	X	X	X	X	X
1,4-Naphthoquinone	130-15-4	X	ND	ND	ND	X
1-Naphthylamine	134-32-7	OS(44)	ND	ND	ND	X
2-Naphthylamine	91-59-8	X	ND	ND	ND	X
Nicotine	54-11-5	DE(67)	ND	ND	ND	X
5-Nitroacenaphthene	602-87-9	X	ND	ND	ND	X
2-Nitroaniline	88-74-4	X	X	ND	X	X
3-Nitroaniline	99-09-2	X	X	ND	X	X
4-Nitroaniline	100-01-6	X	X	ND	X	X
5-Nitro-o-anisidine	99-59-2	X	ND	ND	ND	X
Nitrobenzene	98-95-3	X	X	X	X	X
4-Nitrobiphenyl	92-93-3	X	ND	ND	ND	X
Nitrofen	1836-75-5	X	ND	ND	ND	X
2-Nitrophenol	88-75-5	X	X	X	X	X

Compounds	CAS No[a]	Appropriate Preparation Techniques[b]				
		3510	3520	3540/ 3541	3550	3580
4-Nitrophenol	100-02-7	X	X	X	X	X
5-Nitro-o-toluidine	99-55-8	X	X	ND	ND	X
Nitroquinoline-1-oxide	56-57-5	X	ND	ND	ND	X
N-Nitrosodi-n-butylamine	924-16-3	X	ND	ND	ND	X
N-Nitrosodiethylamine	55-18-5	X	ND	ND	ND	X
N-Nitrosodimethylamine	62-75-9	X	X	X	X	X
N-Nitrosomethylethylamine	10595-95-6	X	ND	ND	ND	X
N-Nitrosodiphenylamine	86-30-6	X	X	X	X	X
N-Nitrosodi-n-propylamine	621-64-7	X	X	X	X	X
N-Nitrosomorpholine	59-89-2	ND	ND	ND	ND	X
N-Nitrosopiperidine	100-75-4	X	ND	ND	ND	X
N-Nitrosopyrrolidine	930-55-2	X	ND	ND	ND	X
Octamethyl pyrophosphoramide	152-16-9	LR	ND	ND	ND	LR
4,4'-Oxydianiline	101-80-4	X	ND	ND	ND	X
Parathion	56-38-2	X	X	ND	ND	X
Pentachlorobenzene	608-93-5	X	ND	ND	ND	X
Pentachloronitrobenzene	82-68-8	X	ND	ND	ND	X
Pentachlorophenol	87-86-5	X	X	X	X	X
Phenacetin	62-44-2	X	ND	ND	ND	X
Phenanthrene	85-01-8	X	X	X	X	X
Phenobarbital	50-06-6	X	ND	ND	ND	X
Phenol	108-95-2	DC(28)	X	X	X	X
1,4-Phenylenediamine	106-50-3	X	ND	ND	ND	X
Phorate	298-02-2	X	ND	ND	ND	X
Phosalone	2310-17-0	HS(65)	ND	ND	ND	X
Phosmet	732-11-6	HS(15)	ND	ND	ND	X
Phosphamidon	13171-21-6	HE(63)	ND	ND	ND	X
Phthalic anhydride	85-44-9	CP,HE(1)	ND	ND	ND	CP
2-Picoline (2-Methylpyridine)	109-06-8	X	X	ND	ND	ND
Piperonyl sulfoxide	120-62-7	X	ND	ND	ND	X
Pronamide	23950-58-5	X	ND	ND	ND	X
Propylthiouracil	51-52-5	LR	ND	ND	ND	LR
Pyrene	129-00-0	X	X	X	X	X
Resorcinol	108-46-3	DC,OE(10)	ND	ND	ND	X
Safrole	94-59-7	X	ND	ND	ND	X
Strychnine	57-24-9	AW,0S(55)	ND	ND	ND	X
Sulfallate	95-06-7	X	ND	ND	ND	X
Terbufos	13071-79-9	X	ND	ND	ND	X
1,2,4,5-Tetrachlorobenzene	95-94-3	X	ND	ND	ND	X

Compounds	CAS No[a]	Appropriate Preparation Techniques[b] 3510	3520	3540/ 3541	3550	3580
2,3,4,6-Tetrachlorophenol	58-90-2	X	ND	ND	ND	X
Tetrachlorvinphos	961-11-5	X	ND	ND	ND	X
Tetraethyl dithiopyrophosphate	3689-24-5	X	X	ND	ND	ND
Tetraethyl pyrophosphate	107-49-3	X	ND	ND	ND	X
Thionazine	297-97-2	X	ND	ND	ND	X
Thiophenol (Benzenethiol)	108-98-5	X	ND	ND	ND	X
Toluene diisocyanate	584-84-9	HE(6)	ND	ND	ND	X
o-Toluidine	95-53-4	X	ND	ND	ND	X
Toxaphene	8001-35-2	X	X	X	X	X
1,2,4-Trichlorobenzene	120-82-1	X	X	X	X	X
2,4,5-Trichlorophenol	95-95-4	X	X	ND	X	X
2,4,6-Trichlorophenol	88-06-2	X	X	X	X	X
Trifluralin	1582-09-8	X	ND	ND	ND	X
2,4,5-Trimethylaniline	137-17-7	X	ND	ND	ND	X
Trimethyl phosphate	512-56-1	HE(60)	ND	ND	ND	X
1,3,5-Trinitrobenzene	99-35-4	X	ND	ND	ND	X
Tris(2,3-dibromopropyl) phosphate	126-72-7	X	ND	ND	ND	LR
Tri-p-tolyl phosphate	78-32-0	X	ND	ND	ND	X
O,O,O-Triethyl phosphorothioate	126-68-1	X	ND	ND	ND	X

[a] Chemical Abstract Service Registry Number
[b] See Sec. 1.2 for other acceptable preparation methods.

KEY TO ANALYTE LIST

AW = Adsorption to walls of glassware during extraction and storage.
CP = Nonreproducible chromatographic performance.
DC = Unfavorable distribution coefficient (number in parenthesis is percent recovery).
HE = Hydrolysis during extraction accelerated by acidic or basic conditions (number in parenthesis is percent recovery).
HS = Hydrolysis during storage (number in parenthesis is percent stability).
LR = Low response.
ND = Not determined.
OE = Oxidation during extraction accelerated by basic conditions (number in parenthesis is percent recovery).
OS = Oxidation during storage (number in parenthesis is percent stability).
X = Greater than 70 percent recovery by this technique.

1.2 In addition to the sample preparation methods listed in the above analyte list, Method 3535 describes a solid-phase extraction procedure that may be applied to the extraction of semivolatiles from TCLP leachates (Tables 16 and 17 contain performance data). Method 3542

describes sample preparation for semivolatile organic compounds in air sampled by Method 0010 (Table 11 contains surrogate performance data), Method 3545 describes an automated solvent extraction device for semivolatiles in solids (Table 12 contains performance data), and Method 3561 describes a supercritical fluid device for the extraction of PAHs from solids (see Tables 13, 14, and 15 for performance data).

1.3 Method 8270 can be used to quantitate most neutral, acidic, and basic organic compounds that are soluble in methylene chloride and capable of being eluted, without derivatization, as sharp peaks from a gas chromatographic fused-silica capillary column coated with a slightly polar silicone. Such compounds include polynuclear aromatic hydrocarbons, chlorinated hydrocarbons and pesticides, phthalate esters, organophosphate esters, nitrosamines, haloethers, aldehydes, ethers, ketones, anilines, pyridines, quinolines, aromatic nitro compounds, and phenols, including nitrophenols. See Table 1 for a list of compounds and their characteristic ions that have been evaluated.

In most cases, Method 8270 is not appropriate for the quantitation of multicomponent analytes, e.g., Aroclors, Toxaphene, Chlordane, etc., because of limited sensitivity for those analytes. When these analytes have been identified by another technique, Method 8270 may be appropriate for confirmation of the identification of these analytes when concentration in the extract permits. Refer to Sec. 7.0 of Methods 8081 and 8082 for guidance on calibration and quantitation of multicomponent analytes such as the Aroclors, Toxaphene, and Chlordane.

1.4 The following compounds may require special treatment when being determined by this method:

1.4.1 Benzidine may be subject to oxidative losses during solvent concentration and its chromatographic behavior is poor.

1.4.2 Under the alkaline conditions of the extraction step from aqueous matrices, α-BHC, γ-BHC, Endosulfan I and II, and Endrin are subject to decomposition. Neutral extraction should be performed if these compounds are expected.

1.4.3 Hexachlorocyclopentadiene is subject to thermal decomposition in the inlet of the gas chromatograph, chemical reaction in acetone solution, and photochemical decomposition.

1.4.4 N-nitrosodimethylamine is difficult to separate from the solvent under the chromatographic conditions described.

1.4.5 N-nitrosodiphenylamine decomposes in the gas chromatographic inlet and cannot be separated from diphenylamine.

1.4.6 Pentachlorophenol, 2,4-dinitrophenol, 4-nitrophenol, benzoic acid, 4,6-dinitro-2-methylphenol, 4-chloro-3-methylphenol, 2-nitroaniline, 3-nitroaniline, 4-chloroaniline, and benzyl alcohol are subject to erratic chromatographic behavior, especially if the GC system is contaminated with high boiling material.

1.4.7 Pyridine may perform poorly at the GC injection port temperatures listed in the method. Lowering the injection port temperature may reduce the amount of degradation. However, the analyst must use caution in modifying the injection port temperature, as the performance of other analytes may be adversely affected. Therefore, if pyridine is to be determined in addition to other target analytes, it may be necessary to perform separate

analyses. In addition, pyridine may be lost during the evaporative concentration of the sample extract. As a result, many of the extraction methods listed above may yield low recoveries unless great care is exercised during the concentration steps. For this reason, analysts may wish to consider the use of extraction techniques such as pressurized fluid extraction (Method 3545) or supercritical fluid extraction, which involve smaller extract volumes, thereby reducing or eliminating the need for evaporative concentration techniques for many applications.

1.4.8 Toluene diisocyanate rapidly hydrolyses in water (half-life of less then 30 min.). Therefore, recoveries of this compound from aqueous matrices should not be expected. In addition, in solid matrices, toluene diisocyanate often reacts with alcohols and amines to produce urethane and ureas and consequently cannot usually coexist in a solution containing these materials.

1.4.9 In addition, analytes in the list provided above are flagged when there are limitations caused by sample preparation and/or chromatographic problems.

1.5 The estimated quantitation limit (EQL) of Method 8270 for determining an individual compound is approximately 660 µg/kg (wet weight) for soil/sediment samples, 1-200 mg/kg for wastes (dependent on matrix and method of preparation), and 10 µg/L for ground water samples (see Table 2). EQLs will be proportionately higher for sample extracts that require dilution to avoid saturation of the detector.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatograph/mass spectrometers and skilled in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 The samples are prepared for analysis by gas chromatography/mass spectrometry (GC/MS) using the appropriate sample preparation (refer to Method 3500) and, if necessary, sample cleanup procedures (refer to Method 3600).

2.2 The semivolatile compounds are introduced into the GC/MS by injecting the sample extract into a gas chromatograph (GC) with a narrow-bore fused-silica capillary column. The GC column is temperature-programmed to separate the analytes, which are then detected with a mass spectrometer (MS) connected to the gas chromatograph.

2.3 Analytes eluted from the capillary column are introduced into the mass spectrometer via a jet separator or a direct connection. Identification of target analytes is accomplished by comparing their mass spectra with the electron impact (or electron impact-like) spectra of authentic standards. Quantitation is accomplished by comparing the response of a major (quantitation) ion relative to an internal standard using a five-point calibration curve.

2.4 The method includes specific calibration and quality control steps that supersede the general requirements provided in Method 8000.

3.0 INTERFERENCES

3.1 Raw GC/MS data from all blanks, samples, and spikes must be evaluated for interferences. Determine if the source of interference is in the preparation and/or cleanup of the samples and take corrective action to eliminate the problem.

3.2 Contamination by carryover can occur whenever high-concentration and low-concentration samples are sequentially analyzed. To reduce carryover, the sample syringe must be rinsed with solvent between sample injections. Whenever an unusually concentrated sample is encountered, it should be followed by the analysis of solvent to check for cross-contamination.

4.0 APPARATUS AND MATERIALS

4.1 Gas chromatograph/mass spectrometer system

4.1.1 Gas chromatograph - An analytical system complete with a temperature-programmable gas chromatograph suitable for splitless injection and all required accessories, including syringes, analytical columns, and gases. The capillary column should be directly coupled to the source.

4.1.2 Column - 30-m x 0.25-mm ID (or 0.32-mm ID) 1-µm film thickness silicone-coated fused-silica capillary column (J&W Scientific DB-5 or equivalent).

4.1.3 Mass spectrometer

4.1.3.1 Capable of scanning from 35 to 500 amu every 1 sec or less, using 70 volts (nominal) electron energy in the electron impact ionization mode. The mass spectrometer must be capable of producing a mass spectrum for decafluorotriphenylphosphine (DFTPP) which meets the criteria in Table 3 when 1 µL of the GC/MS tuning standard is injected through the GC (50 ng of DFTPP).

4.1.3.2 An ion trap mass spectrometer may be used if it is capable of axial modulation to reduce ion-molecule reactions and can produce electron impact-like spectra that match those in the EPA/NIST Library. The mass spectrometer must be capable of producing a mass spectrum for DFTPP which meets the criteria in Table 3 when 5 or 50 ng are introduced.

4.1.4 GC/MS interface - Any GC-to-MS interface may be used that gives acceptable calibration points at 50 ng per injection for each compound of interest and achieves acceptable tuning performance criteria. For a narrow-bore capillary column, the interface is usually capillary-direct into the mass spectrometer source.

4.1.5 Data system - A computer system should be interfaced to the mass spectrometer. The system must allow the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program. The computer should have software that can search any GC/MS data file for ions of a specific mass and that can plot such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software should also be available that allows integrating the abundances in any EICP between specified time or scan-number limits. The most recent version of the EPA/NIST Mass Spectral Library should also be available.

4.1.6 Guard column (optional) - (J&W deactivated fused-silica, 0.25-mm ID x 6-m, or equivalent) between the injection port and the analytical column joined with column joiners (Hewlett-Packard Catalog No. 5062-3556, or equivalent).

4.2 Syringe - 10-µL.

4.3 Volumetric flasks, Class A - Appropriate sizes with ground-glass stoppers.

4.4 Balance - Analytical, capable of weighing 0.0001 g.

4.5 Bottles - glass with polytetrafluoroethylene (PTFE)-lined screw caps or crimp tops.

5.0 REAGENTS

5.1 Reagent grade inorganic chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Stock standard solutions (1000 mg/L) - Standard solutions can be prepared from pure standard materials or purchased as certified solutions.

5.3.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide quality acetone or other suitable solvent and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is assayed to be 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially-prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

5.3.2 Transfer the stock standard solutions into bottles with PTFE-lined screw-caps. Store, protected from light, at -10°C or less or as recommended by the standard manufacturer. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

5.3.3 Stock standard solutions must be replaced after 1 year or sooner if comparison with quality control check samples indicates a problem.

5.3.4 It is recommended that nitrosamine compounds be placed together in a separate calibration mix and not combined with other calibration mixes. When using a premixed certified standard, consult the manufacturer's instructions for additional guidance.

5.3.5 Mixes with hydrochloride salts may contain hydrochloric acid, which can cause analytical difficulties. When using a premixed certified standard, consult the manufacturer's instructions for additional guidance.

5.4 Internal standard solutions - The internal standards recommended are 1,4-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12} (see Table 5). Other compounds may be used as internal standards as long as the specifications in Sec. 7.3.2 are met.

5.4.1 Dissolve 0.200 g of each compound with a small volume of carbon disulfide. Transfer to a 50 mL volumetric flask and dilute to volume with methylene chloride so that the final solvent is approximately 20% carbon disulfide. Most of the compounds are also soluble in small volumes of methanol, acetone, or toluene, except for perylene-d_{12}. The resulting solution will contain each standard at a concentration of 4,000 ng/µL. Each 1-mL sample extract undergoing analysis should be spiked with 10 µL of the internal standard solution, resulting in a concentration of 40 ng/µL of each internal standard. Store at -10°C or less when not in use. When using premixed certified solutions, store according to the manufacturer's documented holding time and storage temperature recommendations.

5.4.2 If a more sensitive mass spectrometer is employed to achieve lower detection levels, a more dilute internal standard solution may be required. Area counts of the internal standard peaks should be between 50-200% of the area of the target analytes in the mid-point calibration analysis.

5.5 GC/MS tuning standard - A methylene chloride solution containing 50 ng/µL of decafluorotriphenylphosphine (DFTPP) should be prepared. The standard should also contain 50 ng/µL each of 4,4'-DDT, pentachlorophenol, and benzidine to verify injection port inertness and GC column performance. Store at -10°C or less when not in use. If a more sensitive mass spectrometer is employed to achieve lower detection levels, a more dilute tuning solution may be necessary. When using premixed certified solutions, store according to the manufacturer's documented holding time and storage temperature recommendations.

5.6 Calibration standards - A minimum of five calibration standards should be prepared at five different concentrations. At least one of the calibration standards should correspond to a sample concentration at or below that necessary to meet the data quality objectives of the project. The remaining standards should correspond to the range of concentrations found in actual samples but should not exceed the working range of the GC/MS system. Each standard should contain each analyte for detection by this method.

5.6.1 It is the intent of EPA that all target analytes for a particular analysis be included in the calibration standard(s). These target analytes may not include the entire list of analytes (Sec. 1.1) for which the method has been demonstrated. However, the laboratory shall not report a quantitative result for a target analyte that was not included in the calibration standard(s).

5.6.2 Each 1-mL aliquot of calibration standard should be spiked with 10 µL of the internal standard solution prior to analysis. All standards should be stored at -10°C or less, and should be freshly prepared once a year, or sooner if check standards indicate a problem. The calibration verification standard should be prepared weekly and stored at 4°C. When using premixed certified solutions, store according to the manufacturer's documented holding time and storage temperature recommendations.

5.7 Surrogate standards - The recommended surrogates are phenol-d_6, 2-fluorophenol, 2,4,6-tribromophenol, nitrobenzene-d_5, 2-fluorobiphenyl, and p-terphenyl-d_{14}. See Method 3500 for instructions on preparing the surrogate solutions.

5.7.1 Surrogate standard check - Determine what the appropriate concentration should be for the blank extracts after all extraction, cleanup, and concentration steps. Inject this concentration into the GC/MS to determine recovery of surrogate standards. It is recommended that this check be done whenever a new surrogate spiking solution is prepared.

NOTE: Method 3561 (SFE Extraction of PAHs) recommends the use of bromobenzene and p-quaterphenyl to better cover the range of PAHs listed in the method.

5.7.2 If a more sensitive mass spectrometer is employed to achieve lower detection levels, a more dilute surrogate solution may be necessary.

5.8 Matrix spike and laboratory control standards - See Method 3500 for instructions on preparing the matrix spike standard. The same standard may be used as the laboratory control standard (LCS).

5.8.1 Matrix spike check - Determine what concentration should be in the blank extracts after all extraction, cleanup, and concentration steps. Inject this concentration into the GC/MS to determine recovery. It is recommended that this check be done whenever a new matrix spiking solution is prepared.

5.8.2 If a more sensitive mass spectrometer is employed to achieve lower detection levels, a more dilute matrix and LCS spiking solution may be necessary.

5.8.3 Some projects may require the spiking of the specific compounds of interest, since the spiking compounds listed in Method 3500 would not be representative of the compounds of interest required for the project. When this occurs, the matrix and LCS spiking standards should be prepared in methanol, with each compound present at a concentration appropriate for the project.

5.9 Solvents - Acetone, hexane, methylene chloride, isooctane, carbon disulfide, toluene, and other appropriate solvents. All solvents should be pesticide quality or equivalent.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

6.2 Store the sample extracts at -10°C, protected from light, in sealed vials (e.g., screw-cap vials or crimp-capped vials) equipped with unpierced PTFE-lined septa.

7.0 PROCEDURE

7.1 Sample preparation

7.1.1 Samples are normally prepared by one of the following methods prior to GC/MS analysis.

Matrix	Methods
Air (particulates and sorbent resin)	3542
Water (including TCLP leachates)	3510, 3520, 3535
Soil/sediment	3540, 3541, 3545, 3550, 3560, 3561
Waste	3540, 3541, 3545, 3550, 3560, 3561, 3580

7.1.2 In very limited applications, direct injection of the sample into the GC/MS system with a 10-µL syringe may be appropriate. The detection limit is very high (approximately 10,000 µg/L). Therefore, it is only permitted where concentrations in excess of 10,000 µg/L are expected.

7.2 Extract cleanup - Extracts may be cleaned up by any of the following methods prior to GC/MS analysis.

Analytes of interest	Methods
Aniline & aniline derivatives	3620
Phenols	3630, 3640, 8041[a]
Phthalate esters	3610, 3620, 3640
Nitrosamines	3610, 3620, 3640
Organochlorine pesticides & PCBs	3610, 3620, 3630, 3660, 3665
Nitroaromatics and cyclic ketones	3620, 3640
Polynuclear aromatic hydrocarbons	3611, 3630, 3640
Haloethers	3620, 3640
Chlorinated hydrocarbons	3620, 3640
Organophosphorus pesticides	3620
Petroleum waste	3611, 3650
All base, neutral, and acid priority pollutants	3640

[a] Method 8041 includes a derivatization technique and a GC/ECD analysis, if interferences are encountered on GC/FID.

7.3 Initial calibration

Establish the GC/MS operating conditions, using the following recommendations as guidance.

Mass range:	35-500 amu
Scan time:	1 sec/scan
Initial temperature:	40°C, hold for 4 minutes
Temperature program:	40-270°C at 10°C/min
Final temperature:	270°C, hold until benzo[g,h,i]perylene elutes
Injector temperature:	250-300°C
Transfer line temperature:	250-300°C
Source temperature:	According to manufacturer's specifications
Injector:	Grob-type, splitless
Injection volume:	1-2 µL
Carrier gas:	Hydrogen at 50 cm/sec or helium at 30 cm/sec
Ion trap only:	Set axial modulation, manifold temperature, and emission current to manufacturer's recommendations

Split injection is allowed if the sensitivity of the mass spectrometer is sufficient.

7.3.1 The GC/MS system must be hardware-tuned using a 50-ng injection of DFTPP. Analyses must not begin until the tuning criteria are met.

7.3.1.1 In the absence of specific recommendations on how to acquire the mass spectrum of DFTPP from the instrument manufacturer, the following approach has been shown to be useful: Three scans (the peak apex scan and the scans immediately preceding and following the apex) are acquired and averaged. Background subtraction is required, and must be accomplished using a single scan acquired no more than 20 scans prior to the elution of DFTPP. The background subtraction should be designed only to eliminate column bleed or instrument background ions. Do not subtract part of the DFTPP peak.

7.3.1.2 Use the DFTPP mass intensity criteria in Table 3 as tuning acceptance criteria. Alternatively, other documented tuning criteria may be used (e.g. CLP, Method 525, or manufacturer's instructions), provided that method performance is not adversely affected.

NOTE: All subsequent standards, samples, MS/MSDs, and blanks associated with a DFTPP analysis must use the identical mass spectrometer instrument conditions.

7.3.1.3 The GC/MS tuning standard solution should also be used to assess GC column performance and injection port inertness. Degradation of DDT to DDE and DDD should not exceed 20%. (See Sec. 8.0 of Method 8081 for the percent breakdown calculation). Benzidine and pentachlorophenol should be present at their normal responses, and no peak tailing should be visible.

7.3.1.4 If degradation is excessive and/or poor chromatography is noted, the injection port may require cleaning. It may also be necessary to break off the first 6-12 in. of the capillary column. The use of a guard column (Sec. 4.1.6) between the injection port and the analytical column may help prolong analytical column performance.

7.3.2 The internal standards selected in Sec. 5.4 should permit most of the components of interest in a chromatogram to have retention times of 0.80-1.20 relative to one of the internal standards. Use the base peak ion from the specific internal standard as the primary ion for quantitation (see Table 1). If interferences are noted, use the next most intense ion as the quantitation ion (i.e. for 1,4-dichlorobenzene-d_4, use 152 m/z for quantitation).

7.3.3 Analyze 1-2 µL of each calibration standard (containing internal standards) and tabulate the area of the primary characteristic ion against concentration for each target analyte (as indicated in Table 1). A set of at least five calibration standards is necessary (see Sec. 5.6 and Method 8000). The injection volume must be the same for all standards and sample extracts. Figure 1 shows a chromatogram of a calibration standard containing base/neutral and acid analytes.

Calculate response factors (RFs) for each target analyte relative to one of the internal standards as follows:

$$RF = \frac{A_s \times C_{is}}{A_{is} \times C_s}$$

where:

A_s = Peak area (or height) of the analyte or surrogate.
A_{is} = Peak area (or height) of the internal standard.
C_s = Concentration of the analyte or surrogate, in µg/L.
C_{is} = Concentration of the internal standard, in µg/L.

7.3.4 System performance check compounds (SPCCs)

7.3.4.1 A system performance check must be performed to ensure that minimum average RFs are met before the calibration curve is used. For semivolatiles, the System performance check compounds (SPCCs) are: N-nitroso-di-n-propylamine; hexachlorocyclopentadiene; 2,4-dinitrophenol; and 4-nitrophenol.

7.3.4.2 The minimum acceptable average RF for these compounds is 0.050. These SPCCs typically have very low RFs (0.1-0.2) and tend to decrease in response as the chromatographic system begins to deteriorate or the standard material begins to deteriorate. They are usually the first to show poor performance. Therefore, they must meet the minimum requirement when the system is calibrated.

7.3.4.3 If the minimum response factors are not met, the system must be evaluated, and corrective action must be taken before sample analysis begins. Possible problems include standard mixture degradation, injection port inlet contamination, contamination at the front end of the analytical column, and active sites in the column or chromatographic system. This check must be met before sample analysis begins.

7.3.5 Calibration check compounds (CCCs)

7.3.5.1 The purpose of the CCCs are to evaluate the calibration from the standpoint of the integrity of the system. High variability for these compounds may be indicative of system leaks or reactive sites on the column. Meeting the CCC criteria is not a substitute for successful calibration of the target analytes using one of the approaches described in Sec. 7.0 of Method 8000.

7.3.5.2 Calculate the mean response factor and the relative standard deviation (RSD) of the response factors for each target analyte. The RSD should be less than or equal to 15% for each target analyte. However, the RSD for each individual CCC (see Table 4) must be less than or equal to 30%.

$$\text{mean RF} = \overline{RF} = \frac{\sum_{i=1}^{n} RF_i}{n} \qquad SD = \sqrt{\frac{\sum_{i=1}^{n} (RF_i - \overline{RF})^2}{n-1}}$$

$$RSD = \frac{SD}{\overline{RF}} \times 100$$

7.3.5.3 If the RSD of any CCC is greater than 30%, then the chromatographic system is too reactive for analysis to begin. Clean or replace the injector liner and/or capillary column, then repeat the calibration procedure beginning with Sec. 7.3.

7.3.5.4 If the CCCs are not included in the list of analytes for a project, and therefore not included in the calibration standards, then refer to Sec. 7.0 of Method 8000.

7.3.6 Evaluation of retention times - The relative retention time (RRT) of each target analyte in each calibration standard should agree within 0.06 RRT units. Late-eluting target analytes usually have much better agreement.

$$RRT = \frac{\text{Retention time of the analyte}}{\text{Retention time of the internal standard}}$$

7.3.7 Linearity of target analytes - If the RSD of any target analytes is 15% or less, then the relative response factor is assumed to be constant over the calibration range, and the average relative response factor may be used for quantitation (Sec. 7.6.2).

7.3.7.1 If the RSD of any target analyte is greater than 15%, refer to Sec. 7.0 in Method 8000 for additional calibration options. One of the options must be applied to GC/MS calibration in this situation, or a new initial calibration must be performed.

NOTE: Method 8000 designates a linearity criterion of 20% RSD. That criterion pertains to GC and HPLC methods other than GC/MS. Method 8270 requires 15% RSD as evidence of sufficient linearity to employ an average response factor.

7.3.7.2 When the RSD exceeds 15%, the plotting and visual inspection of a calibration curve can be a useful diagnostic tool. The inspection may indicate analytical problems, including errors in standard preparation, the presence of active sites in the chromatographic system, analytes that exhibit poor chromatographic behavior, etc.

7.4 GC/MS calibration verification - Calibration verification consists of three steps that are performed at the beginning of each 12-hour analytical shift.

7.4.1 Prior to the analysis of samples or calibration standards, inject 50 ng of the DFTPP standard into the GC/MS system. The resultant mass spectrum for DFTPP must meet the criteria given in Table 3 before sample analysis begins. These criteria must be demonstrated each 12-hour shift during which samples are analyzed.

7.4.2 The initial calibration (Sec. 7.3) for each compound of interest should be verified once every 12 hours prior to sample analysis, using the introduction technique and

conditions used for samples. This is accomplished by analyzing a calibration standard at a concentration near the midpoint concentration for the calibrating range of the GC/MS. The results from the calibration standard analysis should meet the verification acceptance criteria provided in Secs. 7.4.4 through 7.4.7.

NOTE: The DFTPP and calibration verification standard may be combined into a single standard as long as both tuning and calibration verification acceptance criteria for the project can be met without interferences.

7.4.3 A method blank should be analyzed either after the calibration standard, or at any other time during the analytical shift, to ensure that the total system (introduction device, transfer lines and GC/MS system) is free of contaminants. If the method blank indicates contamination, then it may be appropriate to analyze a solvent blank to demonstrate that the contamination is not a result of carryover from standards or samples. See Sec. 8.0 of Method 8000B for method blank performance criteria.

7.4.4 System performance check compounds (SPCCs)

7.4.4.1 A system performance check must be made during every 12-hour analytical shift. Each SPCC in the calibration verification standard must meet a minimum response factor of 0.050. This is the same check that is applied during the initial calibration.

7.4.4.2 If the minimum response factors are not met, the system must be evaluated, and corrective action must be taken before sample analysis begins. Possible problems include standard mixture degradation, injection port inlet contamination, contamination at the front end of the analytical column, and active sites in the column or chromatographic system. This check must be met before sample analysis begins.

7.4.5 Calibration check compounds (CCCs)

7.4.5.1 After the system performance check is met, the CCCs listed in Table 4 are used to check the validity of the initial calibration. Use percent difference when performing the average response factor model calibration. Use percent drift when calibrating using a regression fit model. Refer to Sec. 7.0 of Method 8000 for guidance on calculating percent difference and drift.

7.4.5.2 If the percent difference for each CCC is less than or equal to 20%, then the initial calibration is assumed to be valid. If the criterion is not met (i.e., greater than 20% difference or drift) for any one CCC, then corrective action must be taken prior to the analysis of samples. If the CCCs are not included in the list of analytes for a project, and therefore not included in the calibration standards, then all analytes must meet the 20% difference or drift criterion.

7.4.5.3 Problems similar to those listed under SPCCs could affect the CCCs. If the problem cannot be corrected by other measures, a new initial calibration must be generated. The CCC criteria must be met before sample analysis begins.

7.4.6 Internal standard retention time - The retention times of the internal standards in the calibration verification standard must be evaluated immediately after or during data acquisition. If the retention time for any internal standard changes by more than 30 seconds

from that in the mid-point standard level of the most recent initial calibration sequence, then the chromatographic system must be inspected for malfunctions and corrections must be made, as required. When corrections are made, reanalysis of samples analyzed while the system was malfunctioning is required.

7.4.7 Internal standard response - If the EICP area for any of the internal standards in the calibration verification standard changes by a factor of two (-50% to +100%) from that in the mid-point standard level of the most recent initial calibration sequence, the mass spectrometer must be inspected for malfunctions and corrections must be made, as appropriate. When corrections are made, reanalysis of samples analyzed while the system was malfunctioning is required.

7.5 GC/MS analysis of samples

7.5.1 It is highly recommended that sample extracts be screened on a GC/FID or GC/PID using the same type of capillary column used in the GC/MS system. This will minimize contamination of the GC/MS system from unexpectedly high concentrations of organic compounds.

7.5.2 Allow the sample extract to warm to room temperature. Just prior to analysis, add 10 µL of the internal standard solution to the 1-mL concentrated sample extract obtained from sample preparation.

7.5.3 Inject a 1-2 µL aliquot of the sample extract into the GC/MS system, using the same operating conditions that were used for the calibration (Sec. 7.3). The volume to be injected should contain 100 ng of base/neutral and 200 ng of acid surrogates (assuming 100% recovery), unless a more sensitive GC/MS system is being used and the surrogate solution is less concentrated then that listed in Sec. 5.7. The injection volume must be the same volume used for the calibration standards.

7.5.4 If the response for any quantitation ion exceeds the initial calibration range of the GC/MS system, the sample extract must be diluted and reanalyzed. Additional internal standard solution must be added to the diluted extract to maintain the same concentration as in the calibration standards (40 ng/µL, unless a more sensitive GC/MS system is being used). Secondary ion quantitation should be used only when there are sample interferences with the primary ion.

NOTE: It may be a useful diagnostic tool to monitor internal standard retention times and responses (area counts) in all samples, spikes, blanks, and standards to effectively check drifting method performance, poor injection execution, and anticipate the need for system inspection and/or maintenance.

7.5.4.1 When ions from a compound in the sample saturate the detector, this analysis must be followed by the analysis of an instrument blank consisting of clean solvent. If the blank analysis is not free of interferences, then the system must be decontaminated. Sample analysis may not resume until the blank analysis is demonstrated to be free of interferences.

7.5.4.2 All dilutions should keep the response of the major constituents (previously saturated peaks) in the upper half of the linear range of the curve.

7.5.5 The use of selected ion monitoring (SIM) is acceptable for applications requiring detection limits below the normal range of electron impact mass spectrometry. However, SIM may provide a lesser degree of confidence in the compound identification unless multiple ions are monitored for each compound.

7.6 Qualitative analysis

7.6.1 The qualitative identification of compounds determined by this method is based on retention time and on comparison of the sample mass spectrum, after background correction, with characteristic ions in a reference mass spectrum. The reference mass spectrum must be generated by the laboratory using the conditions of this method. The characteristic ions from the reference mass spectrum are defined as the three ions of greatest relative intensity, or any ions over 30% relative intensity, if less than three such ions occur in the reference spectrum. Compounds are identified when the following criteria are met.

7.6.1.1 The intensities of the characteristic ions of a compound must maximize in the same scan or within one scan of each other. Selection of a peak by a data system target compound search routine where the search is based on the presence of a target chromatographic peak containing ions specific for the target compound at a compound-specific retention time will be accepted as meeting this criterion.

7.6.1.2 The RRT of the sample component is within ± 0.06 RRT units of the RRT of the standard component.

7.6.1.3 The relative intensities of the characteristic ions agree within 30% of the relative intensities of these ions in the reference spectrum. (Example: For an ion with an abundance of 50% in the reference spectrum, the corresponding abundance in a sample spectrum can range between 20% and 80%.)

7.6.1.4 Structural isomers that produce very similar mass spectra should be identified as individual isomers if they have sufficiently different GC retention times. Sufficient GC resolution is achieved if the height of the valley between two isomer peaks is less than 25% of the sum of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs. Diastereomeric pairs (e.g., Aramite and Isosafrol) that may be separable by the GC should be identified, quantitated and reported as the sum of both compounds by the GC.

7.6.1.5 Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. When gas chromatographic peaks obviously represent more than one sample component (i.e., a broadened peak with shoulder(s) or a valley between two or more maxima), appropriate selection of analyte spectra and background spectra is important.

7.6.1.6 Examination of extracted ion current profiles of appropriate ions can aid in the selection of spectra and in qualitative identification of compounds. When analytes coelute (i.e., only one chromatographic peak is apparent), the identification criteria may be met, but each analyte spectrum will contain extraneous ions contributed by the coeluting compound.

7.6.2 For samples containing components not associated with the calibration standards, a library search may be made for the purpose of tentative identification. The necessity to perform this type of identification will be determined by the purpose of the analyses being conducted. Data system library search routines should not use normalization routines that would misrepresent the library or unknown spectra when compared to each other.

For example, the RCRA permit or waste delisting requirements may require the reporting of non-target analytes. Only after visual comparison of sample spectra with the nearest library searches may the analyst assign a tentative identification. Guidelines for tentative identification are:

(1) Relative intensities of major ions in the reference spectrum (ions > 10% of the most abundant ion) should be present in the sample spectrum.

(2) The relative intensities of the major ions should agree within ± 20%. (Example: For an ion with an abundance of 50% in the standard spectrum, the corresponding sample ion abundance must be between 30 and 70%.)

(3) Molecular ions present in the reference spectrum should be present in the sample spectrum.

(4) Ions present in the sample spectrum but not in the reference spectrum should be reviewed for possible background contamination or presence of coeluting compounds.

(5) Ions present in the reference spectrum but not in the sample spectrum should be reviewed for possible subtraction from the sample spectrum because of background contamination or coeluting peaks. Data system library reduction programs can sometimes create these discrepancies.

7.7 Quantitative analysis

7.7.1 Once a compound has been identified, the quantitation of that compound will be based on the integrated abundance of the primary characteristic ion from the EICP.

7.7.2 If the RSD of a compound's response factor is 15% or less, then the concentration in the extract may be determined using the average response factor ($\overline{RF}$) from initial calibration data (Sec. 7.3.5). See Method 8000, Sec. 7.0, for the equations describing internal standard calibration and either linear or non-linear calibrations.

7.7.3 Where applicable, the concentration of any non-target analytes identified in the sample (Sec. 7.6.2) should be estimated. The same formulae should be used with the following modifications: The areas A_x and A_{is} should be from the total ion chromatograms, and the RF for the compound should be assumed to be 1.

7.7.4 The resulting concentration should be reported indicating: (1) that the value is an estimate, and (2) which internal standard was used to determine concentration. Use the nearest internal standard free of interferences.

7.7.5 Quantitation of multicomponent compounds (e.g., Toxaphene, Aroclors, etc.) is beyond the scope of Method 8270. Normally, quantitation is performed using a GC/ECD, by Methods 8081 or 8082. However, Method 8270 may be used to confirm the identification

of these compounds, when the concentrations are at least 10 ng/µL in the concentrated sample extract.

7.7.6 Structural isomers that produce very similar mass spectra should be quantitated as individual isomers if they have sufficiently different GC retention times. Sufficient GC resolution is achieved if the height of the valley between two isomer peaks is less than 25% of the sum of the two peak heights. Otherwise, structural isomers are quantitated as isomeric pairs. Diastereomeric pairs (e.g., Aramite and Isosafrol) that may be separable by the GC should be summed and reported as the sum of both compounds.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Quality control procedures to ensure the proper operation of the various sample preparation and/or sample introduction techniques can be found in Method 3500. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures necessary to evaluate the GC system operation are found in Sec. 7.0 of Method 8000 and include calibration verification and chromatographic analysis of samples. In addition, instrument QC requirements may be found in the following sections of Method 8270:

8.2.1 The GC/MS system must be tuned to meet the DFTPP criteria discussed in Secs. 7.3.1 and 7.4.1.

8.2.2 There must be an initial calibration of the GC/MS system as described in Sec. 7.3.

8.2.3 The GC/MS system must meet the calibration verification acceptance criteria in Sec. 7.4, each 12 hours.

8.2.4 The RRT of the sample component must fall within the RRT window of the standard component provided in Sec. 7.6.1.

8.3 Initial demonstration of proficiency - Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Method 8000, Sec. 8.0 for information on how to accomplish this demonstration.

8.4 Sample quality control for preparation and analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch and the addition of surrogates to each field sample and QC sample.

8.4.1 Before processing any samples, the analyst should demonstrate, through the analysis of a method blank, that interferences from the analytical system, glassware, and reagents are under control. Each time a set of samples is analyzed or there is a change in

reagents, a method blank should be analyzed as a safeguard against chronic laboratory contamination. The blanks should be carried through all stages of sample preparation and measurement.

8.4.2 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair.

8.4.3 A laboratory control sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

8.4.4 See Method 8000, Sec. 8.0 for the details on carrying out sample quality control procedures for preparation and analysis.

8.5 Surrogate recoveries - The laboratory must evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000, Sec. 8.0 for information on evaluating surrogate data and developing and updating surrogate limits.

8.6 The experience of the analyst performing GC/MS analyses is invaluable to the success of the methods. Each day that analysis is performed, the calibration verification standard should be evaluated to determine if the chromatographic system is operating properly. Questions that should be asked are: Do the peaks look normal? Is the response obtained comparable to the response from previous calibrations? Careful examination of the standard chromatogram can indicate whether the column is still performing acceptably, the injector is leaking, the injector septum needs replacing, etc. If any changes are made to the system (e.g., the column changed, a septum is changed), see the guidance in Sec 8.2 of Method 8000 regarding whether recalibration of the system must take place.

8.7 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 Method 8250 (the packed column version of Method 8270) was tested by 15 laboratories using organic-free reagent water, drinking water, surface water, and industrial wastewaters spiked at six concentrations ranging from 5 to 1,300 µg/L. Single operator accuracy and precision, and method accuracy were found to be directly related to the concentration of the analyte and essentially independent of the sample matrix. Linear equations to describe these relationships are presented in Table 7. These values are presented as guidance only and are not

intended as absolute acceptance criteria. Laboratories should generate their own acceptance criteria for capillary column method performance. (See Method 8000.)

9.2 Chromatograms from calibration standards analyzed with Day 0 and Day 7 samples were compared to detect possible deterioration of GC performance. These recoveries (using Method 3510 extraction) are presented in Table 8.

9.3 Method performance data using Method 3541 (automated Soxhlet extraction) are presented in Table 9. Single laboratory accuracy and precision data were obtained for semivolatile organics in a clay soil by spiking at a concentration of 6 mg/kg for each compound. The spiking solution was mixed into the soil during addition and then allowed to equilibrate for approximately 1 hour prior to extraction. The spiked samples were then extracted by Method 3541 (Automated Soxhlet). Three extractions were performed and each extract was analyzed by gas chromatography/mass spectrometry following Method 8270. The low recovery of the more volatile compounds is probably due to volatilization losses during equilibration. These data are listed in Table 10 and were taken from Reference 7.

9.4 Surrogate precision and accuracy data are presented in Table 11 from a field dynamic spiking study based on air sampling by Method 0010. The trapping media were prepared for analysis by Method 3542 and subsequently analyzed by Method 8270.

9.5 Single laboratory precision and bias data using Method 3545 (pressurized fluid extraction) for semivolatile organic compounds are presented in Table 12. The samples were conditioned spiked samples prepared and certified by a commercial supplier that contained 57 semivolatile organics at three concentrations (250, 2500, and 12,500 µg/kg) on three types of soil (clay, loam and sand). Spiked samples were extracted both by the Dionex Accelerated Solvent Extraction system and by the Perstorp Environmental Soxtec™ (automated Soxhlet). The data in Table 12 represent seven replicate extractions and analyses for each individual sample and were taken from reference 9. The average recoveries from the three matrices for all analytes and all replicates relative to the automated Soxhlet data are as follows: clay 96.8%, loam 98.7% and sand 102.1%. The average recoveries from the three concentrations also relative to the automated Soxhlet data are as follows: low-101.2%, mid-97.2% and high-99.2%.

9.6 Single laboratory precision and bias data using Method 3561 (SFE extraction of PAHs with a variable restrictor and solid trapping material) were obtained for the method analytes by the extraction of two certified reference materials (EC-1, a lake sediment from Environment Canada and HS-3, a marine sediment from the National Science and Engineering Research Council of Canada, both naturally-contaminated with PAHs). The SFE instrument used for these extractions was a Hewlett-Packard Model 7680. Analysis was by GC/MS. Average recoveries from six replicate extractions range from 85 to 148% (overall average of 100%) based on the certified value (or a Soxhlet value if a certified value was unavailable for a specific analyte) for the lake sediment. Average recoveries from three replicate extractions range from 73 to 133% (overall average of 92%) based on the certified value for the marine sediment. The data are found in Tables 13 and 14 and were taken from Reference 10.

9.7 Single laboratory precision and accuracy data using Method 3561 (SFE extraction of PAHs with a fixed restrictor and liquid trapping) were obtained for twelve of the method analytes by the extraction of a certified reference material (a soil naturally contaminated with PAHs). The SFE instrument used for these extractions was a Dionex Model 703-M. Analysis was by GC/MS. Average recoveries from four replicate extractions range from 60 to 122% (overall average of 89%) based on the certified value. Following are the instrument conditions that were utilized to extract a 3.4 g sample: Pressure - 300 atm; Time - 60 min.; Extraction fluid - CO_2; Modifier - 10% 1:1 (v/v)

methanol/methylene chloride; Oven temperature - 80°C; Restrictor temperature - 120°C; and, Trapping fluid - chloroform (methylene chloride has also been used). The data are found in Table 15 and were taken from Reference 11.

9.8 Tables 16 and 17 contain single-laboratory precision and accuracy data for solid-phase extraction of TCLP buffer solutions spiked at two levels and extracted using Method 3535.

9.9 Table 18 contains multiple-laboratory data for solid-phase extraction of spiked TCLP soil leachates extracted using Method 3535.

10.0 REFERENCES

1. Eichelberger, J.W., Harris, L.E., and Budde, W.L., "Reference Compound to Calibrate Ion Abundance Measurement in Gas Chromatography-Mass Spectrometry Systems", Analytical Chemistry, 47, 995-1000, 1975.

2. "Method Detection Limit for Methods 624 and 625", Olynyk, P., Budde, W.L., and Eichelberger, J.W., unpublished report, October 1980.

3. "Interlaboratory Method Study for EPA Method 625-Base/Neutrals, Acids, and Pesticides", Final Report for EPA Contract 68-03-3102.

4. Burke, J.A., "Gas Chromatography for Pesticide Residue Analysis: Some Practical Aspects", Journal of the Association of Official Analytical Chemists (AOAC), 48, 1037, 1965.

5. Lucas, S.V., Komfeld, R.A., "GC-MS Suitability Testing of RCRA Appendix VIII and Michigan List Analytes ", U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268, February 20, 1987, Contract No. 68-03-3224.

6. Engel, T.M., Komfeld, R.A., Warner, J.S., and Andrews, K.D., "Screening of Semivolatile Organic Compounds for Extractability and Aqueous Stability by SW-846, Method 3510", U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268, June 5, 1987, Contract 68-03-3224.

7. Lopez-Avila, V. (W. Beckert, Project Officer); "Development of a Soxtec Extraction Procedure for Extraction of Organic Compounds from Soils and Sediments"; U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Las Vegas, NV, October 1991; EPA 600/X-91/140.

8. Bursey, J., Merrill, R., McAllister, R., and McGaughey, J., "Laboratory Validation of VOST and SemiVOST for Halogenated Hydrocarbons from the Clean Air Act Amendments List", Vol. 1 and 2, U.S. Environmental Protection Agency, EPA 600/R-93/123a and b, (NTIS PB 93-227163 and 93-27171), Research Triangle Park, NC, July 1993.

9. Richter, B., Ezzell, J., and Felix, D., "Single Laboratory Method Validation Report: Extraction of Target Compound List/Priority Pollutant List BNAs and Pesticides using Accelerated Solvent Extraction (ASE) with Analytical Validation by GC/MS and GC/ECD", Document 101124, Dionex Corporation, Salt Lake City, UT, June 16, 1994.

10. Lee, H.B., Peart, T.E., Hong-You, R.L., and Gere, D.R., "Supercritical Carbon Dioxide Extraction of Polycyclic Aromatic Hydrocarbons from Sediments", J. Chromatography, A 653 83-91 (1993).

11. Warner, S., "SFE Extraction of PNAs from Solid Matrices Using the Dionex 703M SFE Extractor and a Liquid Trap," EPA Region III, Central Regional Laboratory, 839 Bestgate Road, Annapolis, MD 21401, December 12, 1994.

12. Markell, C., "3M Data Submission to EPA," letter to B. Lesnik, June 27, 1995.

TABLE 1

CHARACTERISTIC IONS FOR SEMIVOLATILE COMPOUNDS

Compound	Retention Time (min)	Primary Ion	Secondary Ion(s)
2-Picoline	3.75[a]	93	66,92
Aniline	5.68	93	66,65
Phenol	5.77	94	65,66
Bis(2-chloroethyl) ether	5.82	93	63,95
2-Chlorophenol	5.97	128	64,130
1,3-Dichlorobenzene	6.27	146	148,111
1,4-Dichlorobenzene-d_4 (IS)	6.35	152	150,115
1,4-Dichlorobenzene	6.40	146	148,111
Benzyl alcohol	6.78	108	79,77
1,2-Dichlorobenzene	6.85	146	148,111
N-Nitrosomethylethylamine	6.97	88	42,43,56
Bis(2-chloroisopropyl) ether	7.22	45	77,121
Ethyl carbamate	7.27	62	44,45,74
Thiophenol (Benzenethiol)	7.42	110	66,109,84
Methyl methanesulfonate	7.48	80	79,65,95
N-Nitrosodi-n-propylamine	7.55	70	42,101,130
Hexachloroethane	7.65	117	201,199
Maleic anhydride	7.65	54	98,53,44
Nitrobenzene	7.87	77	123,65
Isophorone	8.53	82	95,138
N-Nitrosodiethylamine	8.70	102	42,57,44,56
2-Nitrophenol	8.75	139	109,65
2,4-Dimethylphenol	9.03	122	107,121
p-Benzoquinone	9.13	108	54,82,80
Bis(2-chloroethoxy)methane	9.23	93	95,123
Benzoic acid	9.38	122	105,77
2,4-Dichlorophenol	9.48	162	164,98
Trimethyl phosphate	9.53	110	79,95,109,140
Ethyl methanesulfonate	9.62	79	109,97,45,65
1,2,4-Trichlorobenzene	9.67	180	182,145
Naphthalene-d_8 (IS)	9.75	136	68
Naphthalene	9.82	128	129,127
Hexachlorobutadiene	10.43	225	223,227
Tetraethyl pyrophosphate	11.07	99	155,127,81,109
Diethyl sulfate	11.37	139	45,59,99,111,125
4-Chloro-3-methylphenol	11.68	107	144,142
2-Methylnaphthalene	11.87	142	141
2-Methylphenol	12.40	107	108,77,79,90
Hexachloropropene	12.45	213	211,215,117,106,141
Hexachlorocyclopentadiene	12.60	237	235,272
N-Nitrosopyrrolidine	12.65	100	41,42,68,69
Acetophenone	12.67	105	71,51,120

TABLE 1
(continued)

Compound	Retention Time (min)	Primary Ion	Secondary Ion(s)
4-Methylphenol	12.82	107	108,77,79,90
2,4,6-Trichlorophenol	12.85	196	198,200
o-Toluidine	12.87	106	107,77,51,79
3-Methylphenol	12.93	107	108,77,79,90
2-Chloronaphthalene	13.30	162	127,164
N-Nitrosopiperidine	13.55	114	42,55,56,41
1,4-Phenylenediamine	13.62	108	80,53,54,52
1-Chloronaphthalene	13.65[a]	162	127,164
2-Nitroaniline	13.75	65	92,138
5-Chloro-2-methylaniline	14.28	106	141,140,77,89
Dimethyl phthalate	14.48	163	194,164
Acenaphthylene	14.57	152	151,153
2,6-Dinitrotoluene	14.62	165	63,89
Phthalic anhydride	14.62	104	76,50,148
o-Anisidine	15.00	108	80,123,52
3-Nitroaniline	15.02	138	108,92
Acenaphthene-d_{10} (IS)	15.05	164	162,160
Acenaphthene	15.13	154	153,152
2,4-Dinitrophenol	15.35	184	63,154
2,6-Dinitrophenol	15.47	162	164,126,98,63
4-Chloroaniline	15.50	127	129,65,92
Isosafrole	15.60	162	131,104,77,51
Dibenzofuran	15.63	168	139
2,4-Diaminotoluene	15.78	121	122,94,77,104
2,4-Dinitrotoluene	15.80	165	63,89
4-Nitrophenol	15.80	139	109,65
2-Naphthylamine	16.00[a]	143	115,116
1,4-Naphthoquinone	16.23	158	104,102,76,50,130
p-Cresidine	16.45	122	94,137,77,93
Dichlorovos	16.48	109	185,79,145
Diethyl phthalate	16.70	149	177,150
Fluorene	16.70	166	165,167
2,4,5-Trimethylaniline	16.70	120	135,134,91,77
N-Nitrosodi-n-butylamine	16.73	84	57,41,116,158
4-Chlorophenyl phenyl ether	16.78	204	206,141
Hydroquinone	16.93	110	81,53,55
4,6-Dinitro-2-methylphenol	17.05	198	51,105
Resorcinol	17.13	110	81,82,53,69
N-Nitrosodiphenylamine	17.17	169	168,167
Safrole	17.23	162	104,77,103,135
Hexamethyl phosphoramide	17.33	135	44,179,92,42
3-(Chloromethyl)pyridine hydrochloride	17.50	92	127,129,65,39
Diphenylamine	17.54[a]	169	168,167

TABLE 1
(continued)

Compound	Retention Time (min)	Primary Ion	Secondary Ion(s)
1,2,4,5-Tetrachlorobenzene	17.97	216	214,179,108,143,218
1-Naphthylamine	18.20	143	115,89,63
1-Acetyl-2-thiourea	18.22	118	43,42,76
4-Bromophenyl phenyl ether	18.27	248	250,141
Toluene diisocyanate	18.42	174	145,173,146,132,91
2,4,5-Trichlorophenol	18.47	196	198,97,132,99
Hexachlorobenzene	18.65	284	142,249
Nicotine	18.70	84	133,161,162
Pentachlorophenol	19.25	266	264,268
5-Nitro-o-toluidine	19.27	152	77,79,106,94
Thionazine	19.35	107	96,97,143,79,68
4-Nitroaniline	19.37	138	65,108,92,80,39
Phenanthrene-d_{10} (IS)	19.55	188	94,80
Phenanthrene	19.62	178	179,176
Anthracene	19.77	178	176,179
1,4-Dinitrobenzene	19.83	168	75,50,76,92,122
Mevinphos	19.90	127	192,109,67,164
Naled	20.03	109	145,147,301,79,189
1,3-Dinitrobenzene	20.18	168	76,50,75,92,122
Diallate (cis or trans)	20.57	86	234,43,70
1,2-Dinitrobenzene	20.58	168	50,63,74
Diallate (trans or cis)	20.78	86	234,43,70
Pentachlorobenzene	21.35	250	252,108,248,215,254
5-Nitro-o-anisidine	21.50	168	79,52,138,153,77
Pentachloronitrobenzene	21.72	237	142,214,249,295,265
4-Nitroquinoline-1-oxide	21.73	174	101,128,75,116
Di-n-butyl phthalate	21.78	149	150,104
2,3,4,6-Tetrachlorophenol	21.88	232	131,230,166,234,168
Dihydrosaffrole	22.42	135	64,77
Demeton-O	22.72	88	89,60,61,115,171
Fluoranthene	23.33	202	101,203
1,3,5-Trinitrobenzene	23.68	75	74,213,120,91,63
Dicrotophos	23.82	127	67,72,109,193,237
Benzidine	23.87	184	92,185
Trifluralin	23.88	306	43,264,41,290
Bromoxynil	23.90	277	279,88,275,168
Pyrene	24.02	202	200,203
Monocrotophos	24.08	127	192,67,97,109
Phorate	24.10	75	121,97,93,260
Sulfallate	24.23	188	88,72,60,44
Demeton-S	24.30	88	60,81,89,114,115
Phenacetin	24.33	108	180,179,109,137,80
Dimethoate	24.70	87	93,125,143,229

TABLE 1
(continued)

Compound	Retention Time (min)	Primary Ion	Secondary Ion(s)
Phenobarbital	24.70	204	117,232,146,161
Carbofuran	24.90	164	149,131,122
Octamethyl pyrophosphoramide	24.95	135	44,199,286,153,243
4-Aminobiphenyl	25.08	169	168,170,115
Dioxathion	25.25	97	125,270,153
Terbufos	25.35	231	57,97,153,103
α,α-Dimethylphenylamine	25.43	58	91,65,134,42
Pronamide	25.48	173	175,145,109,147
Aminoazobenzene	25.72	197	92,120,65,77
Dichlone	25.77	191	163,226,228,135,193
Dinoseb	25.83	211	163,147,117,240
Disulfoton	25.83	88	97,89,142,186
Fluchloralin	25.88	306	63,326,328,264,65
Mexacarbate	26.02	165	150,134,164,222
4,4'-Oxydianiline	26.08	200	108,171,80,65
Butyl benzyl phthalate	26.43	149	91,206
4-Nitrobiphenyl	26.55	199	152,141,169,151
Phosphamidon	26.85	127	264,72,109,138
2-Cyclohexyl-4,6-Dinitrophenol	26.87	231	185,41,193,266
Methyl parathion	27.03	109	125,263,79,93
Carbaryl	27.17	144	115,116,201
Dimethylaminoazobenzene	27.50	225	120,77,105,148,42
Propylthiouracil	27.68	170	142,114,83
Benz(a)anthracene	27.83	228	229,226
Chrysene-d_{12} (IS)	27.88	240	120,236
3,3'-Dichlorobenzidine	27.88	252	254,126
Chrysene	27.97	228	226,229
Malathion	28.08	173	125,127,93,158
Kepone	28.18	272	274,237,178,143,270
Fenthion	28.37	278	125,109,169,153
Parathion	28.40	109	97,291,139,155
Anilazine	28.47	239	241,143,178,89
Bis(2-ethylhexyl) phthalate	28.47	149	167,279
3,3'-Dimethylbenzidine	28.55	212	106,196,180
Carbophenothion	28.58	157	97,121,342,159,199
5-Nitroacenaphthene	28.73	199	152,169,141,115
Methapyrilene	28.77	97	50,191,71
Isodrin	28.95	193	66,195,263,265,147
Captan	29.47	79	149,77,119,117
Chlorfenvinphos	29.53	267	269,323,325,295
Crotoxyphos	29.73	127	105,193,166
Phosmet	30.03	160	77,93,317,76
EPN	30.11	157	169,185,141,323

TABLE 1
(continued)

Compound	Retention Time (min)	Primary Ion	Secondary Ion(s)
Tetrachlorvinphos	30.27	329	109,331,79,333
Di-n-octyl phthalate	30.48	149	167,43
2-Aminoanthraquinone	30.63	223	167,195
Barban	30.83	222	51,87,224,257,153
Aramite	30.92	185	191,319,334,197,321
Benzo(b)fluoranthene	31.45	252	253,125
Nitrofen	31.48	283	285,202,139,253
Benzo(k)fluoranthene	31.55	252	253,125
Chlorobenzilate	31.77	251	139,253,111,141
Fensulfothion	31.87	293	97,308,125,292
Ethion	32.08	231	97,153,125,121
Diethylstilbestrol	32.15	268	145,107,239,121,159
Famphur	32.67	218	125,93,109,217
Tri-p-tolyl phosphate[b]	32.75	368	367,107,165,198
Benzo(a)pyrene	32.80	252	253,125
Perylene-d_{12} (IS)	33.05	264	260,265
7,12-Dimethylbenz(a)anthracene	33.25	256	241,239,120
5,5-Diphenylhydantoin	33.40	180	104,252,223,209
Captafol	33.47	79	77,80,107
Dinocap	33.47	69	41,39
Methoxychlor	33.55	227	228,152,114,274,212
2-Acetylaminofluorene	33.58	181	180,223,152
4,4'-Methylenebis(2-chloroaniline)	34.38	231	266,268,140,195
3,3'-Dimethoxybenzidine	34.47	244	201,229
3-Methylcholanthrene	35.07	268	252,253,126,134,113
Phosalone	35.23	182	184,367,121,379
Azinphos-methyl	35.25	160	132,93,104,105
Leptophos	35.28	171	377,375,77,155,379
Mirex	35.43	272	237,274,270,239,235
Tris(2,3-dibromopropyl) phosphate	35.68	201	137,119,217,219,199
Dibenz(a,j)acridine	36.40	279	280,277,250
Mestranol	36.48	277	310,174,147,242
Coumaphos	37.08	362	226,210,364,97,109
Indeno(1,2,3-cd)pyrene	39.52	276	138,227
Dibenz(a,h)anthracene	39.82	278	139,279
Benzo(g,h,i)perylene	41.43	276	138,277
1,2:4,5-Dibenzopyrene	41.60	302	151,150,300
Strychnine	45.15	334	334,335,333
Piperonyl sulfoxide	46.43	162	135,105,77
Hexachlorophene	47.98	196	198,209,211,406,408
Aldrin	--	66	263,220
Aroclor 1016	--	222	260,292
Aroclor 1221	--	190	224,260

TABLE 1
(continued)

Compound	Retention Time (min)	Primary Ion	Secondary Ion(s)
Aroclor 1232	--	190	224,260
Aroclor 1242	--	222	256,292
Aroclor 1248	--	292	362,326
Aroclor 1254	--	292	362,326
Aroclor 1260	--	360	362,394
α-BHC	--	183	181,109
β-BHC	--	181	183,109
δ-BHC	--	183	181,109
γ-BHC (Lindane)	--	183	181,109
4,4'-DDD	--	235	237,165
4,4'-DDE	--	246	248,176
4,4'-DDT	--	235	237,165
Dieldrin	--	79	263,279
1,2-Diphenylhydrazine	--	77	105,182
Endosulfan I	--	195	339,341
Endosulfan II	--	337	339,341
Endosulfan sulfate	--	272	387,422
Endrin	--	263	82,81
Endrin aldehyde	--	67	345,250
Endrin ketone	--	317	67,319
2-Fluorobiphenyl (surr)	--	172	171
2-Fluorophenol (surr)	--	112	64
Heptachlor	--	100	272,274
Heptachlor epoxide	--	353	355,351
Nitrobenzene-d_5 (surr)	--	82	128,54
N-Nitrosodimethylamine	--	42	74,44
Phenol-d_6 (surr)	--	99	42,71
Terphenyl-d_{14} (surr)	--	244	122,212
2,4,6-Tribromophenol (surr)	--	330	332,141
Toxaphene	--	159	231,233

IS = internal standard
surr = surrogate
[a]Estimated retention times
[b]Substitute for the non-specific mixture, tricresyl phosphate

TABLE 2

ESTIMATED QUANTITATION LIMITS (EQLs) FOR SEMIVOLATILE ORGANICS

Compound	Estimated Quantitation Limits[a] Ground water (µg/L)	Low Soil/Sediment[b] (µg/kg)
Acenaphthene	10	660
Acenaphthylene	10	660
Acetophenone	10	ND
2-Acetylaminofluorene	20	ND
1-Acetyl-2-thiourea	1000	ND
2-Aminoanthraquinone	20	ND
Aminoazobenzene	10	ND
4-Aminobiphenyl	20	ND
Anilazine	100	ND
o-Anisidine	10	ND
Anthracene	10	660
Aramite	20	ND
Azinphos-methyl	100	ND
Barban	200	ND
Benz(a)anthracene	10	660
Benzo(b)fluoranthene	10	660
Benzo(k)fluoranthene	10	660
Benzoic acid	50	3300
Benzo(g,h,i)perylene	10	660
Benzo(a)pyrene	10	660
p-Benzoquinone	10	ND
Benzyl alcohol	20	1300
Bis(2-chloroethoxy)methane	10	660
Bis(2-chloroethyl) ether	10	660
Bis(2-chloroisopropyl) ether	10	660
4-Bromophenyl phenyl ether	10	660
Bromoxynil	10	ND
Butyl benzyl phthalate	10	660
Captafol	20	ND
Captan	50	ND
Carbaryl	10	ND
Carbofuran	10	ND
Carbophenothion	10	ND
Chlorfenvinphos	20	ND
4-Chloroaniline	20	1300
Chlorobenzilate	10	ND
5-Chloro-2-methylaniline	10	ND

TABLE 2
(continued)

Compound	Estimated Quantitation Limits[a] Ground water (µg/L)	Low Soil/Sediment[b] (µg/kg)
4-Chloro-3-methylphenol	20	1300
3-(Chloromethyl)pyridine hydrochloride	100	ND
2-Chloronaphthalene	10	660
2-Chlorophenol	10	660
4-Chlorophenyl phenyl ether	10	660
Chrysene	10	660
Coumaphos	40	ND
p-Cresidine	10	ND
Crotoxyphos	20	ND
2-Cyclohexyl-4,6-dinitrophenol	100	ND
Demeton-O	10	ND
Demeton-S	10	ND
Diallate (cis or trans)	10	ND
Diallate (trans or cis)	10	ND
2,4-Diaminotoluene	20	ND
Dibenz(a,j)acridine	10	ND
Dibenz(a,h)anthracene	10	660
Dibenzofuran	10	660
Dibenzo(a,e)pyrene	10	ND
Di-n-butyl phthalate	10	ND
Dichlone	NA	ND
1,2-Dichlorobenzene	10	660
1,3-Dichlorobenzene	10	660
1,4-Dichlorobenzene	10	660
3,3'-Dichlorobenzidine	20	1300
2,4-Dichlorophenol	10	660
2,6-Dichlorophenol	10	ND
Dichlorovos	10	ND
Dicrotophos	10	ND
Diethyl phthalate	10	660
Diethylstilbestrol	20	ND
Diethyl sulfate	100	ND
Dimethoate	20	ND
3,3'-Dimethoxybenzidine	100	ND
Dimethylaminoazobenzene	10	ND
7,12-Dimethylbenz(a)anthracene	10	ND
3,3'-Dimethylbenzidine	10	ND
2,4-Dimethylphenol	10	660

TABLE 2
(continued)

Compound	Estimated Quantitation Limits[a]	
	Ground water (µg/L)	Low Soil/Sediment[b] (µg/kg)
Dimethyl phthalate	10	660
1,2-Dinitrobenzene	40	ND
1,3-Dinitrobenzene	20	ND
1,4-Dinitrobenzene	40	ND
4,6-Dinitro-2-methylphenol	50	3300
2,4-Dinitrophenol	50	3300
2,4-Dinitrotoluene	10	660
2,6-Dinitrotoluene	10	660
Dinocap	100	ND
Dinoseb	20	ND
5,5-Diphenylhydantoin	20	ND
Di-n-octyl phthalate	10	660
Disulfoton	10	ND
EPN	10	ND
Ethion	10	ND
Ethyl carbamate	50	ND
Bis(2-ethylhexyl) phthalate	10	660
Ethyl methanesulfonate	20	ND
Famphur	20	ND
Fensulfothion	40	ND
Fenthion	10	ND
Fluchloralin	20	ND
Fluoranthene	10	660
Fluorene	10	660
Hexachlorobenzene	10	660
Hexachlorobutadiene	10	660
Hexachlorocyclopentadiene	10	660
Hexachloroethane	10	660
Hexachlorophene	50	ND
Hexachloropropene	10	ND
Hexamethylphosphoramide	20	ND
Indeno(1,2,3-cd)pyrene	10	660
Isodrin	20	ND
Isophorone	10	660
Isosafrole	10	ND
Kepone	20	ND
Leptophos	10	ND
Malathion	50	ND

TABLE 2
(continued)

Compound	Estimated Quantitation Limits[a]	
	Ground water (µg/L)	Low Soil/Sediment[b] (µg/kg)
Mestranol	20	ND
Methapyrilene	100	ND
Methoxychlor	10	ND
3-Methylcholanthrene	10	ND
Methyl methanesulfonate	10	ND
2-Methylnaphthalene	10	660
Methyl parathion	10	ND
2-Methylphenol	10	660
3-Methylphenol	10	ND
4-Methylphenol	10	660
Mevinphos	10	ND
Mexacarbate	20	ND
Mirex	10	ND
Monocrotophos	40	ND
Naled	20	ND
Naphthalene	10	660
1,4-Naphthoquinone	10	ND
1-Naphthylamine	10	ND
2-Naphthylamine	10	ND
Nicotine	20	ND
5-Nitroacenaphthene	10	ND
2-Nitroaniline	50	3300
3-Nitroaniline	50	3300
4-Nitroaniline	20	ND
5-Nitro-o-anisidine	10	ND
Nitrobenzene	10	660
4-Nitrobiphenyl	10	ND
Nitrofen	20	ND
2-Nitrophenol	10	660
4-Nitrophenol	50	3300
5-Nitro-o-toluidine	10	ND
4-Nitroquinoline-1-oxide	40	ND
N-Nitrosodi-n-butylamine	10	ND
N-Nitrosodiethylamine	20	ND
N-Nitrosodiphenylamine	10	660
N-Nitroso-di-n-propylamine	10	660
N-Nitrosopiperidine	20	ND
N-Nitrosopyrrolidine	40	ND

TABLE 2
(continued)

Compound	Estimated Quantitation Limits[a]	
	Ground water (µg/L)	Low Soil/Sediment[b] (µg/kg)
Octamethyl pyrophosphoramide	200	ND
4,4'-Oxydianiline	20	ND
Parathion	10	ND
Pentachlorobenzene	10	ND
Pentachloronitrobenzene	20	ND
Pentachlorophenol	50	3300
Phenacetin	20	ND
Phenanthrene	10	660
Phenobarbital	10	ND
Phenol	10	660
1,4-Phenylenediamine	10	ND
Phorate	10	ND
Phosalone	100	ND
Phosmet	40	ND
Phosphamidon	100	ND
Phthalic anhydride	100	ND
2-Picoline	ND	ND
Piperonyl sulfoxide	100	ND
Pronamide	10	ND
Propylthiouracil	100	ND
Pyrene	10	660
Resorcinol	100	ND
Safrole	10	ND
Strychnine	40	ND
Sulfallate	10	ND
Terbufos	20	ND
1,2,4,5-Tetrachlorobenzene	10	ND
2,3,4,6-Tetrachlorophenol	10	ND
Tetrachlorvinphos	20	ND
Tetraethyl pyrophosphate	40	ND
Thionazine	20	ND
Thiophenol (Benzenethiol)	20	ND
o-Toluidine	10	ND
1,2,4-Trichlorobenzene	10	660
2,4,5-Trichlorophenol	10	660
2,4,6-Trichlorophenol	10	660
Trifluralin	10	ND
2,4,5-Trimethylaniline	10	ND

TABLE 2
(continued)

Compound	Estimated Quantitation Limits[a] Ground water (µg/L)	Low Soil/Sediment[b] (µg/kg)
Trimethyl phosphate	10	ND
1,3,5-Trinitrobenzene	10	ND
Tris(2,3-dibromopropyl) phosphate	200	ND
Tri-p-tolyl phosphate(h)	10	ND

[a] Sample EQLs are highly matrix-dependent. The EQLs listed here are provided for guidance and may not always be achievable.

[b] EQLs listed for soil/sediment are based on wet weight. Normally, data are reported on a dry weight basis, therefore, EQLs will be higher based on the % dry weight of each sample. These EQLs are based on a 30-g sample and gel permeation chromatography cleanup.

ND = Not Determined

NA = Not Applicable

Other Matrices	Factor[c]
High-concentration soil and sludges by ultrasonic extractor	7.5
Non-water miscible waste	75

[c]EQL = (EQL for Low Soil/Sediment given above in Table 2) x (Factor)

TABLE 3

DFTPP KEY IONS AND ION ABUNDANCE CRITERIA[a,b]

Mass	Ion Abundance Criteria
51	30-60% of mass 198
68	< 2% of mass 69
70	< 2% of mass 69
127	40-60% of mass 198
197	< 1% of mass 198
198	Base peak, 100% relative abundance
199	5-9% of mass 198
275	10-30% of mass 198
365	> 1% of mass 198
441	Present but less than mass 443
442	> 40% of mass 198
443	17-23% of mass 442

[a] Data taken from Reference 3.
[b] Alternate tuning criteria may be employed, (e.g., CLP, Method 525, or manufacturers' instructions), provided that method performance is not adversely affected.

TABLE 4

CALIBRATION CHECK COMPOUNDS (CCC)

Base/Neutral Fraction	Acid Fraction
Acenaphthene	4-Chloro-3-methylphenol
1,4-Dichlorobenzene	2,4-Dichlorophenol
Hexachlorobutadiene	2-Nitrophenol
Diphenylamine	Phenol
Di-n-octyl phthalate	Pentachlorophenol
Fluoranthene	2,4,6-Trichlorophenol
Benzo(a)pyrene	

TABLE 5

SEMIVOLATILE INTERNAL STANDARDS WITH CORRESPONDING ANALYTES ASSIGNED FOR QUANTITATION

1,4-Dichlorobenzene-d_4	Naphthalene-d_8	Acenaphthene-d_{10}
Aniline	Acetophenone	Acenaphthene
Benzyl alcohol	Benzoic acid	Acenaphthylene
Bis(2-chloroethyl) ether	Bis(2-chloroethoxy)methane	1-Chloronaphthalene
Bis(2-chloroisopropyl) ether	4-Chloroaniline	2-Chloronaphthalene
2-Chlorophenol	4-Chloro-3-methylphenol	4-Chlorophenyl phenyl ether
1,3-Dichlorobenzene	2,4-Dichlorophenol	Dibenzofuran
1,4-Dichlorobenzene	2,6-Dichlorophenol	Diethyl phthalate
1,2-Dichlorobenzene	α,α-Dimethyl-	Dimethyl phthalate
Ethyl methanesulfonate	phenethylamine	2,4-Dinitrophenol
2-Fluorophenol (surr)	2,4-Dimethylphenol	2,4-Dinitrotoluene
Hexachloroethane	Hexachlorobutadiene	2,6-Dinitrotoluene
Methyl methanesulfonate	Isophorone	Fluorene
2-Methylphenol	2-Methylnaphthalene	2-Fluorobiphenyl (surr)
4-Methylphenol	Naphthalene	Hexachlorocyclopentadiene
N-Nitrosodimethylamine	Nitrobenzene	1-Naphthylamine
N-Nitroso-di-n-propylamine	Nitrobenzene-d_8 (surr)	2-Naphthylamine
Phenol	2-Nitrophenol	2-Nitroaniline
Phenol-d_6 (surr)	N-Nitrosodi-n-butylamine	3-Nitroaniline
2-Picoline	N-Nitrosopiperidine	4-Nitroaniline
	1,2,4-Trichlorobenzene	4-Nitrophenol
		Pentachlorobenzene
		1,2,4,5-Tetrachlorobenzene
		2,3,4,6-Tetrachlorophenol
		2,4,6-Tribromophenol (surr)
		2,4,6-Trichlorophenol
		2,4,5-Trichlorophenol

(surr) = surrogate

TABLE 5
(continued)

Phenanthrene-d_{10}	Chrysene-d_{12}	Perylene-d_{12}
4-Aminobiphenyl	Benzidine	Benzo(b)fluoranthene
Anthracene	Benzo(a)anthracene	Benzo(k)fluoranthene
4-Bromophenyl phenyl ether	Bis(2-ethylhexyl) phthalate	Benzo(g,h,i)perylene
Di-n-butyl phthalate	Butyl benzyl phthalate	Benzo(a)pyrene
4,6-Dinitro-2-methylphenol	Chrysene	Dibenz(a,j)acridine
Diphenylamine	3,3'-Dichlorobenzidine	Dibenz(a,h)anthracene
Fluoranthene	p-Dimethyl aminoazobenzene	
Hexachlorobenzene	Pyrene	
N-Nitrosodiphenylamine	Terphenyl-d_{14} (surr)	
Pentachlorophenol	7,12-Dimethylbenz(a) anthracene	
Pentachloronitrobenzene	Di-n-octyl phthalate	
Phenacetin	Indeno(1,2,3-cd) pyrene	
Phenanthrene	3-Methylcholanthrene	
Pronamide		

(surr) = surrogate

TABLE 6

MULTILABORATORY PERFORMANCE DATA[a]

Compound	Test conc. (µg/L)	Limit for s (µg/L)	Range for $\bar{x}$ (µg/L)	Range p, p_s (%)
Acenaphthene	100	27.6	60.1-132.3	47-145
Acenaphthylene	100	40.2	53.5-126.0	33-145
Aldrin	100	39.0	7.2-152.2	D-166
Anthracene	100	32.0	43.4-118.0	27-133
Benz(a)anthracene	100	27.6	41.8-133.0	33-143
Benzo(b)fluoranthene	100	38.8	42.0-140.4	24-159
Benzo(k)fluoranthene	100	32.3	25.2-145.7	11-162
Benzo(a)pyrene	100	39.0	31.7-148.0	17-163
Benzo(g,h,i)perylene	100	58.9	D-195.0	D-219
Benzyl butyl phthalate	100	23.4	D-139.9	D-152
β-BHC	100	31.5	41.5-130.6	24-149
δ-BHC	100	21.6	D-100.0	D-110
Bis(2-chloroethyl) ether	100	55.0	42.9-126.0	12-158
Bis(2-chloroethoxy)methane	100	34.5	49.2-164.7	33-184
Bis(2-chloroisopropyl) ether	100	46.3	62.8-138.6	36-166
Bis(2-ethylhexyl) phthalate	100	41.1	28.9-136.8	8-158
4-Bromophenyl phenyl ether	100	23.0	64.9-114.4	53-127
2-Chloronaphthalene	100	13.0	64.5-113.5	60-118
4-Chlorophenyl phenyl ether	100	33.4	38.4-144.7	25-158
Chrysene	100	48.3	44.1-139.9	17-168
4,4'-DDD	100	31.0	D-134.5	D-145
4,4'-DDE	100	32.0	19.2-119.7	4-136
4,4'-DDT	100	61.6	D-170.6	D-203
Dibenzo(a,h)anthracene	100	70.0	D-199.7	D-227
Di-n-butyl phthalate	100	16.7	8.4-111.0	1-118
1,2-Dichlorobenzene	100	30.9	48.6-112.0	32-129
1,3-Dichlorobenzene	100	41.7	16.7-153.9	D-172
1,4-Dichlorobenzene	100	32.1	37.3-105.7	20-124
3,3'-Dichlorobenzidine	100	71.4	8.2-212.5	D-262
Dieldrin	100	30.7	44.3-119.3	29-136
Diethyl phthalate	100	26.5	D-100.0	D-114
Dimethyl phthalate	100	23.2	D-100.0	D-112
2,4-Dinitrotoluene	100	21.8	47.5-126.9	39-139
2,6-Dinitrotoluene	100	29.6	68.1-136.7	50-158
Di-n-octyl phthalate	100	31.4	18.6-131.8	4-146
Endosulfan sulfate	100	16.7	D-103.5	D-107
Endrin aldehyde	100	32.5	D-188.8	D-209
Fluoranthene	100	32.8	42.9-121.3	26-137

TABLE 6
(continued)

Compound	Test conc. (µg/L)	Limit for s (µg/L)	Range for $\bar{x}$ (µg/L)	Range p, $p_{s(\%)}$
Fluorene	100	20.7	71.6-108.4	59-121
Heptachlor	100	37.2	D-172.2	D-192
Heptachlor epoxide	100	54.7	70.9-109.4	26.155
Hexachlorobenzene	100	24.9	7.8-141.5	D-152
Hexachlorobutadiene	100	26.3	37.8-102.2	24-116
Hexachloroethane	100	24.5	55.2-100.0	40-113
Indeno(1,2,3-cd)pyrene	100	44.6	D-150.9	D-171
Isophorone	100	63.3	46.6-180.2	21-196
Naphthalene	100	30.1	35.6-119.6	21-133
Nitrobenzene	100	39.3	54.3-157.6	35-180
N-Nitrosodi-n-propylamine	100	55.4	13.6-197.9	D-230
Aroclor 1260	100	54.2	19.3-121.0	D-164
Phenanthrene	100	20.6	65.2-108.7	54-120
Pyrene	100	25.2	69.6-100.0	52-115
1,2,4-Trichlorobenzene	100	28.1	57.3-129.2	44-142
4-Chloro-3-methylphenol	100	37.2	40.8-127.9	22-147
2-Chlorophenol	100	28.7	36.2-120.4	23-134
2,4-Chlorophenol	100	26.4	52.5-121.7	39-135
2,4-Dimethylphenol	100	26.1	41.8-109.0	32-119
2,4-Dinitrophenol	100	49.8	D-172.9	D-191
2-Methyl-4,6-dinitrophenol	100	93.2	53.0-100.0	D-181
2-Nitrophenol	100	35.2	45.0-166.7	29-182
4-Nitrophenol	100	47.2	13.0-106.5	D-132
Pentachlorophenol	100	48.9	38.1-151.8	14-176
Phenol	100	22.6	16.6-100.0	5-112
2,4,6-Trichlorophenol	100	31.7	52.4-129.2	37-144

s = Standard deviation of four recovery measurements, in µg/L

$\bar{x}$ = Average recovery for four recovery measurements, in µg/L

p, p_s = Measured percent recovery

D = Detected; result must be greater than zero

[a] Criteria from 40 CFR Part 136 for Method 625, using a packed GC column. These criteria are based directly on the method performance data in Table 7. Where necessary, the limits for recovery have been broadened to assure applicability of the limits to concentrations below those used to develop Table 7. These values are for guidance only. Appropriate derivation of acceptance criteria for capillary columns should result in much narrower ranges. See Method 8000 for information on developing and updating acceptance criteria for method performance.

TABLE 7

METHOD ACCURACY AND PRECISION AS FUNCTIONS OF CONCENTRATION[a]

Compound	Accuracy, as recovery, x' (µg/L)	Single analyst precision, s_r' (µg/L)	Overall precision, S' (µg/L)
Acenaphthene	0.96C+0.19	$0.15\bar{x}$-0.12	$0.21\bar{x}$-0.67
Acenaphthylene	0.89C+0.74	$0.24\bar{x}$-1.06	$0.26\bar{x}$-0.54
Aldrin	0.78C+1.66	$0.27\bar{x}$-1.28	$0.43\bar{x}$+1.13
Anthracene	0.80C+0.68	$0.21\bar{x}$-0.32	$0.27\bar{x}$-0.64
Benz(a)anthracene	0.88C-0.60	$0.15\bar{x}$+0.93	$0.26\bar{x}$-0.21
Benzo(b)fluoranthene	0.93C-1.80	$0.22\bar{x}$+0.43	$0.29\bar{x}$+0.96
Benzo(k)fluoranthene	0.87C-1.56	$0.19\bar{x}$+1.03	$0.35\bar{x}$+0.40
Benzo(a)pyrene	0.90C-0.13	$0.22\bar{x}$+0.48	$0.32\bar{x}$+1.35
Benzo(g,h,i)perylene	0.98C-0.86	$0.29\bar{x}$+2.40	$0.51\bar{x}$-0.44
Benzyl butyl phthalate	0.66C-1.68	$0.18\bar{x}$+0.94	$0.53\bar{x}$+0.92
β-BHC	0.87C-0.94	$0.20\bar{x}$-0.58	$0.30\bar{x}$+1.94
δ-BHC	0.29C-1.09	$0.34\bar{x}$+0.86	$0.93\bar{x}$-0.17
Bis(2-chloroethyl) ether	0.86C-1.54	$0.35\bar{x}$-0.99	$0.35\bar{x}$+0.10
Bis(2-chloroethoxy)methane	1.12C-5.04	$0.16\bar{x}$+1.34	$0.26\bar{x}$+2.01
Bis(2-chloroisopropyl) ether	1.03C-2.31	$0.24\bar{x}$+0.28	$0.25\bar{x}$+1.04
Bis(2-ethylhexyl) phthalate	0.84C-1.18	$0.26\bar{x}$+0.73	$0.36\bar{x}$+0.67
4-Bromophenyl phenyl ether	0.91C-1.34	$0.13\bar{x}$+0.66	$0.16\bar{x}$+0.66
2-Chloronaphthalene	0.89C+0.01	$0.07\bar{x}$+0.52	$0.13\bar{x}$+0.34
4-Chlorophenyl phenyl ether	0.91C+0.53	$0.20\bar{x}$-0.94	$0.30\bar{x}$-0.46
Chrysene	0.93C-1.00	$0.28\bar{x}$+0.13	$0.33\bar{x}$-0.09
4,4'-DDD	0.56C-0.40	$0.29\bar{x}$-0.32	$0.66\bar{x}$-0.96
4,4'-DDE	0.70C-0.54	$0.26\bar{x}$-1.17	$0.39\bar{x}$-1.04
4,4'-DDT	0.79C-3.28	$0.42\bar{x}$+0.19	$0.65\bar{x}$-0.58
Dibenzo(a,h)anthracene	0.88C+4.72	$0.30\bar{x}$+8.51	$0.59\bar{x}$+0.25
Di-n-butyl phthalate	0.59C+0.71	$0.13\bar{x}$+1.16	$0.39\bar{x}$+0.60
1,2-Dichlorobenzene	0.80C+0.28	$0.20\bar{x}$+0.47	$0.24\bar{x}$+0.39
1,3-Dichlorobenzene	0.86C-0.70	$0.25\bar{x}$+0.68	$0.41\bar{x}$+0.11
1,4-Dichlorobenzene	0.73C-1.47	$0.24\bar{x}$+0.23	$0.29\bar{x}$+0.36
3,3'-Dichlorobenzidine	1.23C-12.65	$0.28\bar{x}$+7.33	$0.47\bar{x}$+3.45
Dieldrin	0.82C-0.16	$0.20\bar{x}$-0.16	$0.26\bar{x}$-0.07
Diethyl phthalate	0.43C+1.00	$0.28\bar{x}$+1.44	$0.52\bar{x}$+0.22
Dimethyl phthalate	0.20C+1.03	$0.54\bar{x}$+0.19	$1.05\bar{x}$-0.92
2,4-Dinitrotoluene	0.92C-4.81	$0.12\bar{x}$+1.06	$0.21\bar{x}$+1.50
2,6-Dinitrotoluene	1.06C-3.60	$0.14\bar{x}$+1.26	$0.19\bar{x}$+0.35
Di-n-octyl phthalate	0.76C-0.79	$0.21\bar{x}$+1.19	$0.37\bar{x}$+1.19
Endosulfan sulfate	0.39C+0.41	$0.12\bar{x}$+2.47	$0.63\bar{x}$-1.03
Endrin aldehyde	0.76C-3.86	$0.18\bar{x}$+3.91	$0.73\bar{x}$-0.62
Fluoranthene	0.81C+1.10	$0.22\bar{x}$-0.73	$0.28\bar{x}$-0.60
Fluorene	0.90C-0.00	$0.12\bar{x}$+0.26	$0.13\bar{x}$+0.61

TABLE 7
(continued)

Compound	Accuracy, as recovery, x' (µg/L)	Single analyst precision, s_r' (µg/L)	Overall precision, S' (µg/L)
Heptachlor	0.87C-2.97	$0.24\bar{x}$-0.56	$0.50\bar{x}$-0.23
Heptachlor epoxide	0.92C-1.87	$0.33\bar{x}$-0.46	$0.28\bar{x}$+0.64
Hexachlorobenzene	0.74C+0.66	$0.18\bar{x}$-0.10	$0.43\bar{x}$-0.52
Hexachlorobutadiene	0.71C-1.01	$0.19\bar{x}$+0.92	$0.26\bar{x}$+0.49
Hexachloroethane	0.73C-0.83	$0.17\bar{x}$+0.67	$0.17\bar{x}$+0.80
Indeno(1,2,3-cd)pyrene	0.78C-3.10	$0.29\bar{x}$+1.46	$0.50\bar{x}$-0.44
Isophorone	1.12C+1.41	$0.27\bar{x}$+0.77	$0.33\bar{x}$+0.26
Naphthalene	0.76C+1.58	$0.21\bar{x}$-0.41	$0.30\bar{x}$-0.68
Nitrobenzene	1.09C-3.05	$0.19\bar{x}$+0.92	$0.27\bar{x}$+0.21
N-Nitrosodi-n-propylamine	1.12C-6.22	$0.27\bar{x}$+0.68	$0.44\bar{x}$+0.47
Aroclor 1260	0.81C-10.86	$0.35\bar{x}$+3.61	$0.43\bar{x}$+1.82
Phenanthrene	0.87C+0.06	$0.12\bar{x}$+0.57	$0.15\bar{x}$+0.25
Pyrene	0.84C-0.16	$0.16\bar{x}$+0.06	$0.15\bar{x}$+0.31
1,2,4-Trichlorobenzene	0.94C-0.79	$0.15\bar{x}$+0.85	$0.21\bar{x}$+0.39
4-Chloro-3-methylphenol	0.84C+0.35	$0.23\bar{x}$+0.75	$0.29\bar{x}$+1.31
2-Chlorophenol	0.78C+0.29	$0.18\bar{x}$+1.46	$0.28\bar{x}$+0.97
2,4-Dichlorophenol	0.87C-0.13	$0.15\bar{x}$+1.25	$0.21\bar{x}$+1.28
2,4-Dimethylphenol	0.71C+4.41	$0.16\bar{x}$+1.21	$0.22\bar{x}$+1.31
2,4-Dinitrophenol	0.81C-18.04	$0.38\bar{x}$+2.36	$0.42\bar{x}$+26.29
2-Methyl-4,6-dinitrophenol	1.04C-28.04	$0.10\bar{x}$+42.29	$0.26\bar{x}$+23.10
2-Nitrophenol	0.07C-1.15	$0.16\bar{x}$+1.94	$0.27\bar{x}$+2.60
4-Nitrophenol	0.61C-1.22	$0.38\bar{x}$+2.57	$0.44\bar{x}$+3.24
Pentachlorophenol	0.93C+1.99	$0.24\bar{x}$+3.03	$0.30\bar{x}$+4.33
Phenol	0.43C+1.26	$0.26\bar{x}$+0.73	$0.35\bar{x}$+0.58
2,4,6-Trichlorophenol	0.91C-0.18	$0.16\bar{x}$+2.22	$0.22\bar{x}$+1.81

x' = Expected recovery for one or more measurements of a sample containing a concentration of C, in µg/L.

s_r' = Expected single analyst standard deviation of measurements at an average concentration of $\bar{x}$, in µg/L.

S' = Expected interlaboratory standard deviation of measurements at an average concentration found of $\bar{x}$, in µg/L.

C = True value for the concentration, in µg/L.

$\bar{x}$ = Average recovery found for measurements of samples containing a concentration of C, in µg/L.

[a] Criteria from 40 CFR Part 136 for Method 625, using a packed GC column. These criteria are based directly on the method performance data in Table 7. These values are for guidance only. Appropriate derivation of acceptance criteria for capillary columns should result in much narrower ranges. See Method 8000 for information on developing and updating acceptance criteria for method performance.

TABLE 8

EXTRACTION EFFICIENCY AND AQUEOUS STABILITY RESULTS

	Percent Recovery, Day 0		Percent Recovery, Day 7	
Compound	Mean	RSD	Mean	RSD
3-Amino-9-ethylcarbazole	80	8	73	3
4-Chloro-1,2-phenylenediamine	91	1	108	4
4-Chloro-1,3-phenylenediamine	84	3	70	3
1,2-Dibromo-3-chloropropane	97	2	98	5
Dinoseb	99	3	97	6
Parathion	100	2	103	4
4,4'-Methylenebis(N,N-dimethylaniline)	108	4	90	4
5-Nitro-o-toluidine	99	10	93	4
2-Picoline	80	4	83	4
Tetraethyl dithiopyrophosphate	92	7	70	1

Data taken from Reference 6.

TABLE 9

MEAN PERCENT RECOVERIES AND PERCENT RSD VALUES FOR SEMIVOLATILE ORGANICS FROM SPIKED CLAY SOIL AND TOPSOIL BY AUTOMATED SOXHLET EXTRACTION (METHOD 3541) WITH HEXANE-ACETONE (1:1)[a]

	Clay Soil		Topsoil	
Compound	Mean Recovery	RSD	Mean Recovery	RSD
1,3-Dichlorobenzene	0	--	0	--
1,2-Dichlorobenzene	0	--	0	--
Nitrobenzene	0	--	0	--
Benzal chloride	0	--	0	--
Benzotrichloride	0	--	0	--
4-Chloro-2-nitrotoluene	0	--	0	--
Hexachlorocyclopentadiene	4.1	15	7.8	23
2,4-Dichloronitrobenzene	35.2	7.6	21.2	15
3,4-Dichloronitrobenzene	34.9	15	20.4	11
Pentachlorobenzene	13.7	7.3	14.8	13
2,3,4,5-Tetrachloronitrobenzene	55.9	6.7	50.4	6.0
Benefin	62.6	4.8	62.7	2.9
alpha-BHC	58.2	7.3	54.8	4.8
Hexachlorobenzene	26.9	13	25.1	5.7
delta-BHC	95.8	4.6	99.2	1.3
Heptachlor	46.9	9.2	49.1	6.3
Aldrin	97.7	12	102	7.4
Isopropalin	102	4.3	105	2.3
Heptachlor epoxide	90.4	4.4	93.6	2.4
trans-Chlordane	90.1	4.5	95.0	2.3
Endosulfan I	96.3	4.4	101	2.2
Dieldrin	129	4.7	104	1.9
2,5-Dichlorophenyl-4-nitrophenyl ether	110	4.1	112	2.1
Endrin	102	4.5	106	3.7
Endosulfan II	104	4.1	105	0.4
p,p'-DDT	134	2.1	111	2.0
2,3,6-Trichlorophenyl-4'-nitrophenyl ether	110	4.8	110	2.8
2,3,4-Trichlorophenyl-4'-nitrophenyl ether	112	4.4	112	3.3
Mirex	104	5.3	108	2.2

[a] The operating conditions for the Soxtec apparatus were as follows: immersion time 45 min; extraction time 45 min; the sample size was 10 g; the spiking concentration was 500 ng/g, except for the surrogate compounds at 1000 ng/g, 2,5-Dichlorophenyl-4-nitrophenyl ether, 2,3,6-Trichlorophenyl-4-nitrophenyl ether, and 2,3,4-Trichlorophenyl-4-nitrophenyl ether at 1500 ng/g, Nitrobenzene at 2000 ng/g, and 1,3-Dichlorobenzene and 1,2-Dichlorobenzene at 5000 ng/g.

TABLE 10

SINGLE LABORATORY ACCURACY AND PRECISION DATA FOR THE EXTRACTION OF SEMIVOLATILE ORGANICS FROM SPIKED CLAY BY AUTOMATED SOXHLET (METHOD 3541)[a]

Compound	Mean Recovery	RSD
Phenol	47.8	5.6
Bis(2-chloroethyl)ether	25.4	13
2-Chlorophenol	42.7	4.3
Benzyl alcohol	55.9	7.2
2-Methylphenol	17.6	6.6
Bis(2-chloroisopropyl)ether	15.0	15
4-Methylphenol	23.4	6.7
N-Nitroso-di-n-propylamine	41.4	6.2
Nitrobenzene	28.2	7.7
Isophorone	56.1	4.2
2-Nitrophenol	36.0	6.5
2,4-Dimethylphenol	50.1	5.7
Benzoic acid	40.6	7.7
Bis(2-chloroethoxy)methane	44.1	3.0
2,4-Dichlorophenol	55.6	4.6
1,2,4-Trichlorobenzene	18.1	31
Naphthalene	26.2	15
4-Chloroaniline	55.7	12
4-Chloro-3-methylphenol	65.1	5.1
2-Methylnaphthalene	47.0	8.6
Hexachlorocyclopentadiene	19.3	19
2,4,6-Trichlorophenol	70.2	6.3
2,4,5-Trichlorophenol	26.8	2.9
2-Chloronaphthalene	61.2	6.0
2-Nitroaniline	73.8	6.0
Dimethyl phthalate	74.6	5.2
Acenaphthylene	71.6	5.7
3-Nitroaniline	77.6	5.3
Acenaphthene	79.2	4.0
2,4-Dinitrophenol	91.9	8.9
4-Nitrophenol	62.9	16
Dibenzofuran	82.1	5.9
2,4-Dinitrotoluene	84.2	5.4
2,6-Dinitrotoluene	68.3	5.8
Diethyl phthalate	74.9	5.4
4-Chlorophenyl-phenyl ether	67.2	3.2
Fluorene	82.1	3.4

TABLE 10
(continued)

Compound	Mean Recovery	RSD
4-Nitroaniline	79.0	7.9
4,6-Dinitro-2-methylphenol	63.4	6.8
N-Nitrosodiphenylamine	77.0	3.4
4-Bromophenyl-phenyl ether	62.4	3.0
Hexachlorobenzene	72.6	3.7
Pentachlorophenol	62.7	6.1
Phenanthrene	83.9	5.4
Anthracene	96.3	3.9
Di-n-butyl phthalate	78.3	40
Fluoranthene	87.7	6.9
Pyrene	102	0.8
Butyl benzyl phthalate	66.3	5.2
3,3'-Dichlorobenzidine	25.2	11
Benzo(a)anthracene	73.4	3.8
Bis(2-ethylhexyl) phthalate	77.2	4.8
Chrysene	76.2	4.4
Di-n-octyl phthalate	83.1	4.8
Benzo(b)fluoranthene	82.7	5.0
Benzo(k)fluoranthene	71.7	4.1
Benzo(a)pyrene	71.7	4.1
Indeno(1,2,3-cd)pyrene	72.2	4.3
Dibenzo(a,h)anthracene	66.7	6.3
Benzo(g,h,i)perylene	63.9	8.0
1,2-Dichlorobenzene	0	--
1,3-Dichlorobenzene	0	--
1,4-Dichlorobenzene	0	--
Hexachloroethane	0	--
Hexachlorobutadiene	0	--

[a] Number of determinations was three. The operating conditions for the Soxtec apparatus were as follows: immersion time 45 min; extraction time 45 min; the sample size was 10 g clay soil; the spike concentration was 6 mg/kg per compound. The sample was allowed to equilibrate 1 hour after spiking.

Data taken from Reference 7.

TABLE 11

PRECISION AND BIAS VALUES FOR METHOD 3542[1]

Compound	Mean Recovery	Standard Deviation	% RSD
2-Fluorophenol	74.6	28.6	38.3
Phenol-d_5	77.8	27.7	35.6
Nitrobenzene-d_5	65.6	32.5	49.6
2-Fluorobiphenyl	75.9	30.3	39.9
2,4,6-Tribromophenol	67.0	34.0	50.7
Terphenyl-d_{14}	78.6	32.4	41.3

[1] The surrogate values shown in Table 11 represent mean recoveries for surrogates in all Method 0010 matrices in a field dynamic spiking study.

TABLE 12

ACCELERATED SOLVENT EXTRACTION (METHOD 3545) RECOVERY VALUES AS PERCENT OF SOXTEC™

	Clay			Loam			Sand			Mean
Compound	Low	Mid	High	Low	Mid	High	Low	Mid	High	Rec.
Phenol	93.3	78.7	135.9	73.9	82.8	124.6	108.8	130.6	89.7	102.0
Bis(2-chloroethyl) ether	102.1	85.1	109.1	96.0	88.0	103.6	122.3	119.9	90.8	101.9
2-Chlorophenol	100.8	82.6	115.0	93.8	88.9	111.1	115.0	115.3	91.9	101.6
1,3-Dichlorobenzene	127.7	129.7	110.0	*364.2	129.9	119.0	*241.3	*163.7	107.1	120.6
1,4-Dichlorobenzene	127.9	127.0	110.5	*365.9	127.8	116.4	*309.6	*164.1	105.8	119.2
1,2-Dichlorobenzene	116.8	115.8	101.3	*159.2	113.4	105.5	*189.3	134.0	100.4	112.5
2-Methylphenol	98.9	82.1	119.7	87.6	89.4	111.0	133.2	128.0	92.1	104.7
Bis(2-chloroisopropyl)ether	109.4	71.5	108.0	81.8	81.0	88.6	118.1	148.3	94.8	100.2
o-Toluidine	100.0	89.7	117.2	100.0	*152.5	120.3	100.0	*199.5	102.7	110.3
N-Nitroso-di-n-propylamine	103.0	79.1	107.7	83.9	88.1	96.2	109.9	123.3	91.4	98.1
Hexachloroethane	97.1	125.1	111.0	*245.4	117.1	128.1	*566.7	147.9	103.7	118.6
Nitrobenzene	104.8	82.4	106.6	86.8	84.6	101.7	119.7	122.1	93.3	100.2
Isophorone	100.0	86.4	98.2	87.1	87.5	109.7	135.5	118.4	92.7	101.7
2,4-Dimethylphenol	100.0	104.5	140.0	100.0	114.4	123.1	100.0	*180.6	96.3	109.8
2-Nitrophenol	80.7	80.5	107.9	91.4	86.7	103.2	122.1	107.1	87.0	96.3
Bis(chloroethoxy)methane	94.4	80.6	94.7	86.5	84.4	99.6	130.6	110.7	93.2	97.2
2,4-Dichlorophenol	88.9	87.8	111.4	85.9	87.6	103.5	123.3	107.0	92.1	98.6
1,2,4-Trichlorobenzene	98.0	97.8	98.8	123.0	93.7	94.5	137.0	99.4	95.3	104.2
Naphthalene	101.7	97.2	123.6	113.2	102.9	129.5	*174.5	114.0	89.8	106.1
4-Chloroaniline	100.0	*150.2	*162.4	100.0	125.5	*263.6	100.0	*250.8	114.9	108.1
Hexachlorobutadiene	101.1	98.7	102.2	124.1	90.3	98.0	134.9	96.1	96.8	104.7
4-Chloro-3-methylphenol	90.4	80.2	114.7	79.0	85.2	109.8	131.6	116.2	90.1	99.7
2-Methylnaphthalene	93.2	89.9	94.6	104.1	92.2	105.9	146.2	99.1	93.3	102.1
Hexachlorocyclopentadiene	100.0	100.0	0.0	100.0	100.0	6.8	100.0	100.0	*238.3	75.8
2,4,6-Trichlorophenol	94.6	90.0	112.0	84.2	91.2	103.6	101.6	95.9	89.8	95.9
2,4,5-Trichlorophenol	84.4	91.9	109.6	96.1	80.7	103.6	108.9	83.9	87.9	94.1
2-Chloronaphthalene	100.0	91.3	93.6	97.6	93.4	98.3	106.8	93.0	92.0	96.2
2-Nitroaniline	90.0	83.4	97.4	71.3	88.4	89.9	112.1	113.3	87.7	92.6
2,6-Dinitrotoluene	83.1	90.6	91.6	86.4	90.6	90.3	104.3	84.7	90.9	90.3
Acenaphthylene	104.9	95.9	100.5	99.0	97.9	108.8	118.5	97.8	92.0	101.7
3-Nitroaniline	*224.0	115.6	97.6	100.0	111.8	107.8	0.0	111.7	99.0	92.9
Acenaphthene	102.1	92.6	97.6	97.2	96.9	104.4	114.2	92.0	89.0	98.4
4-Nitrophenol	0.0	93.2	121.5	18.1	87.1	116.6	69.1	90.5	84.5	75.6
2,4-Dinitrotoluene	73.9	91.9	100.2	84.7	93.8	98.9	100.9	84.3	87.3	90.7

TABLE 12
(continued)

Compound	Clay			Loam			Sand			Mean
	Low	Mid	High	Low	Mid	High	Low	Mid	High	Rec.
Dibenzofuran	89.5	91.7	109.3	98.5	92.2	111.4	113.8	92.7	90.4	98.8
4-Chlorophenyl phenyl ether	83.0	94.5	98.7	95.7	94.3	94.2	111.4	87.7	90.3	94.4
Fluorene	85.2	94.9	89.2	102.0	95.5	93.8	121.3	85.7	90.9	95.4
4-Nitroaniline	77.8	114.8	94.5	129.6	103.6	95.4	*154.1	89.3	87.5	99.1
N-Nitrosodiphenylamine	82.6	96.7	93.8	92.9	93.4	116.4	97.5	110.9	86.7	96.8
4-Bromophenyl phenyl ether	85.6	92.9	92.8	91.1	107.6	89.4	118.0	97.5	87.1	95.8
Hexachlorobenzene	95.4	91.7	92.3	95.4	93.6	83.7	106.8	94.3	90.0	93.7
Pentachlorophenol	68.2	85.9	107.7	53.2	89.8	88.1	96.6	59.8	81.3	81.2
Phenanthrene	92.1	93.7	93.3	100.0	97.8	113.3	124.4	101.0	89.9	100.6
Anthracene	101.6	95.0	93.5	92.5	101.8	118.4	123.0	94.5	90.6	101.2
Carbazole	94.4	99.3	96.6	105.5	96.7	111.4	115.7	83.2	88.9	99.1
Fluoranthene	109.9	101.4	94.3	111.6	96.6	109.6	123.2	85.4	92.7	102.7
Pyrene	106.5	105.8	107.6	116.7	90.7	127.5	103.4	95.5	93.2	105.2
3,3'-Dichlorobenzidine	100.0	*492.3	131.4	100.0	*217.6	*167.6	100.0	*748.8	100.0	116.5
Benzo(a)anthracene	98.1	107.0	98.4	119.3	98.6	104.0	105.0	93.4	89.3	101.5
Chrysene	100.0	108.5	100.2	116.8	93.0	117.0	106.7	93.6	90.2	102.9
Benzo(b)fluoranthene	106.6	109.9	75.6	121.7	100.7	93.9	106.9	81.9	93.6	99.0
Benzo(k)fluoranthene	102.4	105.2	88.4	125.5	99.4	95.1	144.7	89.2	78.1	103.1
Benzo(a)pyrene	107.9	105.5	80.8	122.3	97.7	104.6	101.7	86.2	92.0	99.9
Indeno(1,2,3-cd)pyrene	95.1	105.7	93.8	126.0	105.2	90.4	133.6	82.6	91.9	102.7
Dibenz(a,h)anthracene	85.0	102.6	82.0	118.8	100.7	91.9	142.3	71.0	93.1	98.6
Benzo(g,h,i)perylene	98.0	0.0	81.2	0.0	33.6	78.6	128.7	83.0	94.2	66.4
Mean	95.1	94.3	101.0	95.5	96.5	104.1	113.0	100.9	92.5	

* Values greater than 150% were not used to determine the averages, but the 0% values were used.

TABLE 13

SINGLE LABORATORY ACCURACY AND PRECISION FOR THE EXTRACTION OF PAHs FROM A CERTIFIED REFERENCE SEDIMENT EC-1, USING METHOD 3561 (SFE - SOLID TRAP)

Compound	Certified Value (mg/kg)	SFE Value[a] (mg/kg)	Percent of Certified Value	SFE RSD
Naphthalene	(27.9)[b]	41.3 ± 3.6	(148)	8.7
Acenaphthylene	(0.8)	0.9 ± 0.1	(112)	11.1
Acenaphthene	(0.2)	0.2 ± 0.01	(100)	0.05
Fluorene	(15.3)	15.6 ± 1.8	(102)	11.5
Phenanthrene	15.8 ± 1.2	16.1 ± 1.8	102	11.2
Anthracene	(1.3)	1.1 ± 0.2	(88)	18.2
Fluoranthene	23.2 ± 2.0	24.1 ± 2.1	104	8.7
Pyrene	16.7 ± 2.0	17.2 ± 1.9	103	11.0
Benz(a)anthracene	8.7 ± 0.8	8.8 ± 1.0	101	11.4
Chrysene	(9.2)	7.9 ± 0.9	(86)	11.4
Benzo(b)fluoranthene	7.9 ± 0.9	8.5 ± 1.1	108	12.9
Benzo(k)fluoranthene	4.4 ± 0.5	4.1 ± 0.5	91	12.2
Benzo(a)pyrene	5.3 ± 0.7	5.1 ± 0.6	96	11.8
Indeno(1,2,3-cd)pyrene	5.7 ± 0.6	5.2 ± 0.6	91	11.5
Benzo(g,h,i)perylene	4.9 ± 0.7	4.3 ± 0.5	88	11.6
Dibenz(a,h)anthracene	(1.3)	1.1 ± 0.2	(85)	18.2

[a] Relative standard deviations for the SFE values are based on six replicate extractions.

[b] Values in parentheses were obtained from, or compared to, Soxhlet extraction results which were not certified.

Data are taken from Reference 10.

TABLE 14

SINGLE LABORATORY ACCURACY AND PRECISION FOR THE EXTRACTION OF PAHs FROM A CERTIFIED REFERENCE SEDIMENT HS-3, USING METHOD 3561 (SFE - SOLID TRAP)

Compound	Certified Value (mg/kg)	SFE Value[a] (mg/kg)	Percent of Certified Value	SFE RSD
Naphthalene	9.0 ± 0.7	7.4 ± 0.6	82	8.1
Acenaphthylene	0.3 ± 0.1	0.4 ± 0.1	133	25.0
Acenaphthene	4.5 ± 1.5	3.3 ± 0.3	73	9.0
Fluorene	13.6 ± 3.1	10.4 ± 1.3	77	12.5
Phenanthrene	85.0 ± 20.0	86.2 ± 9.5	101	11.0
Anthracene	13.4 ± 0.5	12.1 ± 1.5	90	12.4
Fluoranthene	60.0 ± 9.0	54.0 ± 6.1	90	11.3
Pyrene	39.0 ± 9.0	32.7 ± 3.7	84	11.3
Benz(a)anthracene	14.6 ± 2.0	12.1 ± 1.3	83	10.7
Chrysene	14.1 ± 2.0	12.0 ± 1.3	85	10.8
Benzo(b)fluoranthene	7.7 ± 1.2	8.4 ± 0.9	109	10.7
Benzo(k)fluoranthene	2.8 ± 2.0	3.2 ± 0.5	114	15.6
Benzo(a)pyrene	7.4 ± 3.6	6.6 ± 0.8	89	12.1
Indeno(1,2,3-cd)pyrene	5.0 ± 2.0	4.5 ± 0.6	90	13.3
Benzo(g,h,i)perylene	5.4 ± 1.3	4.4 ± 0.6	82	13.6
Dibenz(a,h)anthracene	1.3 ± 0.5	1.1 ± 0.3	85	27.3

[a] Relative standard deviations for the SFE values are based on three replicate extractions.

Data are taken from Reference 10.

TABLE 15

SINGLE LABORATORY ACCURACY AND PRECISION FOR THE EXTRACTION OF PAHs FROM A CERTIFIED REFERENCE SOIL SRS103-100, USING METHOD 3561 (SFE - LIQUID TRAP)

Compound	Certified Value (mg/kg)	SFE Value[a] (mg/kg)	Percent of Certified Value	SFE RSD
Naphthalene	32.4 ± 8.2	29.55	91	10.5
2-Methylnaphthalene	62.1 ± 11.5	76.13	122	2.0
Acenaphthene	632 ± 105	577.28	91	2.9
Dibenzofuran	307 ± 49	302.25	98	4.1
Fluorene	492 ± 78	427.15	87	3.0
Phenanthrene	1618 ± 340	1278.03	79	3.4
Anthracene	422 ± 49	400.80	95	2.6
Fluoranthene	1280 ± 220	1019.13	80	4.5
Pyrene	1033 ± 285	911.82	88	3.1
Benz(a)anthracene	252 ± 8	225.50	89	4.8
Chrysene	297 ± 26	283.00	95	3.8
Benzo(a)pyrene	97.2 ± 17.1	58.28	60	6.5
Benzo(b)fluoranthene + Benzo(k)fluoranthene	153 ± 22	130.88	86	10.7

[a] Relative standard deviations for the SFE values are based on four replicate extractions.

Data are taken from Reference 11.

TABLE 16

SINGLE LABORATORY RECOVERY DATA FOR SOLID-PHASE EXTRACTION OF BASE/NEUTRAL/ACID EXTRACTABLES FROM SPIKED TCLP BUFFERS LOW SPIKE LEVEL

Analyte	Spike Level (µg/L)	Buffer 1 (pH = 2.886)		Buffer 2 (pH = 4.937)	
		Recovery (%)	RSD	Recovery (%)	RSD
1,4-Dichlorobenzene	3,750	63	10	63	9
Hexachloroethane	1,500	55	6	77	4
Nitrobenzene	1,000	82	10	100	5
Hexachlorobutadiene	250	65	3	56	4
2,4-Dinitrotoluene	65	89	4	101	5
Hexachlorobenzene	65	98	5	95	6
o-Cresol	100,000	83	10	85	5
m-Cresol*	100,000	86	8	85	3
p-Cresol*	100,000	*	*	*	*
2,4,6-Trichlorophenol	1,000	84	12	95	12
2,4,5-Trichlorophenol	200,000	83	11	88	3
Pentachlorophenol	50,000	82	9	78	9

Results from seven replicate spiked buffer samples.

* In this study, m-cresol and p-cresol co-eluted and were quantitated as a mixture of both isomers.

Data from Reference 12.

TABLE 17

SINGLE LABORATORY RECOVERY DATA FOR SOLID-PHASE EXTRACTION OF BASE/NEUTRAL/ACID EXTRACTABLES FROM SPIKED TCLP BUFFERS HIGH SPIKE LEVEL

Analyte	Spike Level (µg/L)	Buffer 1 (pH = 2.886)		Buffer 2 (pH = 4.937)	
		Recovery (%)	RSD	Recovery (%)	RSD
1,4-Dichlorobenzene	15,000	63	10	63	9
Hexachloroethane	6,000	54	7	46	7
Nitrobenzene	4,000	81	4	81	13
Hexachlorobutadiene	1,000	81	5	70	11
2,4-Dinitrotoluene	260	99	8	98	3
Hexachlorobenzene	260	89	8	91	9
o-Cresol*	400,000	92	15	90	4
m-Cresol*	400,000	95	8	82	6
p-Cresol*	400,000	82	14	84	7
2,4,6-Trichlorophenol	4,000	93	12	104	12
2,4,5-Trichlorophenol	800,000	93	14	97	23
Pentachlorophenol	200,000	84	9	73	8

Results from seven replicate spiked buffer samples.

* In this study, recoveries of these compounds were determined from triplicate spikes of the individual compounds into separate buffer solutions.

Data from Reference 12.

TABLE 18

RECOVERY DATA FROM THREE LABORATORIES FOR SOLID-PHASE EXTRACTION
OF BASE/NEUTRAL/ACID EXTRACTABLES FROM SPIKED TCLP LEACHATES FROM SOIL SAMPLES

Buffer 1 pH = 2.886		Lab 1			Lab 2			Lab 3		
Analyte	Spike Level (µg/L)*	%R	RSD	n	%R	RSD	n	%R	RSD	n
o-Cresol	200,000	86	8	7	35.3	0.7	3	7.6	6	3
m-Cresol**	--	77	8	7	--	--	--	--	--	--
p-Cresol**	--	--	--	--	--	--	--	7.7	11	3
2,4,6-Trichlorophenol	2,000	106	6	7	96.3	3.9	3	44.8	5	3
2,4,5-Trichlorophenol	400,000	93	3	7	80.5	4.5	3	63.3	11	3
Pentachlorophenol	100,000	79	2	7	33.8	12.2	3	29.2	13	3
1,4-Dichlorobenzene	7,500	51	5	7	81.3	5.3	3	19.2	7	3
Hexachloroethane	3,000	50	5	7	66.2	2.1	3	12.6	11	3
Nitrobenzene	2,000	80	8	7	76.3	5.3	3	63.9	12	3
Hexachlorobutadiene	500	53	8	7	63.3	4.8	3	9.6	9	3
2,4-Dinitrotoluene	130	89	8	7	35.7	2.6	3	58.2	17	3
Hexachlorobenzene	130	84	21	7	92.3	1.6	3	71.7	9	3

(continued)

TABLE 18
(continued)

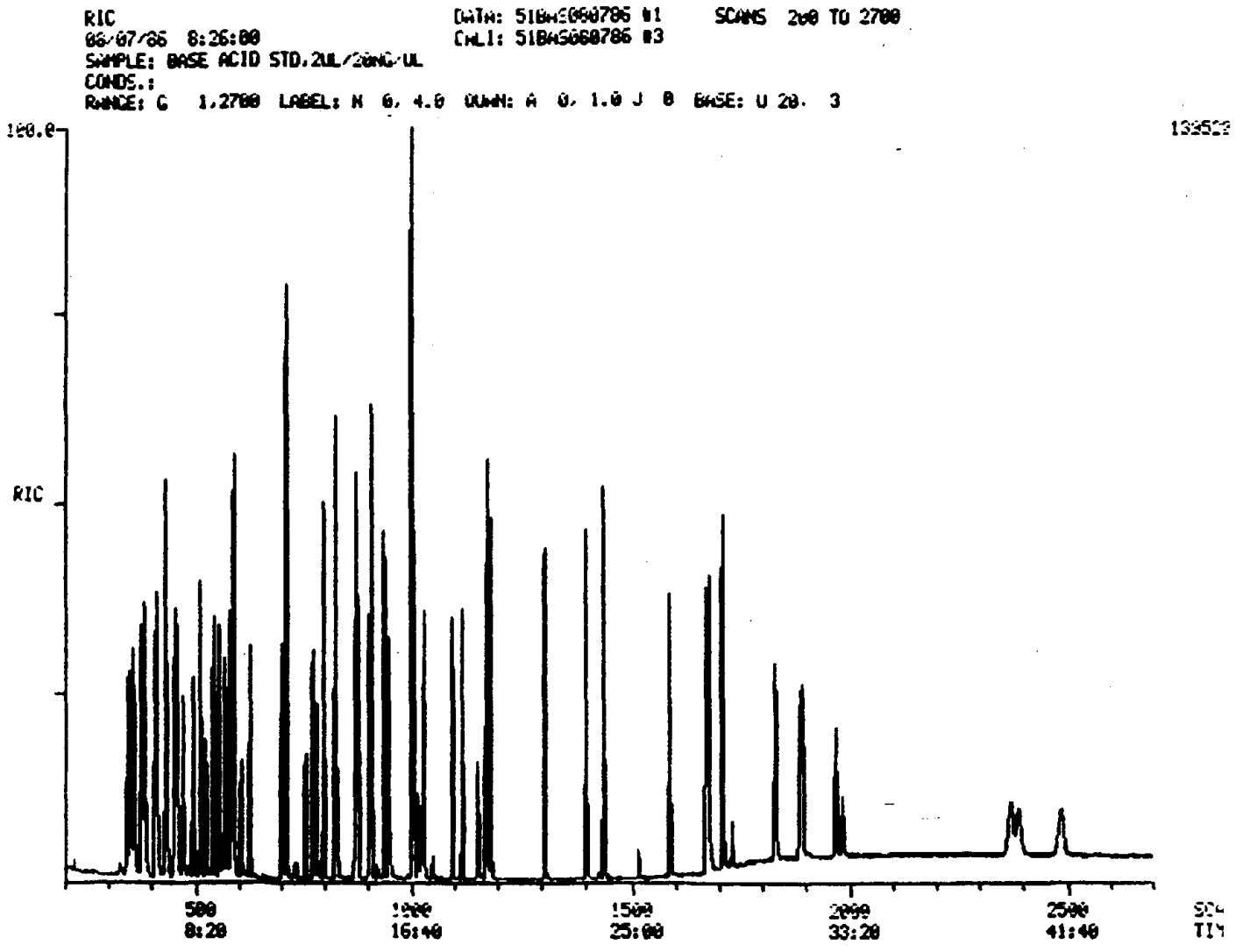
Buffer 2 pH = 4.937		Lab 1			Lab 2			Lab 3		
Analyte	Spike Level (µg/L)*	%R	RSD	n	%R	RSD	n	%R	RSD	n
o-Cresol	200,00	97	13	7	37.8	4.5	3	6.1	24	3
m-Cresol**	--	83	4	7	--	--	--	6.0	25	3
p-Cresol**	--	--	--	--	--	--	--	--	--	--
2,4,6-Trichlorophenol	2,000	104	4	7	91.7	8.0	3	37.7	25	3
2,4,5-Trichlorophenol	400,000	94	4	7	85.2	0.4	3	64.4	10	3
Pentachlorophenol	100,000	109	11	7	41.9	28.2	3	36.6	32	3
1,4-Dichlorobenzene	7,500	50	5	7	79.7	1.0	3	26.5	68	3
Hexachloroethane	3,000	51	3	7	64.9	2.0	3	20.3	90	3
Nitrobenzene	2,000	80	4	7	79.0	2.3	3	59.4	6	3
Hexachlorobutadiene	500	57	5	7	60	3.3	3	16.6	107	3
2,4-Dinitrotoluene	130	86	6	7	38.5	5.2	3	62.2	6	3
Hexachlorobenzene	130	86	7	7	91.3	0.9	3	75.5	5	3

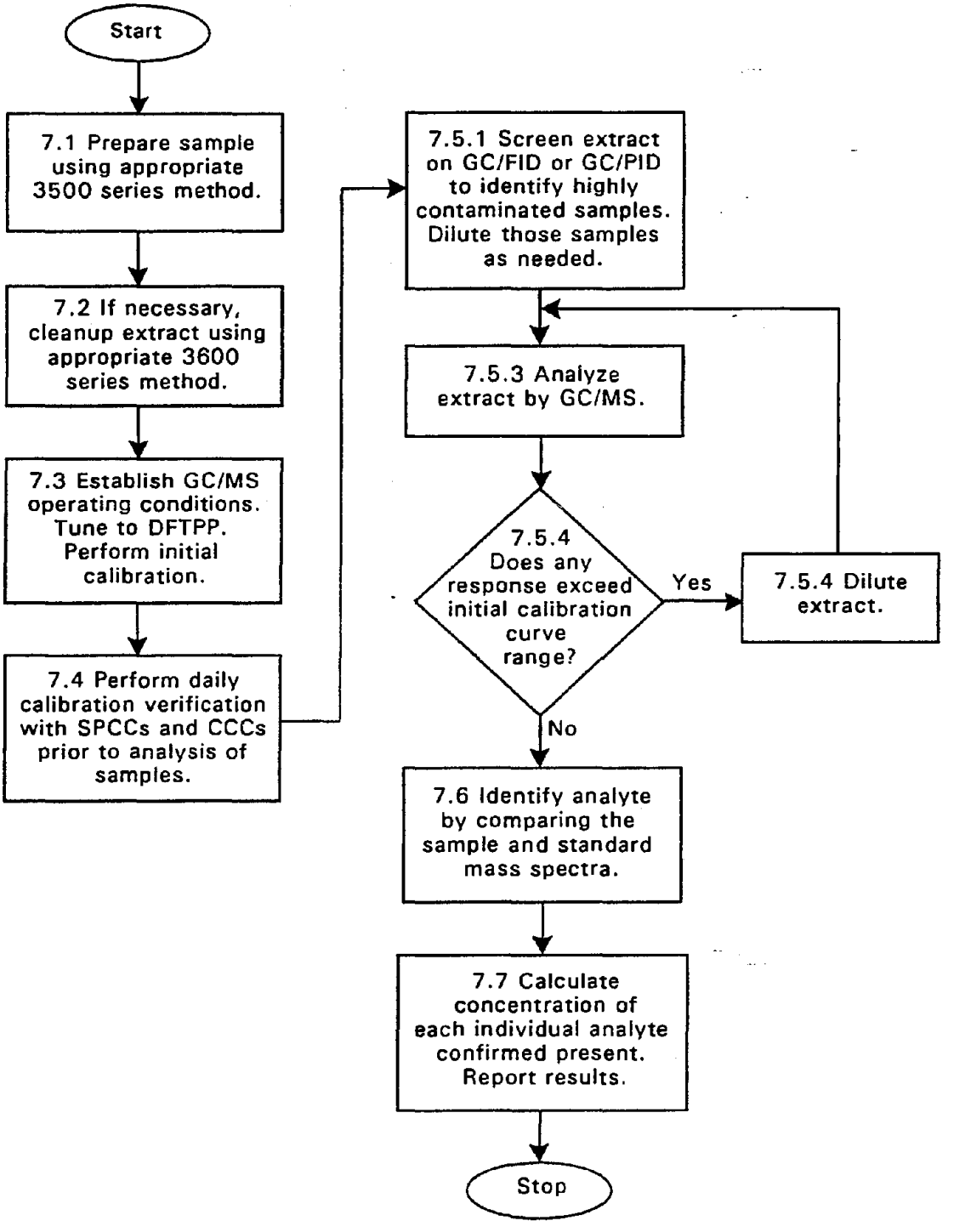
* 250-mL aliquots of leachate were spiked. Lab 1 spiked at one-half these levels.

** m-Cresol and p-Cresol coelute. Lab 1 and Lab 3 reported o-Cresol and the sum of m- and p-Cresol. Lab 2 reported the sum of all three isomers of Cresol.

Data from Reference 12.

FIGURE 1
GAS CHROMATOGRAM OF BASE/NEUTRAL AND ACID CALIBRATION STANDARD




Start
7.1 Prepare sample using appropriate 3500 series method.
7.2 If necessary, cleanup extract using appropriate 3600 series method.
7.3 Establish GC/MS operating conditions. Tune to DFTPP. Perform initial calibration.
7.4 Perform daily calibration verification with SPCCs and CCCs prior to analysis of samples.
7.5.1 Screen extract on GC/FID or GC/PID to identify highly contaminated samples. Dilute those samples as needed.
7.5.3 Analyze extract by GC/MS.
7.5.4 Does any response exceed initial calibration curve range?
Yes
7.5.4 Dilute extract.
No
7.6 Identify analyte by comparing the sample and standard mass spectra.
7.7 Calculate concentration of each individual analyte confirmed present. Report results.
Stop

METHOD 8280B

POLYCHLORINATED DIBENZO-*p*-DIOXINS AND POLYCHLORINATED DIBENZOFURANS BY HIGH RESOLUTION GAS CHROMATOGRAPHY/LOW RESOLUTION MASS SPECTROMETRY (HRGC/LRMS)

1.0 SCOPE AND APPLICATION

1.1 This method is appropriate for the detection and quantitative measurement of 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (2,3,7,8-TCDD), 2,3,7,8-tetrachlorinated dibenzofuran (2,3,7,8-TCDF), and the 2,3,7,8-substituted penta-, hexa-, hepta-, and octachlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) (Figure 1) in water (at part-per-trillion concentrations), soil, fly ash, and chemical waste samples, including stillbottoms, fuel oil, and sludge matrices (at part-per-billion concentrations). The following compounds can be determined by this method (see Sec. 1.4 for a discussion of "total" concentrations).

Compound	CAS Registry No.
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	1746-01-6
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin (PeCDD)	40321-76-4
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)	39227-28-6
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)	57653-85-7
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin (HxCDD)	19408-74-3
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin (HpCDD)	35822-46-9
1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin (OCDD)	3268-87-9
2,3,7,8-Tetrachlorodibenzofuran (TCDF)	51207-31-9
1,2,3,7,8-Pentachlorodibenzofuran (PeCDF)	57117-41-6
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)	57117-31-4
1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)	70648-26-9
1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF)	57117-44-9
1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF)	72918-21-9
2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF)	60851-34-5
1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)	67562-39-4
1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF)	55673-89-7
1,2,3,4,5,6,7,8-Octachlorodibenzofuran (OCDF)	39001-02-0
Total Tetrachlorodibenzo-*p*-dioxin (TCDD)	41903-57-5
Total Pentachlorodibenzo-*p*-dioxin (PeCDD)	36088-22-9
Total Hexachlorodibenzo-*p*-dioxin (HxCDD)	34465-46-8
Total Heptachlorodibenzo-*p*-dioxin (HpCDD)	37871-00-4
Total Tetrachlorodibenzofuran (TCDF)	55722-27-5
Total Pentachlorodibenzofuran (PeCDF)	30402-15-4
Total Hexachlorodibenzofuran (HxCDF)	55684-94-1
Total Heptachlorodibenzofuran (HpCDF)	38998-75-3

1.2 The analytical method requires the use of high resolution gas chromatography and low resolution mass spectrometry (HRGC/LRMS) on sample extracts that have been subjected to specified cleanup procedures. The calibration range is dependent on the compound and the sample size. The sample size varies by sample matrix. Table 2 lists the quantitation limits for the various matrices.

1.3 This method requires the calculation of the 2,3,7,8-TCDD toxicity equivalence according to the procedures given in the U.S. Environmental Protection Agency "Update of Toxicity Equivalency Factors (TEFs) for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-*p*-Dioxins and Dibenzofurans (CDDs/CDFs)" February 1989 (EPA 625/3-89/016). If the toxicity equivalence is greater than or equal to 0.7 ppb (soil or fly ash), 7 ppt (aqueous), or 7 ppb (chemical waste), analysis on a column capable of resolving all 2,3,7,8-substituted PCDDs/PCDFs may be necessary. If the expected concentrations of the PCDDs and PCDFs are below the quantitation limits in Table 2, use of Method 8290 may be more appropriate.

1.4 This method contains procedures for reporting the total concentration of all PCDDs/PCDFs in a given level of chlorination (i.e., Total TCDD, Total PeCDF, etc.), although complete chromatographic separation of all 210 possible PCDDs/PCDFs is not possible under the instrumental conditions described here.

1.5 This method is restricted for use only by analysts experienced with residue analysis and skilled in HRGC/LRMS. Each analyst must demonstrate the ability to generate acceptable results with this method.

1.6 Because of the extreme toxicity of these compounds, the analyst must take necessary precautions to prevent the exposure of laboratory personnel or others to materials known or believed to contain PCDDs or PCDFs. Typical infectious waste incinerators are not satisfactory devices for disposal of materials highly contaminated with PCDDs or PCDFs. A laboratory planning to use these compounds should prepare a disposal plan. Additional safety instructions are outlined in Sec. 11.0.

2.0 SUMMARY OF THE METHOD

2.1 This procedure uses a matrix-specific extraction, analyte-specific cleanup, and high-resolution capillary column gas chromatography/low resolution mass spectrometry (HRGC/LRMS) techniques.

2.2 If interferants are encountered, the method provides selected cleanup procedures to aid the analyst in their elimination. The analysis flow chart is shown at the end of this procedure.

2.3 A specified amount of water, soil, fly ash, or chemical waste samples is spiked with internal standards and extracted according to a matrix-specific extraction procedure. Aqueous samples are filtered, and solid samples that show an aqueous phase are centrifuged before extraction. The extraction procedures and solvents are:

2.3.1 Soil, fly ash, or chemical waste samples are extracted with the combination of a Dean-Stark water trap and a Soxhlet extractor using toluene. Soil, fly ash, and other solids may also be extracted using pressurized fluid extraction (PFE) by Method 3545.

2.3.2 Water samples are extracted with a separatory funnel or liquid-liquid extractor using methylene chloride, and the particulate fraction that results from filtering the water samples are extracted separately in a Soxhlet extractor using toluene.

2.4 The extracts are spiked with $^{37}Cl_4$-2,3,7,8-TCDD and submitted to an acid-base washing treatment, dried and concentrated. The extracts are cleaned up by column chromatography on alumina, silica gel, and activated carbon on Celite 545® and concentrated again.

2.5 An aliquot of the concentrated extract is injected into an HRGC/LRMS system capable of performing the selected ion monitoring.

2.6 The identification of the target compounds is based on their ordered elution and comparison to standard solutions (Table 1) from an appropriate GC column and MS identification. Isomer specificity for all 2,3,7,8-substituted PCDDs/PCDFs cannot be achieved on a single column. The use of both DB-5 and SP2331 (or equivalent) columns is advised. No analyses can proceed unless all the criteria for retention times, peak identification, signal-to-noise and ion abundance ratios are met by the GC/MS system after the initial calibration and calibration verification.

2.7 A calculation of the toxicity equivalent concentration (TEQ) of each sample is made using international consensus toxicity equivalence factors (TEFs), and the TEQ is used to determine if the concentrations of target compounds in the sample are high enough to warrant confirmation of the results on a second GC column.

3.0 INTERFERENCES

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines which may cause misinterpretation of chromatographic data. All of these materials must be demonstrated to be free from interferents under the conditions of analysis by running laboratory method blanks.

3.2 The use of high purity reagents and pesticide grade solvents helps to minimize interference problems. Purification of solvents by distillation, in all glass systems, may be required.

3.3 Interferants co-extracted from the sample will vary considerably from source to source, depending upon the industrial process being sampled. PCDDs and PCDFs are often associated with other interfering chlorinated compounds such as PCBs and polychlorinated diphenyl ethers (PCDPEs) which may be found at concentrations several orders of magnitude higher than that of the analytes of interest. Retention times of target analytes must be verified using reference standards. While certain cleanup techniques are provided as part of this method, unique samples may require additional cleanup techniques to achieve the sensitivity specified in this method.

3.4 High resolution capillary columns are used to resolve as many isomers as possible; however, no single column is known to resolve all of the 210 isomers. The columns employed by the laboratory in these analyses must be capable of resolving all 17 of the 2,3,7,8-substituted PCDDs/PCDFs sufficiently to meet the method specifications.

4.0 APPARATUS AND MATERIALS

4.1 Gas chromatograph/mass spectrometer system:

4.1.1 Gas chromatograph - An analytical system with a temperature-programmable gas chromatograph and all necessary accessories including syringes, analytical columns, and gases. The GC injection port shall be designed for capillary columns; a splitless or an on-column injection technique is recommended. A 2-µL injection volume is assumed throughout

this method; however, with some GC injection ports, other volumes may be more appropriate. A 1-µL injection volume may be used if adequate sensitivity and precision can be demonstrated.

4.1.2 GC column - Fused silica capillary columns are needed. The columns shall demonstrate the required separation of all 2,3,7,8-specific isomers whether a dual column or a single column analysis is chosen. Column operating conditions shall be evaluated at the beginning and end of each 12-hour period during which samples or concentration calibration solutions are analyzed.

4.1.2.1 Isomer specificity for all 2,3,7,8-substituted PCDDs/PCDFs cannot be achieved on the 60 m DB-5 column. Problems have been associated with the separation of 2,3,7,8-TCDD from 1,2,3,7-TCDD and 1,2,6,8-TCDD, and separation of 2,3,7,8-TCDF from 1,2,4,9-, 1,2,7,9-, 2,3,4,6-, 2,3,4,7-, and 2,3,4,8-TCDF. Because of the toxicologic concern associated with 2,3,7,8-TCDD and 2,3,7,8-TCDF, additional analyses may be necessary for some samples, as described in Sec. 7.15.8. In instances where the toxicity equivalent concentration (TEQ) is greater than 0.7 ppb (solids), 7 ppt (aqueous), or 7 ppb (chemical waste), the reanalysis of the sample extract on a 60 m SP-2330 or SP-2331 GC, or DB-225 column (or equivalent column) may be required in order to determine the concentrations of the individual 2,3,7,8-substituted isomers. For the DB-225 column, problems are associated with the separation of 2,3,7,8-TCDF from 2,3,4,7-TCDF and a combination of 1,2,3,9- and 2,3,4,8-TCDF.

4.1.2.2 For any sample analyzed on a DB-5 or equivalent column in which 2,3,7,8-TCDF is reported as an Estimated Maximum Possible Concentration (Sec. 7.15.7) that is above the quantitation limit for the matrix, analysis of the extract is recommended on a second GC column which provides better specificity for 2,3,7,8-TCDF.

4.1.2.3 Analysis on a single column is acceptable if the required separation of all the 2,3,7,8-specific isomers is demonstrated, and the minimum acceptance criteria outlined in Sec. 7.12 are met. See Sec. 7.14.5 for the specifications for the analysis of the 2,3,7,8-specific isomers using both dual columns and single columns.

4.2 Mass spectrometer - A low resolution instrument is employed, utilizing 70 volts (nominal) electron energy in the electron impact ionization mode. The system must be capable of selected ion monitoring (SIM). The recommended configuration is for at least 18 ions per cycle, with a cycle time of 1 sec or less, and a minimum integration time of 25 msec per m/z. Other cycle times and integration times may be employed, provided that the analyst can demonstrate acceptable performance for the calibration standards and window defining mixes. The integration time used to analyze samples shall be identical to the time used to analyze the initial and continuing calibration solutions and quality control samples.

4.2.1 Interfaces - GC/MS interfaces constructed of all glass or glass-lined materials are necessary. Glass can be deactivated by silanizing with dichlorodimethylsilane. Inserting a fused silica column directly into the MS source is recommended. Care must be taken not to expose the end of the column to the electron beam.

4.2.2 Data system - An interfaced data system is necessary to acquire, store, reduce and output mass spectral data.

4.3 Miscellaneous equipment

4.3.1 Nitrogen evaporation apparatus (N-Evap* Analytical Evaporator Model 111, Organomation Association Inc., Northborough, MA, or equivalent).

4.3.2 Balance capable of accurately weighing ±0.01 g.

4.3.3 Water bath - Equipped with concentric ring cover and temperature controlled within ± 2°C.

4.3.4 Stainless steel (or glass) pan large enough to hold contents of 1-pint sample containers.

4.3.5 Glove box - For use in preparing standards from neat materials and in handling soil/sediment samples containing fine particulates that may pose a risk of exposure.

4.3.6 Rotary evaporator, R-110, Buchi/Brinkman - American Scientific No. E5045-10 or equivalent.

4.3.7 Centrifuge - Capable of operating at 400 x G with a 250-300 mL capacity.

4.3.8 Drying oven.

4.3.9 Vacuum oven - Capable of drying solvent-washed solid reagents at 110°C.

4.3.10 Mechanical shaker - A magnetic stirrer, wrist-action or platform-type shaker that produces vigorous agitation. Used for pre-treatment of fly ash samples.

4.4 Miscellaneous laboratory glassware

4.4.1 Extraction jars - Amber glass with polytetrafluoroethylene (PTFE)-lined screw cap; minimum capacity of approximately 200 mL; must be compatible with mechanical shaker to be used.

4.4.2 Kudema-Danish (K-D) Apparatus - 500-mL evaporating flask, 10-mL graduated concentrator tubes with ground glass stoppers, three-ball macro-Snyder column.

NOTE: The use of a solvent vapor recovery system (Kontes K-545000-1006 or K-547300-0000, Ace Glass 6614-30, or equivalent) is recommended for the purpose of solvent recovery during the concentration procedures requiring the use of Kudema-Danish evaporative concentrators. Incorporation of this apparatus may be required by State or local municipality regulations that govern air emissions of volatile organics. EPA recommends the incorporation of this type of reclamation system as a method to implement an emissions reduction program. Solvent recovery is a means to conform with waste minimization and pollution prevention initiatives.

4.4.3 Disposable Pasteur pipets, 150 mm long x 5 mm ID.

4.4.4 Disposable serological pipets, 10-mL for preparation of the carbon column described in Sec. 7.10.

4.4.5 Vials - 0.3-mL and 2-mL amber borosilicate glass with conical shaped reservoir and screw caps lined with PTFE-faced silicone disks.

4.4.6 Funnels - Glass; appropriate size to accommodate filter paper (12.5 cm).

4.4.7 Chromatography columns - 300 mm x 10.5 mm glass chromatographic column fitted with PTFE stopcock.

4.4.8 Soxhlet apparatus, 500-mL flask, all glass - Complete with glass extractor body, condenser, glass extraction thimbles, heating mantle, and variable transformer for heat control.

NOTE: Extraction thimbles must be of sufficient size to hold 100 g of sand, 5 g of silica gel, and at least 10 g of solid sample, with room to mix the sand and sample in the thimble.

4.4.9 Dean-Stark water separator apparatus, with a PTFE stopcock. Must fit between Soxhlet extractor body and condenser.

4.4.10 Concentrator tubes - 15-mL conical centrifuge tubes.

4.4.11 Separatory funnels - 125-mL and 2-L separatory funnels with a PTFE stopcock.

4.4.12 Continuous liquid-liquid extractor - 1-L sample capacity, suitable for use with heavier than water solvents.

4.4.13 PTFE boiling chips - wash with hexane prior to use.

4.4.14 Buchner funnel - 15 cm.

4.4.15 Filtration flask - For use with Buchner funnel, 1-L capacity.

4.5 Filters

4.5.1 Filter paper - Whatman No. 1 or equivalent.

4.5.2 Glass fiber filter - 15-cm, for use with Buchner funnel.

4.5.3 0.7 µm, Whatman GFF, or equivalent material compatible with toluene. Rinse with toluene.

4.6 Glass wool, silanized - Extract with methylene chloride and hexane before use.

4.7 Laboratory glassware cleaning procedures - Reuse of glassware should be minimized to avoid the risk of using contaminated glassware. All glassware that is reused shall be scrupulously cleaned as soon as possible after use, applying the following procedure.

4.7.1 Rinse glassware with the last solvent used in it.

4.7.2 Wash with hot water containing detergent.

4.7.3 Rinse with copious amounts of tap water and several portions of organic-free reagent water. Drain dry.

4.7.4 Rinse with pesticide grade acetone and hexane.

4.7.5 After glassware is dry, store inverted or capped with aluminum foil in a clean environment.

4.7.6 Do not bake reusable glassware as a routine part of cleaning. Baking may be warranted after particularly dirty samples are encountered, but should be minimized, as repeated baking may cause active sites on the glass surface that will irreversibly adsorb PCDDs/PCDFs.

CAUTION: The analysis for PCDDs/PCDFs in water samples is for much lower concentrations than in soil/sediment, fly ash, or chemical waste samples. Extreme care must be taken to prevent cross-contamination between soil/sediment, fly ash, chemical waste and water samples. Therefore, it is strongly recommended that separate glassware be reserved for analyzing water samples.

4.8 Pre-extraction of glassware - All glassware should be rinsed or pre-extracted with solvent immediately before use. Soxhlet-Dean-Stark (SDS) apparatus and continuous liquid-liquid extractors should be pre-extracted for approximately three hours immediately prior to use, using the same solvent and extraction conditions that will be employed for sample extractions. The pooled waste solvent for a set of extractions may be concentrated and analyzed as a method of demonstrating that the glassware was free of contamination.

It is recommended that each piece of reusable glassware be numbered in such a fashion that the laboratory can associate all reusable glassware with the processing of a particular sample. This will assist the laboratory in:

1) Tracking down possible sources of contamination for individual samples,

2) Identifying glassware associated with highly contaminated samples that may require extra cleaning, and

3) Determining when glassware should be discarded.

5.0 REAGENTS

5.1 Solvents - all solvents must be pesticide grade, distilled-in-glass.

5.1.1 Hexane, C_6H_{14}

5.1.2 Methanol, CH_3OH

5.1.3 Methylene chloride, CH_2Cl_2

5.1.4 Toluene, $C_6H_5CH_3$

5.1.5 Isooctane, $(CH_3)_3CCH_2CH(CH_3)_2$

5.1.6 Cyclohexane, C_6H_{12}

5.1.7 Acetone, CH_3COCH_3

5.1.8 Tridecane, $CH_3(CH_2)_{11}CH_3$

5.1.9 Nonane, C_9H_{20}

5.2 White quartz sand - 60/70 mesh, for use in the Soxhlet-Dean-Stark (SDS) extractor. Bake at 450°C for 4 hours minimum.

5.3 Sodium sulfate (granular, anhydrous), Na_2SO_4 - Purify by heating at 400°C for 4 hours in a shallow tray, or by extracting with methylene chloride. If, after heating, the sodium sulfate develops a noticeable grayish cast (due to the presence of carbon in the crystal matrix) that batch of sodium sulfate is not suitable for use and should be discarded. Extraction with methylene chloride may produce sodium sulfate that is suitable for use in such instances, but following extraction, a reagent blank must be analyzed that demonstrates that there is no interference from the sodium sulfate.

5.4 Potassium hydroxide, KOH - ACS reagent grade, prepare a 20% (w/v) solution in organic-free reagent water.

5.5 Sulfuric acid, H_2SO_4, concentrated - ACS reagent grade, specific gravity 1.84.

5.6 Sodium chloride, NaCl - ACS reagent grade, prepare a 5% (w/v) solution in organic-free reagent water.

5.7 Hydrochloric acid, HCl, concentrated - ACS reagent grade, specific gravity 1.17. Prepare a 1N solution in organic-free reagent water for pretreatment of fly ash samples.

5.8 Column chromatography reagents

This section describes the column chromatography reagents employed in this method for cleanup of sample extracts. The quality of two of these reagents, the alumina and silica gel, is critical to a successful analysis. Prior to employing the reagents in Secs. 5.8.1., 5.8.4., 5.8.5., and 5.8.6., the analyst should demonstrate that they meet the performance requirements in Sec. 7.9.2.

5.8.1 Alumina, acidic - Supelco 19996-6C (or equivalent). Soxhlet extract with methylene chloride for 18 hours and activate by heating to 130°C for a minimum of 12 hours.

5.8.2 Charcoal carbon - Activated carbon, Carbopak C (Supelco) or equivalent, prewashed with methanol and dried *in vacuo* at 110°C. (Note: AX-21 [Anderson Development Company] carbon is no longer available, but existing stocks may be utilized).

5.8.3 Celite 545® (Supelco) or equivalent.

5.8.4 Silica gel - High-purity grade, type 60, 70-230 mesh. Soxhlet extract with methylene chloride for 21 hours and activate by heating in a foil covered glass container for 24 hours at 190°C.

5.8.5 Silica gel impregnated with 2% (w/w) sodium hydroxide - Add one part by weight of 1 M NaOH solution to two parts silica gel (extracted and activated) in a screw-cap bottle and mix with a glass rod until free of lumps.

5.8.6 Silica gel impregnated with 40% (w/w) sulfuric acid. Add two parts by weight concentrated sulfuric acid to three parts silica gel (extracted and activated), mix with a glass rod until free of lumps, and store in a screw-cap glass bottle.

5.9 Calibration solutions (Table 1) - Prepare five tridecane (or nonane) solutions (CC1-CC5) containing 10 unlabeled and 7 carbon-labeled PCDDs/PCDFs at known concentrations for use in instrument calibration. One of these five solutions (CC3) is used as the calibration verification solution and contains 7 additional unlabeled 2,3,7,8-isomers. The concentration ranges are homologue-dependent, with the lowest concentrations associated with tetra- and pentachlorinated dioxins and furans (0.1 to 2.0 ng/µL), and the higher concentrations associated with the hexa- through octachlorinated homologues (0.5 to 10.0 ng/µL). Commercially-available standards containing all 17 unlabeled analytes in each solution may also be utilized.

5.10 Internal standard solution (Table 3) - Prepare a solution containing the five internal standards in tridecane (or nonane) at the nominal concentrations listed in Table 3. Mix 10 µL with 1.0 mL of acetone before adding to each sample and blank.

5.11 Recovery standard solution (Table 3) - Prepare a solution in hexane containing the recovery standards, $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD, at concentrations of 5.0 ng/µL, in a solvent other than tridecane or nonane.

5.12 Calibration verification solution - Prepare a solution containing standards to be used for identification and quantitation of target analytes (Table 4).

5.13 Cleanup standard - Prepare a solution containing $^{37}Cl_4$-2,3,7,8-TCDD at a concentration of 5 ng/µL (5 µg/mL) in tridecane (or nonane). Add this solution to all sample extracts prior to cleanup. The solution may be added at this concentration, or diluted into a larger volume of solvent. The recovery of this compound is used to judge the efficiency of the cleanup procedures.

5.14 Matrix spiking standard - Prepare a solution containing ten of the 2,3,7,8-substituted isomers, at the concentrations listed in Table 5 in tridecane (or nonane). Use this solution to prepare the spiked sample aliquot. Dilute 10 µL of this standard to 1.0 mL with acetone and add to the aliquot chosen for spiking.

5.15 Window defining mix - Prepare a solution containing the first and last eluting isomer of each homologue (Table 6). Use this solution to verify that the switching times between the descriptors have been appropriately set.

5.16 Column performance solutions

Chromatographic resolution is verified using a test mixture of PCDDs/PCDFs specific to each column and shown below.

DB-5 test mix: 1,2,3,7-TCDD/1,2,3,8-TCDD
2,3,7,8-TCDD
1,2,3,9-TCDD

DB-225 test mix: 2,3,4,7-TCDF
2,3,7,8-TCDF
1,2,3,9-TCDF

SP-2331 test mix: 2,3,7,8-TCDD
1,4,7,8-TCDD
1,2,3,7-TCDD
1,2,3,8-TCDD

The concentrations of these isomers should be approximately 0.5 ng/µL in tridecane (or nonane).

If the laboratory employs a column that has a different elution order than those specified here, the laboratory must ensure that the isomers eluting closest to 2,3,7,8-TCDD are represented in the column performance solution.

6.0 SAMPLE COLLECTION, HANDLING, AND PRESERVATION

6.1 See the introductory material to this chapter, Organic Analytes.

6.2 Sample collection

6.2.1 Sample collection personnel should, to the extent possible, homogenize samples in the field before filling the sample containers. This should minimize or eliminate the necessity for sample homogenization in the laboratory. The analyst should make a judgment, based on the appearance of the sample, regarding the necessity for additional mixing. If the sample is clearly not homogeneous, the entire contents should be transferred to a glass or stainless steel pan for mixing with a stainless steel spoon or spatula before removal of a sample portion for analysis.

6.2.2 Grab and composite samples must be collected in glass containers. Conventional sampling practices must be followed. The bottle must not be prewashed with sample before collection. Sampling equipment must be free of potential sources of contamination.

6.2.3 If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of sample. If sample pH is greater than 9, adjust to pH 7-9 with sulfuric acid.

6.3 Storage and holding times - All samples should be stored at 4°C in the dark, extracted within 30 days and completely analyzed within 45 days of extraction. Whenever samples are analyzed after the holding time expiration date, the results should be considered to be minimum concentrations and should be identified as such.

NOTE: The holding times listed in Sec. 6.3 are recommendations. PCDDs and PCDFs are very stable in a variety of matrices, and holding times under the conditions listed in Sec. 6.3 may be as high as a year for certain matrices.

7.0 PROCEDURE

Five types of extraction procedures are employed in these analyses, depending on the sample matrix and the available equipment.

1) Chemical waste samples are extracted by refluxing with a Dean-Stark water separator.

2) Fly ash samples and soil/sediment samples may be extracted in a combination of a Soxhlet extractor and a Dean-Stark water separator.

3) Water samples are filtered and then the filtrate is extracted using either a separatory funnel procedure or a continuous liquid-liquid extraction procedure.

4) The filtered particulates are extracted in a combination of a Soxhlet extractor and a Dean-Stark water separator.

5) Fly ash, soil/sediment, and other solid samples may also be extracted using pressurized fluid extraction (PFE), employing Method 3545. (See Method 3545 for the equipment, supplies, reagents, and procedures associated with PFE.)

Sec. 7.1 provides general information on the use of the Soxhlet-Dean-Stark apparatus. The first four matrix-specific extraction procedures are described in Secs. 7.2 - 7.5. Pressurized fluid extraction is described in Method 3545.

NOTE: EPA has not performed a formal evaluation of pressurized fluid extraction (PFE) with respect to Method 8280. However, EPA has received and evaluated data regarding this technique in conjunction with Method 8290, the high resolution mass spectrometric method for PCDDs/PCDFs, and has incorporated those data into that method. Given that Method 8280 addresses a higher concentration range of the target analytes than Method 8290 (rather than a lower range), EPA believes that PFE will also be applicable to analyses employing Method 8280. Analysts wishing to employ PFE are advised to proceed with caution. Consult Method 3545 and the manufacturer of the PFE equipment for additional information regarding PCDD/PCDF extraction. Laboratories wishing to employ PFE in conjunction with Method 8280 should initially demonstrate the applicability of the technique to typical range of concentrations and matrices addressed in Method 8280, focusing on the use of reference materials rather than spiked samples whenever possible. The results of such a demonstration should be maintained on file at the laboratory.

7.1 General considerations for use of the Soxhlet-Dean-Stark (SDS) apparatus

The following procedures apply to use of the SDS apparatus for extracting matrices covered by this protocol.

The combination of a Soxhlet extractor and a Dean-Stark trap is used for the removal of water and extraction of PCDDs/PCDFs from samples of fly ash, soil/sediment, and the particulate fraction of water samples.

For soil/sediment samples, the results of these analyses are reported based on the wet weight of the sample. However, use of the SDS allows the water content of a sample to be determined from the same aliquot of sample that is also extracted for analysis. The amount of water evolved from the sample during extraction is used to approximate the percent solids content of the sample. The percent solids data may be employed by the data user to approximate the dry weight

concentrations. The percent solids determination does not apply to the extraction of particulates from the filtration of water samples or to the extraction of fly ash samples which are treated with an HCl solution prior to extraction.

7.1.1 The extraction of soil/sediment, fly ash, and particulates from water samples will require the use of a Soxhlet thimble. See Sec. 4.6 for a discussion of pre-extraction of glassware such as the SDS. Prior to pre-extraction, prepare the thimble by adding 5 g of 70/230 mesh silica gel to the thimble to produce a thin layer in the bottom of the thimble. This layer will trap fine particles in the thimble. Add 80-100 g of quartz sand on top of the silica gel, and place the thimble in the extractor.

7.1.2 Pre-extract the SDS for three hours with toluene, then allow the apparatus to cool and remove the thimble. Mix the appropriate weight of sample with the sand in the thimble, being careful not to disturb the silica gel layer.

7.1.3 If the sample aliquot to be extracted contains large lumps, or is otherwise not easily mixed in the thimble, the sand and sample may be mixed in another container. Transfer approximately 2/3 of the sand from the thimble to a clean container, being careful not to disturb the silica gel layer when transferring the sand. Thoroughly mix the sand with the sample with a clean spatula, and transfer the sand/sample mixture to the thimble.

7.1.4 If a sample with particularly high moisture content is to be extracted, it may be helpful to leave a small conical depression in the material in the thimble. This will allow the water to drain through the thimble more quickly during the early hours of the extraction. As the moisture is removed during the first few hours of extraction, the depression will collapse, and the sample will be uniformly extracted.

7.2 Chemical waste extraction (including oily sludge/wet fuel oil and stillbottom/oil).

7.2.1 Assemble a flask, a Dean-Stark trap, and a condenser, and pre-extract with toluene for three hours (see Sec. 4.6). After pre-extraction, allow the apparatus to cool, and discard the used toluene, or pool it for later analysis to verify the cleanliness of the glassware.

7.2.2 Weigh about 1 g of the waste sample to two decimal places into a tared pre-extracted 125-mL flask. Add 1 mL of the acetone-diluted internal standard solution (Sec. 5.10) to the sample in the flask. Attach the pre-extracted Dean-Stark water separator and condenser to the flask, and extract the sample by refluxing it with 50 mL of toluene for at least three hours.

Continue refluxing the sample until all the water has been removed. Cool the sample, filter the toluene extract through a rinsed glass fiber filter into a 100-mL round-bottom flask. Rinse the filter with 10 mL of toluene; combine the extract and rinsate. Concentrate the combined solution to approximately 10 mL using a K-D or rotary evaporator as described in Secs. 7.6.1 and 7.6.2. Transfer the concentrated extract to a 125-mL separatory funnel. Rinse the flask with toluene and add the rinse to the separatory funnel. Proceed with acid-base washing treatment per Sec. 7.8, the micro-concentration per Sec. 7.7, the chromatographic procedures per Secs. 7.9 and 7.10, and a final concentration per Sec. 7.11.

7.2.3 Prepare an additional two 1-g aliquots of the sample chosen for spiking. After weighing the sample in a tared pre-extracted flask (Sec. 7.2.2), add 1.0 mL of the acetone-diluted matrix spiking standard solution (Sec. 5.14) to each of the two aliquots. After allowing the matrix spiking solution to equilibrate to approximately 1 hour, add the internal standard solution and extract the aliquots as described in Sec. 7.2.2.

7.3 Fly ash sample extraction

7.3.1 Weigh about 10 g of the fly ash to two decimal places, and transfer to an extraction jar. Add 1 mL of the acetone-diluted internal standard solution to the sample.

7.3.2 Add 150 mL of 1 N HCl to the fly ash sample in the jar. Seal the jar with the PTFE-lined screw cap, place on a mechanical shaker, and shake for 3 hours at room temperature.

7.3.3 Rinse a Whatman #1 (or equivalent) filter paper with toluene, and then filter the sample through the filter paper in a Buchner funnel into a 1 L receiving flask. Wash the fly ash with approximately 500 mL of organic-free reagent water.

7.3.4 Mix the fly ash with the sand in the pre-extracted thimble (Sec. 7.1.2). Place the filter paper from Sec. 7.3.3 on top of the sand. Place the thimble in a SDS extractor, add 200 mL toluene, and extract for 16 hours. The solvent should cycle completely through the system 5-10 times per hour. Cool and filter the toluene extract through a rinsed glass fiber filter into a 500-mL round-bottom flask. Rinse the filter with 10 mL of toluene. Concentrate the extract as described in Secs. 7.6.1 or 7.6.2. Transfer the concentrated extract to a 125-mL separatory funnel. Rinse the flask with toluene and add the rinse to the separatory funnel. Proceed with acid-base washing treatment per Sec. 7.8, the micro-concentration per Sec. 7.7, the chromatographic procedures per Secs. 7.9 and 7.10 and a final concentration per Sec. 7.11.

NOTE: A blank should be analyzed using a piece of filter paper handled in the same manner as the fly ash sample.

7.3.5 Prepare an additional two 10-g aliquots of the sample chosen for spiking for use as the matrix spike and matrix spike duplicate. Transfer each aliquot to a separate extraction jar and add 1.0 mL of the acetone-diluted matrix spiking standard solution (Sec. 5.14) to each of the two aliquots. After allowing the matrix spiking solution to equilibrate for approximately 1 hour, add the internal standard solution and extract the aliquots as described in Sec. 7.3.1.

7.3.6 If pressurized fluid extraction is employed, consult Method 3545.

7.4 Soil/sediment sample extraction

NOTE: Extremely wet samples may require centrifugation to remove standing water before extraction.

7.4.1 Weigh about 10 grams of the soil to two decimal places and transfer to a pre-extracted thimble (Sec. 7.1.2). Mix the sample with the quartz sand, and add 1 mL of the acetone-diluted internal standard solution (Sec. 5.10) to the sample/sand mixture. Add small portions of the solution at several sites on the surface of the sample/sand mixture.

7.4.2 Place the thimble in the SDS apparatus, add 200 to 250 mL toluene, and reflux for 16 hours. The solvent should cycle completely through the system 5-10 times per hour.

7.4.3 Estimate the percent solids content of the soil/sediment sample by measuring the volume of water evolved during the SDS extraction procedure. For extremely wet samples,

the Dean-Stark trap may need to be drained one or more times during the 16-hour extraction. Collect the water from the trap, measure its volume to the nearest 0.1 mL. Assume a density of 1.0 g/mL, and calculate the percent solids content according to the formula below:

$$\text{Percent solids} = \frac{\text{Wet weight of sample} - \text{Weight of water}}{\text{Wet weight of sample}} \times 100$$

7.4.4 Concentrate this extract as described in Secs. 7.6.1 or 7.6.2. Transfer the concentrated extract to a 125 mL separatory funnel. Rinse the flask with toluene and add the rinse to the separatory funnel. Proceed with acid-base washing treatment per Sec. 7.8, the micro concentration per Sec. 7.7, the chromatographic procedures per Secs. 7.9 and 7.10 and a final concentration per Sec. 7.11.

7.4.5 Prepare an additional two 10-g aliquots of the sample chosen for spiking for use as the matrix spike and matrix spike duplicate. After transferring each aliquot to a separate pre-extracted Soxhlet thimble, add 1.0 mL of the acetone-diluted matrix spiking standard solution (Sec. 5.14) to each of the two aliquots. After allowing the matrix spiking solution to equilibrate to approximately 1 hour, add the internal standard solution (Sec. 5.10) and extract the aliquots as described in Sec. 7.4.1.

7.4.6 If pressurized fluid extraction is employed, consult Method 3545.

7.5 Aqueous sample extraction

7.5.1 Allow the sample to come to ambient temperature, then mark the water meniscus on the side of the 1-L sample bottle for determination of the exact sample volume.

7.5.2 Add 1 mL of the acetone-diluted internal standard solution (Sec. 5.10) to the sample bottle. Cap the bottle, and mix the sample by gently shaking for 30 seconds.

7.5.3 Filter the sample through a 0.7-µm filter that has been rinsed with toluene. Collect the aqueous filtrate in a clean flask. If the total dissolved and suspended solids contents are too much to filter through the 0.7-µm filter, centrifuge the sample, decant, and then filter the aqueous phase. Alternatively, other filter configurations, including stacked filters of decreasing pore sizes, may be employed. Procedures for extraction of the particulate fraction are given in Sec. 7.5.4. The aqueous portion may be extracted using either the separatory funnel technique (Sec. 7.5.5.1) or a pre-extracted continuous liquid-liquid extractor (Sec. 7.5.5.2).

NOTE: Organic-free reagent water used as a blank must also be filtered in a similar fashion, and subjected to the same cleanup and analysis as the water samples.

7.5.4 Particulate fraction

7.5.4.1 Combine the particulate on the filter and the filter itself, and if centrifugation was used, the solids from the centrifuge bottle(s), with the quartz sand in the pre-extracted Soxhlet thimble. Place the filter on top of the particulate/sand mixture, and place the thimble into a pre-extracted SDS apparatus.

7.5.4.2 Add 200 to 250 mL of toluene to the SDS apparatus and reflux for 16 hours. The solvent should cycle completely through the system 5-10 times per hour.

7.5.4.3 Allow the Soxhlet to cool, remove the toluene and concentrate this extract as described in Secs. 7.6.1. or 7.6.2.

7.5.4.4 Pressurized fluid extraction has *not* been evaluated for the extraction of the particulate fraction.

7.5.5 Aqueous filtrate

The aqueous filtrate may be extracted by either a separatory funnel procedure (Sec. 7.5.5.1) or a continuous liquid-liquid extraction procedure (Sec. 7.5.5.2).

7.5.5.1 Separatory funnel extraction - Pour the filtered aqueous sample into a 2-L separatory funnel. Add 60 mL methylene chloride to the sample bottle, seal, and shake 60 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. Drain the methylene chloride extract into a 500-mL K-D concentrator (mounted with a 10-mL concentrator tube) by passing the extract through a funnel packed with a glass wool plug and half-filled with anhydrous sodium sulfate. Extract the water sample two more times using 60 mL of fresh methylene chloride each time. Drain each extract through the funnel into the K-D concentrator. After the third extraction, rinse the sodium sulfate with at least 30 mL of fresh methylene chloride. Concentrate this extract as described in Secs. 7.6.1 or 7.6.2.

7.5.5.2 Continuous liquid-liquid extraction - A continuous liquid-liquid extractor may be used in place of a separatory funnel when experience with a sample from a given source indicates that a serious emulsion problem will result or an emulsion is encountered using a separatory funnel. The following procedure is used for a continuous liquid-liquid extractor.

7.5.5.2.1 Pre-extract the continuous liquid-liquid extractor for three hours with methylene chloride and reagent water. Allow the extractor to cool, discard the methylene chloride and the reagent water, and add the filtered aqueous sample to the continuous liquid-liquid extractor. Add 60 mL of methylene chloride to the sample bottle, seal and shake for 30 seconds.

7.5.5.2.2 Transfer the solvent to the extractor. Repeat the sample bottle rinse with an additional 50 to 100 mL portion of methylene chloride and add the rinse to the extractor. Add 200 to 500 mL methylene chloride to the distilling flask and sufficient reagent water to ensure proper operation. Extract for 16 hours. Allow to cool, then detach the flask and dry the sample by running it through a rinsed funnel packed with a glass wool plug and 5 g of anhydrous sodium sulfate into a 500-mL K-D flask. Concentrate the extract according to Secs. 7.6.1 or 7.6.2.

7.5.6 Combination of extracts - The extracts from both the particulate fraction (Sec. 7.5.4) and the aqueous filtrate (Sec. 7.5.5) must be concentrated using the procedures in Sec. 7.6.1 and then combined together prior to the acid-base washing treatment in Sec. 7.8.

7.5.7 Determine the original aqueous sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1-L graduated cylinder. Record the sample volume to the nearest 5 mL.

7.5.8 Prepare an additional two 1-L aliquots of the sample chosen for spiking for use as the matrix spike and matrix spike duplicate. Add 1.0 mL of the acetone-diluted matrix spiking standard solution (Sec. 5.14) to each of the two aliquots in the original sample bottles. After allowing the matrix spiking solution to equilibrate for approximately 1 hour, add the internal standard solution and filter and extract the aliquots as described in Sec. 7.5.2.

7.6 Macro-concentration procedures (all matrices)

Prior to cleanup, extracts from all matrices must be concentrated to approximately 10 mL. In addition, as noted above, the concentrated extracts from the aqueous filtrate and the filtered particulates must be combined prior to cleanup. Two procedures may be used for macro-concentration: rotary evaporator, or Kuderna-Danish (K-D). Concentration of toluene by K-D involves the use of a heating mantle, as toluene boils above the temperature of a water bath. The two procedures are described below.

7.6.1 Concentration by K-D

7.6.1.1 Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Pre-wet the column by adding approximately 1 mL of toluene through the top.

7.6.1.2 Attach the solvent recovery system condenser, place the round-bottom flask in a heating mantle and apply heat as required to complete the concentration in 15-20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

7.6.1.3 When the apparent volume of liquid reaches 10 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

7.6.2 Concentration by rotary evaporator

7.6.2.1 Assemble the rotary evaporator according to manufacturer's instructions, and warm the water bath to 45°C. On a daily basis, preclean the rotary evaporator by concentrating 100 mL of clean extraction solvent through the system. Archive both the concentrated solvent and the solvent in the catch flask for contamination check if necessary. Between samples, three 2-3 mL aliquots of toluene should be rinsed down the feed tube into a waste beaker.

7.6.2.2 Attach the round-bottom flask containing the sample extract to the rotary evaporator. Slowly apply vacuum to the system and begin rotating the sample flask. Lower the sample flask into the water bath and adjust the speed of rotation to complete the concentration in 15-20 minutes. At the proper rate of concentration, the flow of condensed solvent into the receiving flask will be steady, but no bumping or visible boiling will occur.

7.6.2.3 When the apparent volume of the liquid reaches 10 mL, shut off the vacuum and the rotation. Slowly admit air into the system, taking care not to splash the extract out of the sample flask.

7.7 Micro-concentration procedures (all matrices)

When further concentration is required, either a micro-Snyder column technique or a nitrogen evaporation technique is used to adjust the extract to the final volume required.

7.7.1 Micro-Snyder column technique

7.7.1.1 Add another one or two clean boiling chips to the concentrator tube and attach a two-ball micro-Snyder column. Prewet the column by adding about 0.5 mL of toluene to the top of the column.

7.7.1.2 Place the round-bottom flask in a heating mantle and apply heat as required to complete the concentration in 5-10 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood.

7.7.1.3 When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joints with about 0.2 mL of solvent and add to the concentrator tube. Adjust the final volume to 1.0 mL with solvent.

7.7.2 Nitrogen evaporation technique

7.7.2.1 Place the concentrator tube in a warm water bath (approximately 35°C) and evaporate the solvent volume to the required level using a gentle stream of clean, dry nitrogen (filtered through a column of activated carbon).

CAUTION: Do not use plasticized tubing between the carbon trap and the sample.

7.7.2.2 The internal wall of the tube must be rinsed down several times with the appropriate solvent during the operation. During evaporation, the solvent level in the tube must be positioned to prevent water from condensing into the sample (i.e., the solvent level should be below the level of the water bath). Under normal operating conditions, the extract should not be allowed to become dry.

7.7.2.3 When the apparent volume of liquid reaches 0.5 mL, remove the concentrator tube from the water bath. Adjust the final volume to 1.0 mL with solvent.

7.8 Acid-base cleanup procedure (all matrices)

7.8.1 The concentrated extracts from all matrices are subjected to a series of cleanup procedures generally beginning with an acid-base wash, and continuing on with silica gel chromatography, alumina chromatography, and carbon chromatography. The acid-base wash may not be necessary for uncolored extracts, but all the other cleanup procedures should be employed, regardless of the color of the extract. Begin the cleanup procedures by quantitatively transferring each concentrated extract to a separate 125-mL separatory funnel.

7.8.2 Prior to cleanup, all extracts are spiked with the $^{37}Cl_4$-2,3,7,8-TCDD cleanup standard (Sec. 5.13). The recovery of this standard is used to monitor the efficiency of the cleanup procedures. Spike 5 µL of the cleanup standard (or a larger volume of diluted solution containing 25 ng of $^{37}Cl_4$-2,3,7,8-TCDD) into each separatory funnel containing an extract, resulting in a concentration of 0.25 ng/µL in the final extract analyzed by GC/MS.

CAUTION: Concentrated acid and base produce heat when mixed with aqueous solutions, and may cause solutions to boil or splatter. Perform the following extractions carefully, allowing the heat and pressure in the separatory funnel to dissipate before shaking the stoppered funnel.

7.8.3 Partition the concentrated extract against 40 mL of concentrated sulfuric acid. Shake for 2 minutes. Remove and discard the acid layer (bottom). Repeat the acid washing until no color is visible in the acid layer. (Perform acid washing a maximum of 4 times.)

7.8.4 Partition the concentrated extract against 40 mL of 5 percent (w/v) sodium chloride. (Caution: Acid entrained in the extract may produce heat when mixed with the sodium chloride solution). Shake for two minutes. Remove and discard the aqueous layer (bottom).

7.8.5 Partition the concentrated extract against 40 mL of 20 percent (w/v) potassium hydroxide (KOH). (Caution: Allow heat to dissipate before shaking). Shake for 2 minutes. Remove and discard the base layer (bottom). Repeat the base washing until color is not visible in the bottom layer (perform base washing a maximum of four times). Strong base (KOH) is known to degrade certain PCDDs/PCDFs; therefore, contact time should be minimized.

7.8.6 Partition the concentrated extract against 40 mL of 5 percent (w/v) sodium chloride. (Caution: Base entrained in the extract may produce heat when mixed with the sodium chloride solution). Shake for 2 minutes. Remove and discard the aqueous layer (bottom). Dry the organic layer by pouring it through a funnel containing a rinsed filter half-filled with anhydrous sodium sulfate. Collect the extract in an appropriate size (100- to 250-mL) round-bottom flask. Wash the separatory funnel with two 15-mL portions of hexane, pour through the funnel and combine the extracts.

7.8.7 Concentrate the extracts of all matrices to 1.0 mL of hexane using the procedures described in Sec. 7.7. Solvent exchange is accomplished by concentrating the extract to approximately 100 µL, adding 2-3 mL of hexane to the concentrator tube and continuing concentration to a final volume of 1.0 mL.

7.9 Silica gel and alumina column chromatographic procedures

7.9.1 Silica gel column - Insert a glass wool plug into the bottom of a gravity column (1 cm x 30 cm glass column) fitted with a PTFE stopcock. Add 1 g silica gel and tap the column gently to settle the silica gel. Add 2 g sodium hydroxide-impregnated silica gel, 1 g silica gel, 4 g sulfuric acid-impregnated silica gel, and 2 g silica gel (Sec. 5.8). Tap the column gently after each addition. A small positive pressure (5 psi) of clean nitrogen may be used if needed.

7.9.2 Alumina column - Insert a glass wool plug onto the bottom of a gravity column (1 cm x 30 cm glass column) fitted with a PTFE stopcock. Add 6 g of the activated acid alumina (Sec. 5.8.1). Tap the top of the column gently.

NOTE: Check each new batch of silica gel and alumina by combining 50 µL of the continuing calibration solution (CC3) with 950 µL of hexane. Process this solution through both columns in the same manner as a sample extract (Secs. 7.9.5 through 7.9.9). Concentrate the continuing calibration solution to a final volume of 50 µL. Proceed

to Sec. 7.14. If the recovery of any of the analytes is less than 80%, the batch of alumina or silica gel may not be appropriate for use.

7.9.3 Add hexane to each column until the packing is free of air bubbles. A small positive pressure (5 psi) of clean dry nitrogen may be used if needed. Check the columns for channeling. If channeling is present, discard the column. Do not tap a wetted column.

7.9.4 Assemble the two columns such that the eluate from the silica gel column drains directly into the alumina column. Alternatively, the two columns may be eluted separately.

7.9.5 Apply the concentrated extract (in hexane) from Sec. 7.8.7 to the top of the silica gel column. Rinse the vial with enough hexane (1-2 mL) to complete the quantitative transfer of the sample to the surface of the silica.

7.9.6 Using 90 mL of hexane, elute the extract from Column 1 directly onto Column 2 which contains the alumina. Do not allow the alumina column to run dry.

7.9.7 Add 20 mL of hexane to Column 2, and elute until the hexane level is just below the top of the alumina. Do not discard the eluted hexane, but collect in a separate flask and store it for later use, as it may be useful in determining where the labeled analytes are being lost if recoveries are less than 50%.

7.9.8 Add 20 mL of 20% methylene chloride/80% hexane (v/v) to Column 2 and collect the eluate.

7.9.9 Concentrate the extract to 2 to 3 mL using the procedures in Sec. 7.7.

CAUTION: Do not concentrate the eluate to dryness. The sample is now ready to be transferred to the carbon column.

7.10 Carbon column chromatographic procedure

7.10.1 Thoroughly mix 9.0 g activated carbon (Carbopak C, Sec. 5.8.2) and 41.0 g Celite 545® to produce a 18% w/w mixture. Activate the mixture at 130°C for 6 hours, and store in a desiccator.

NOTE: Check each new batch of the carbon/Celite mixture by adding 50 µL of the calibration verification solution to 950 µL of hexane. Process the spiked solution in the same manner as a sample extract (Secs. 7.10.3 through 7.10.5). Concentrate the calibration verification solution to 50 µL and proceed with Sec. 7.14. If the recovery of any of the analytes is less than 80%, this batch of carbon/Celite mixture may not be used.

7.10.2 Prepare a 4-inch long glass column by cutting off each end of a 10-mL disposable serological pipet. Fire polish both ends and flare if desired. Insert a glass wool plug at one end of the column, and pack it with 1 g of the Carbon/Celite mixture. Insert an additional glass wool plug in the other end.

CAUTION: It is very important that the column be packed properly to ensure that carbon fines are not carried into the eluate. PCDDs/PCDFs will adhere to the carbon fines and greatly reduce recovery. If carbon fines are carried into the eluate in

Sec. 7.10.5, filter the eluate, using a 0.7-µm filter (pre-rinsed with toluene), then proceed to Sec. 7.11.

7.10.3 Rinse the column with:

- 4 mL toluene
- 2 mL of methylene chloride/methanol/toluene (75:20:5 v/v)
- 4 mL of cyclohexane/methylene chloride (50:50 v/v)

Discard all the column rinsates.

7.10.4 While the column is still wet, transfer the concentrated eluate from Sec. 7.9.10 to the prepared carbon column. Rinse the eluate container with two 0.5-mL portions of hexane and transfer the rinses to the carbon column. Elute the column with the following sequence of solvents.

- 10 mL of cyclohexane/methylene chloride (50:50 v/v).
- 5 mL of methylene chloride/methanol/toluene (75:20:5 v/v).

NOTE: The above two eluates may be collected and combined, and used as a check on column efficiency.

7.10.5 Once the solvents have eluted through the column, turn the column over, and elute the PCDD/PCDF fraction with 20 mL of toluene, and collect the eluate.

7.11 Final concentration

7.11.1 Evaporate the toluene fraction from Sec. 7.10.5 to approximately 1.0 mL, using the procedures in Secs. 7.6 and 7.7. Transfer the extract to a 2.0-mL conical vial using a toluene rinse.

CAUTION: Do not evaporate the sample extract to dryness.

7.11.2 Add 100 µL tridecane (or nonane) to the extract and reduce the volume to 100 µL using a gentle stream of clean dry nitrogen (Sec. 7.7). The final extract volume should be 100 µL of tridecane (or nonane). Seal the vial and store the sample extract in the dark at ambient temperature until just prior to GC/MS analysis.

7.12 Chromatographic conditions (recommended)

7.12.1 Establish the GC operating conditions necessary to achieve the resolution and sensitivity required for the analyses, using the following conditions as guidance for the DB-5 (or equivalent) column:

Helium Linear Velocity:	35 - 40 cm/sec at 240°C
Initial Temperature:	170°C
Initial Time:	10 minutes
Temperature Program:	increase to 320°C at 8°C/minute
Hold Time:	until OCDF elutes
Total Time:	40-45 minutes

On the DB-5 column, the chromatographic resolution is evaluated using the CC3 calibration standard during both the initial calibration and the calibration verification. The chromatographic peak separation between the $^{13}C_{12}$-2,3,7,8-TCDD peak and the $^{13}C_{12}$-1,2,3,4-TCDD peak must be resolved with a valley of ≤ 25 percent, where:

$$\text{Valley} = \left(\frac{x}{y}\right) \times 10$$

y = the peak height of any TCDD isomer
x = measured as shown in Figure 2

The resolution criteria must be evaluated using measurements made on the selected ion current profile (SICP) for the appropriate ions for each isomer. Measurements are not made from total ion current profiles.

Optimize the operating conditions for sensitivity and resolution, and employ the same conditions for both calibration and sample analyses.

7.12.2 When an SP-2331 (or equivalent) GC column is used to confirm the results for 2,3,7,8-TCDF, the chromatographic resolution is evaluated before the analysis of any calibration standards by the analysis of a commercially-available column performance mixture (Sec. 5.16) that contains the TCDD isomers that elute most closely with 2,3,7,8-TCDD on this GC column (1,4,7,8-TCDD and the 1,2,3,7/1,2,3,8-TCDD pair). Analyze a 2-µL aliquot of this solution, using the column operating conditions and descriptor switching times previously established. The GC operating conditions for this column should be modified from those for the DB-5 (or equivalent) column, focusing on resolution of the closely-eluting TCDD and TCDF isomers.

NOTE: The column performance mixture may be combined with the window defining mix into a single analysis, provided that the combined solution contains the isomers needed to determine that criteria for both analyses can be met.

The chromatographic peak separation between unlabeled 2,3,7,8-TCDD and the peaks representing all other unlabeled TCDD isomers should be resolved with a valley of ≤ 25 percent, where:

$$\%\ \text{Valley} = \left(\frac{x}{y}\right) \times 100$$

y = the peak height of any TCDD isomer
x = measured as shown in Figure 2

The resolution criteria must be evaluated using measurements made on the selected ion current profile (SICP) for the appropriate ions for each isomer. Measurements are not made from total ion current profiles.

Further analyses may not proceed until the GC resolution criteria have been met.

7.13 GC/MS Calibration

Calibration of the GC/MS system involves three separate procedures, mass calibration of the MS, establishment of GC retention time windows, and calibration of the target analytes. These three

procedures are described in Secs. 7.13.1 to 7.13.3. Samples should not be analyzed until acceptable descriptor switching times, chromatographic resolution, and calibrations are achieved and documented. The sequence of analyses is shown in Figure 3.

NOTE: The injection volume for all sample extracts, blanks, quality control samples and calibration solutions must be the same.

7.13.1 Mass calibration - Mass calibration of the MS is recommended prior to analyzing the calibration solutions, blanks, samples and QC samples. It is recommended that the instrument be tuned to greater sensitivity in the high mass range in order to achieve better response for the later eluting compounds. Optimum results using FC-43 for mass calibration may be achieved by scanning from 222-510 amu every 1 second or less, utilizing 70 volts (nominal) electron energy in the electron ionization mode. Under these conditions, m/z 414 and m/z 502 should be 30-50% of m/z 264 (base peak).

7.13.2 Retention time windows - Prior to the calibration of the target analytes, it is necessary to establish the appropriate switching times for the SIM descriptors (Table 7). The switching times are determined by the analysis of the Window Defining Mix, containing the first and last eluting isomers in each homologue (Table 8). Mixes are available for various columns.

The ions in each of the four recommended descriptors are arranged so that there is overlap between the descriptors. The ions for the TCDD, TCDF, PeCDD, and PeCDF isomers are in the first descriptor, the ions for the PeCDD, PeCDF, HxCDD and HxCDF isomers are in the second descriptor, the ions for the HxCDD, HxCDF, HpCDD and HpCDF isomers are in the third, and the ions for the HpCDD, HpCDF, OCDD and OCDF isomers are in the fourth descriptor. The descriptor switching times are set such that the isomers that elute from the GC during a given retention time window will also be those isomers for which the ions are monitored. For the homologues that overlap between descriptors, the laboratory may use discretion in setting the switching times. However, do not set descriptor switching times such that a change in descriptors occurs at or near the expected retention time of any of the 2,3,7,8-substituted isomers.

7.13.3 Calibration of target analytes - Two types of calibration procedures, initial calibration and calibration verification, are necessary (Secs. 7.13.3.1 and 7.13.3.2). The initial calibration is needed before any samples are analyzed for PCDDs/PCDFs, and intermittently throughout sample analysis, as dictated by the results of the calibration verification. The calibration verification is necessary at the beginning of each 12-hour time period during which sample are analyzed.

7.13.3.1 Initial Calibration - Once the Window Defining Mix has been analyzed and the descriptor switching times have been verified (and after the analysis of the column performance solution, if using a GC column other than DB-5), analyze the five concentration calibration solutions (CC1-CC5), described in Table 1, prior to any sample analysis.

7.13.3.1.1 The relative ion abundance criteria for PCDDs/PCDFs presented in Table 9 should be met for all PCDD/PCDF peaks, including the labeled internal and recovery standards, in all solutions. The lower and upper limits of the ion abundance ratios represent a ±15% window around the theoretical abundance ratio for each pair of selected ions.

The $^{37}Cl_4$-2,3,7,8-TCDD cleanup standard contains no ^{35}Cl, thus the ion abundance ratio criterion does not apply to this compound.

7.13.3.1.2 If the laboratory uses a GC column other than those described here, the laboratory must ensure that the isomers eluting closest to 2,3,7,8-TCDD on that column are used to evaluate GC column resolution

7.13.3.2 Calculate the relative response factors (RFs) for the seventeen unlabeled target analytes relative to their appropriate internal standards (RF_n) (Table 10), according to the formulae below. For the seven unlabeled analytes and the $^{37}Cl_4$-2,3,7,8-TCDD cleanup standard that are found only in the CC3 solution, only one RF is calculated for each analyte. For the other 10 unlabeled analytes, calculate the RF of each analyte in each calibration standard.

Calculate the RFs for the five labeled internal standards and the cleanup standard relative to the appropriate recovery standard (RF_{is}) (Table 10), in each calibration standard, according to the following formulae:

$$RF_n = \frac{(A_n^1 + A_n^2) \times Q_{is}}{(A_{is}^1 + A_{is}^2) \times Q_n}$$

$$RF_{is} = \frac{(A_{is}^1 + A_{is}^2) \times Q_{rs}}{(A_{rs}^1 + A_{rs}^2) \times Q_{is}}$$

where:

A_n^1 and A_n^2 = integrated areas of the two quantitation ions of the isomer of interest (Table 8)

A_{is}^1 and A_{is}^2 = integrated areas of the two quantitation ions of the appropriate internal standard (Table 8)

A_{rs}^1 and A_{rs}^2 = integrated areas of the two quantitation ions of the appropriate recovery standard (Table 8)

Q_n = nanograms of unlabeled target analyte injected

Q_{is} = nanograms of appropriate internal standard injected

Q_{rs} = nanograms of appropriate recovery standard injected.

There is only one quantitation ion for the ^{37}Cl cleanup standard. Calculate the relative response factor as described for RF_{is}, using one area for the cleanup standard, and the sum of the areas of the ions from the recovery standard.

The RF_n and xRF_{is} are dimensionless quantities; therefore, the units used to express the Q_n, Q_{is}, and Q_{rs} must be the same.

7.13.3.3 Calculate the relative response factors for the unlabeled PCDDs/PCDFs relative to the recovery standards (RF_{rs}), where:

$$F_{rs} = RF_n \times RF$$

This relative response factor is necessary when the sample is diluted to the extent that the S/N ratio for the internal standard is less than 10.0.

7.13.3.4 Relative Response Factor Criteria - Calculate the mean RF and percent relative standard deviation (%RSD) of the five RFs (CC1 to CC5) for each unlabeled PCDD/PCDF and labeled internal standards present in all five concentration calibration solutions. No mean RF or %RSD calculations are possible for the 2,3,7,8-substituted isomers or the cleanup standard found only in the CC3 solution.

$$\%RSD = \frac{\text{Standard deviation}}{\overline{RF}} \times 100$$

The %RSD of the five RFs (CC1-CC5) for the unlabeled PCDDs/PCDFs and the internal standards should not exceed 15.0%.

7.13.3.5 The response factors to be used for determining the total homologue concentrations are described in Sec. 7.15.2.

7.13.3.6 Calibration Verification - The calibration verification consists of two parts: evaluation of the chromatographic resolution, and verification of the RF values to be used for quantitation. At the beginning of each 12-hour period, the chromatographic resolution is verified in the same fashion as in the initial calibration, through the analysis of the CC3 solution on the DB-5 (or equivalent) column, or through the analysis of the column performance solution on the SP-2331 (or equivalent) column.

Prepare the CC3 solution by combining the volumes of the solutions listed in Table 4 to yield a final volume of 1.0 mL at the concentrations listed for the CC3 solution in Table 1. Alternatively, use a commercially-prepared solution that contains the target analytes at the CC3 concentrations listed in Table 1.

For the DB-5 (or equivalent) column, begin the 12-hour period by analyzing the CC3 solution. Inject a 2-µL aliquot of the calibration verification solution (CC3) into the GC/MS. The identical GC/MS/DS conditions used for the analysis of the initial calibration solutions must be used for the calibration verification solution. Evaluate the chromatographic resolution using the QC criteria in Sec. 7.12.1.

For the SP-2331 (or equivalent) column, or other columns with different elution orders, begin the 12-hour period with the analysis of a 2-µL aliquot of the appropriate column performance solution. Evaluate the chromatographic resolution using the QC criteria in Sec. 7.12.2. If this solution meets the QC criteria, proceed with the analysis of a 2-µL aliquot of the CC3 solution. The identical GC/MS/DS conditions

used for the analysis of the initial calibration solutions must be used for the calibration verification solution.

Calculate the RFs for the seventeen unlabeled target analytes relative to their appropriate internal standards (RF_n) and the response factors for the five labeled internal standards and the cleanup standard relative to the appropriate recovery standard (RF_{is}), according to the formulae in Sec. 7.13.3.2.

Calculate the RFs for the unlabeled PCDDs/PCDFs relative to the recovery standards (RF_{rs}), using the formula in Sec. 7.13.3.3.

Do not proceed with sample analyses until the calibration verification criteria have been met for:

1) GC Column Resolution Criteria - The chromatographic resolution on the DB-5 (or equivalent) and /or the SP-2331 (or equivalent) column must meet the QC criteria in Sec. 7.12. In addition, the chromatographic peak separation between the 1,2,3,4,7,8-HxCDD and the 1,2,3,6,7,8-HxCDD in the CC3 solution shall be resolved with a valley of ≤ 50 percent (Figure 2).

2) Ion Abundance Criteria - The relative ion abundances listed in Table 9 must be met for all PCDD/PCDF peaks, including the labeled internal and recovery standards.

3) Instrument Sensitivity Criteria - For the CC3 solution, the signal-to-noise (S/N) ratio shall be greater than 2.5 for the unlabeled PCDD/PCDF ions, and greater than 10.0 for the labeled internal and recovery standards.

4) Response Factor Criteria - The measured RFs of each analyte and internal standard in the CC3 solution must be within ±30.0% of the mean RFs established during initial calibration for the analytes in all five calibration standards, and within ± 30.0% of the single-point RFs established during initial calibration for those analytes present in only the CC3 standard (see Sec. 7.13.3.2).

$$\% \text{ Difference} = \frac{(\overline{RF} - RF_c)}{\overline{RF}} \times 100$$

where:

$\overline{RF}$ = Mean Relative response factor established during initial calibration.

RF_c = Relative response factor established during calibration verification.

7.13.3.7 In order to demonstrate that the GC/MS system has retained adequate sensitivity during the course of sample analyses, the lowest standard from the initial calibration is analyzed at the end of each 12-hour time period during which samples are analyzed. This analysis must utilize the same injection volume and instrument operating conditions as were used for the preceding sample analyses.

The results of this analysis must meet the acceptance criteria for retention times, ion abundances, and S/N ratio that are listed in Sec 7.13.3.6 for the continuing calibration standard. Response factors do not need to be evaluated in this end-of-shift

standard. If this analysis fails either the ion abundance or S/N ratio criteria, then any samples analyzed during that 12-hour period that indicated the presence of any PCDDs/PCDFs below the method quantitation limit or where estimated maximum possible concentrations were reported must be reanalyzed. Samples with positive results above the method quantitation limit need not be reanalyzed.

7.14 GC/MS analysis of samples

7.14.1 Remove the extract of the sample or blank from storage. Gently swirl the solvent on the lower portion of the vial to ensure complete dissolution of the PCDDs/PCDFs.

7.14.2 Transfer a 50-µL aliquot of the extract to a 0.3-mL vial, and add sufficient recovery standard solution to yield a concentration of 0.5 ng/µL. Reduce the volume of the extract back down to 50 µL using a gentle stream of dry nitrogen.

7.14.3 Inject a 2-µL aliquot of the extract into the GC/MS instrument. Reseal the vial containing the original concentrated extract. Analyze the extract by GC/MS, and monitor all of the ions listed in Table 7. The same MS parameters used to analyze the calibration solutions must be used for the sample extracts.

7.14.4 Dilution of the sample extract is necessary if the concentration of any PCDD/PCDF in the sample has exceeded the calibration range, or the detector has been saturated. An appropriate dilution will result in the largest peak in the diluted sample falling between the mid-point and high-point of the calibration range.

7.14.4.1 Dilutions are performed using an aliquot of the original extract, of which approximately 50 µL remain from Sec. 7.14.2. Remove an appropriate size aliquot from the vial and add it to a sufficient volume of tridecane (or nonane) in a clean 0.3-mL conical vial. Add sufficient recovery standard solution to yield a concentration of 0.5 ng/µL. Reduce the volume of the extract back down to 50 µL using a gentle stream of dry nitrogen.

7.14.4.2 The dilution factor is defined as the total volume of the sample aliquot and clean solvent divided by the volume of the sample aliquot that was diluted.

7.14.4.3 Inject 2 µL of the diluted sample extract into the GC/MS, and analyze according to Secs. 7.14.1 through 7.14.3.

7.14.4.4 Diluted samples in which the MS response of any internal standard is greater than or equal to 10% of the MS response of that internal standard in the most recent calibration verification standard are quantitated using the internal standards.

Diluted samples in which the MS response of any internal standard is less than 10% of the MS response of that internal standard in the most recent calibration verification standard are quantitated using the recovery standards (see Sec. 7.15.3).

7.14.5 Identification Criteria - For a gas chromatographic peak to be unambiguously identified as a PCDD or PCDF, it must meet all of the following criteria.

7.14.5.1 Retention times - In order to make a positive identification of the 2,3,7,8-substituted isomers for which an isotopically labeled internal or recovery

standard is present in the sample extract, the absolute retention time (RT) at the maximum peak height of the analyte must be within -1 to +3 seconds of the retention time of the corresponding labeled standard.

In order to make a positive identification of the 2,3,7,8-substituted isomers for which a labeled standard is *not* available, the relative retention time (RRT) of the analyte must be within 0.05 RRT units of the RRT established by the calibration verification. The RRT is calculated as follows:

$$RT = \frac{\text{retention time of the analyte}}{\text{retention time of the corresponding internal standard}}$$

For non-2,3,7,8-substituted compounds (tetra through hepta), the retention time must be within the retention time windows established by the window defining mix for the corresponding homologue (Sec. 7.13.2).

In order to assure that retention time shifts do not adversely affect the identification of PCDDs/PCDFs, the absolute retention times of the two recovery standards added to every sample extract immediately prior to analysis may not shift by more than ± 10 seconds from their retention times in the calibration verification standard.

7.14.5.2 Peak identification - All of the ions listed in Table 8 for each PCDD/PCDF homologue and labeled standards must be present in the SICP. The ion current response for the two quantitation ions and the M-[COCL]$^+$ ions for the analytes must maximize simultaneously (± 2 seconds). This requirement also applies to the internal standards and recovery standards. For the cleanup standard, only one ion is monitored.

7.14.5.3 Signal-to-noise ratio - The integrated ion current for each analyte ion listed in Table 8 must be at least 2.5 times background noise and must not have saturated the detector (Figure 4). The internal standard ions must be at least 10.0 times background noise and must not have saturated the detector. However, if the M-[COCL]$^+$ ion does not meet the 2.5 times S/N requirement but meets all the other criteria listed in Sec. 7.14.5 and, in the judgement of the GC/MS Interpretation Specialist the peak is a PCDD/PCDF, the peak may be reported as positive and the data flagged on the report form.

7.14.5.4 Ion abundance ratios - The relative ion abundance criteria listed in Table 9 for unlabeled analytes and internal standards must be met using peak areas to calculate ratios.

7.14.5.4.1 If interferences are present, and ion abundance ratios are not met using peak areas, but all other qualitative identification criteria are met (RT, S/N, presence of all 3 ions), then use peak heights to evaluate the ion ratio.

7.14.5.4.2 If, in the judgement of the analyst, the peak is a PCDD/PCDF, then report the ion abundance ratios determined using peak heights, quantitate the peaks using peak heights rather than areas for both

the target analyte and the internal standard, and flag the result on the report form.

7.14.5.5 Polychlorinated diphenyl ether (PCDPE) interferences.

The identification of a GC peak as a PCDF cannot be made if a signal having S/N greater than 2.5 is detected at the same retention time (± 2 seconds) in the corresponding PCDPE channel (Table 8). If a PCDPE is detected, an Estimated Maximum Possible Concentration (EMPC) should be calculated for this GC peak according to Sec. 7.15.7, regardless of the ion abundance ratio, and reported.

7.14.6 When peaks are present that do not meet all of the identification criteria in Sec. 7.14.5 and the reporting of an estimated maximum possible concentration according to Sec. 7.15.7 will not meet the specific project objectives, then the analyst may need to take additional steps to resolve the potential interference problems. However, this decision generally is project-specific and should not be applied without knowledge of the intended application of the results. These steps may be most appropriate when historical data indicate that 2,3,7,8-substituted PCDDs/PCDFs have been detected in samples from the site or facility, yet the results from a specific analysis are inconclusive. The additional steps may include the use of additional or repeated sample cleanup procedures or the use of HRGC/MS/MS (e.g., tandem mass spectrometry).

7.15 Calculations

7.15.1 For GC peaks that have met all the identification criteria outlined in Sec. 7.14.5, calculate the concentration of the individual PCDD or PCDF isomers using the formulae:

ALL MATRICES OTHER THAN WATER:

$$C_n\ (\mu g/kg) = \frac{Q_{is} \times (A_n^1 + A_n^2)}{W \times (A_{is}^1 + A_{is}^2) \times RF_n}$$

WATER:

$$C_n\ (ng/L) = \frac{Q_{is} \times (A_n^1 + A_n^2)}{V \times (A_{is}^1 + A_{is}^2) \times RF_n}$$

where:

A_n^1 and A_n^2 = integrated ion abundances (peak areas) of the quantitation ions of the isomer of interest (Table 8).

A_{is}^1 and A_{is}^2 = integrated ion abundances (peak areas) of the quantitation ions of the appropriate internal standard (Table 8).

C_n = concentration of unlabeled PCDD/PCDF found in the sample.

W = weight of sample extracted, in grams.

V = volume of sample extracted, in liters.

Q_{is} = nanograms of the appropriate internal standard added to the sample prior to extraction.

RF_n = calculated relative response factor from calibration verification (see Sec. 7.13.3.6).

NOTE: In instances where peak heights are used to evaluate ion abundance ratios due to interferences (Sec. 7.14.5.4), substitute peak heights for areas in the formulae above.

For solid matrices, the units of ng/g that result from the formula above are equivalent to µg/kg. Using isotope dilution techniques for quantitation, the concentration data are recovery corrected, and therefore, the volume of the final extract and the injection volume are implicit in the value of Q_{is}.

7.15.1.1 For homologues that contain only one 2,3,7,8-substituted isomer (TCDD, PeCDD, HpCDD, and TCDF), the RF of the 2,3,7,8-substituted isomer from the calibration verification will be used to quantitate both the 2,3,7,8-substituted isomers and the non-2,3,7,8-isomers.

7.15.1.2 For homologues that contain *more than* one 2,3,7,8-substituted isomer (HxCDD, PeCDF, HxCDF, and HpCDF), the RF used to calculate the concentration of each 2,3,7,8-substituted isomers will be the RF determined for that isomer during the calibration verification.

7.15.1.3 For homologues that contain one or more non-2,3,7,8-substituted isomer, the RF used to calculate the concentration of these isomers will be the lowest of the RFs determined during the calibration verification for the 2,3,7,8-substituted isomers in that homologue. This RF will yield the highest possible concentration for the non-2,3,7,8-substituted isomers.

NOTE: The relative response factors of given isomers within any homologue may be different. However, for the purposes of these calculations, it will be assumed that every non-2,3,7,8-substituted isomer for a given homologue has the same relative response factor. In order to minimize the effect of this assumption on risk assessment, the 2,3,7,8-substituted isomer with the lowest RF was chosen as representative of each homologue. All relative response factor calculations for the non-2,3,7,8–substituted isomers in a given homologue are based on that isomer.

7.15.2 In addition to the concentrations of specific isomers, the total homologue concentrations are also reported. Calculate the total concentration of each homologue of PCDDs/PCDFs as follows:

Total concentration = sum of the concentrations of every positively identified isomer of each PCDD/PCDF homologue.

The total concentration must include the non-2,3,7,8-substituted isomers as well as the 2,3,7,8-substituted isomers that are also reported separately. The total number of GC peaks included in the total homologue concentration should be reported.

7.15.3 If the area of any internal standard in a diluted sample is less than 10% of the area of that internal standard in the calibration verification standard, then the unlabeled PCDD/PCDF concentrations in the sample shall be estimated using the recovery standard, using the formulae that follow. The purpose is to ensure that there is an adequate MS response for quantitation in a diluted sample. While use of a smaller aliquot of the sample might require smaller dilutions and therefore yield a larger area for the internal standard in the diluted extract, this practice leads to other concerns about the homogeneity of the sample and the representativeness of the aliquot taken for extraction.

ALL MATRICES OTHER THAN WATER:

$$C_n\ (\mu g/kg) = \frac{Q_{rs} \times (A_n^1 + A_n^2) \times D}{W \times (A_{rs}^1 + A_{rs}^2) \times RF_{rs}}$$

WATER:

$$C_n\ (ng/L) = \frac{Q_{rs} \times (A_n^1 + A_n^2) \times D}{V \times (A_{rs}^1 + A_{rs}^2) \times RF_{rs}}$$

where:

D = the dilution factor (Sec. 7.14.4.2).

A_n^1, A_n^2, A_{rs}^1, A_{rs}^2, Q_{rs}, RF_{rs}, W, and V are defined in Secs. 7.13.3.2 and 7.15.1.

7.15.4 Report results for soil/sediment, fly ash, and chemical waste samples in micrograms per kilogram (µg/kg) and water samples in nanograms per liter (ng/L).

7.15.5 Calculate the percent recovery, R_{is}, for each internal standard and the cleanup standard in the sample extract, using the formula:

$$R_{is}(\%) = \frac{(A_{is}^1 + A_{is}^2) \times Q_{rs}}{(A_{rs}^1 + A_{rs}^2) \times RF_{is} \times Q_{is}} \times 100$$

where:

A_{is}^1, A_{is}^2, A_{rs}^1, A_{rs}^2, Q_{is}, Q_{rs}, and RF_{is} are defined in Secs. 7.13.3.2 and 7.15.1.

NOTE: When calculating the recovery of the $^{37}Cl_4$-2,3,7,8-TCDD cleanup standard, only one m/z is monitored for this standard; therefore, only one peak area will be used in the numerator of this formula. Use both peak areas of the $^{13}C_{12}$-1,2,3,4-TCDD recovery standard in the denominator.

7.15.5.1 The $^{13}C_{12}$-1,2,3,4-TCDD is used to quantitate the TCDD and TCDF internal standards and the cleanup standard, and the $^{13}C_{12}$-1,2,3,7,8,9-HxCDD is used to quantitate the HxCDD, HpCDF and OCDD internal standards (Table 10).

7.15.5.2 If the original sample, prior to any dilutions, has any internal standard with a percent recovery of less than 25% or greater than 150%, re-extraction and reanalysis of that sample is necessary.

7.15.6 Sample specific estimated detection limit - The sample specific estimated detection limit (EDL) is the estimate made by the laboratory of the concentration of a given analyte required to produce a signal with a peak height of at least 2.5 times the background signal level. The estimate is specific to a particular analysis of the sample, and will be affected by sample size, dilution, etc.

7.15.6.1 An EDL is calculated for each 2,3,7,8-substituted isomer that is not identified, regardless of whether or not non-2,3,7,8-substituted isomers in that homologue are present. The EDL is also calculated for 2,3,7,8-substituted isomers giving responses for both the quantitation ions that are less than 2.5 times the background level.

7.15.6.2 Use the formulae below to calculate an EDL for each absent 2,3,7,8-substituted PCDD/PCDF. The background level (H_n) is determined by measuring the height of the noise at the expected retention times of both the quantitation ions of the particular 2,3,7,8-substituted isomer.

ALL MATRICES OTHER THAN WATER:

$$\text{EDL } (\mu g/kg) = \frac{2.5 \times Q_{is} \times (H_n^1 + H_n^2) \times D}{W \times (H_{is}^1 + H_{is}^2) \times RF_n}$$

WATER:

$$\text{EDL } (ng/L) = \frac{2.5 \times Q_{is} \times (H_n^1 + H_n^2) \times D}{V \times (H_{is}^1 + H_{is}^2) \times RF_n}$$

where:

H_n^1 and H_n^2 = The peak heights of the noise for both of the quantitation ions of the 2,3,7,8-substituted isomer of interest

H_{is}^1 and H_{is}^2 = The peak heights of both the quantitation ions of the appropriate internal standards

D = dilution factor (Sec. 7.14.4.2).

Q_{is}, RF_{is}, W and V are defined in Secs. 7.13.3.2 and 7.15.1.

7.15.6.3 If none of the isomers within a homologue are detected, then the EDL for the "total" homologue concentration is the lowest EDL for any of the 2,3,7,8-

substituted isomers that were not detected. Do not add together the EDLs for the various isomers. If a 2,3,7,8-substituted isomer is reported in the homologue, then no EDL for the "total" is calculated.

7.15.7 Estimated maximum possible concentration - An estimated maximum possible concentration (EMPC) is calculated for 2,3,7,8-substituted isomers that are characterized by a response with an S/N of at least 2.5 for both the quantitation ions, and meet all of the identification criteria in Sec. 7.14.5 except the ion abundance ratio criteria in Sec. 7.14.5.4 or when a peak representing a PCDPE has been detected (7.14.5.5). An EMPC is a worst-case estimate of the concentration. Calculate the EMPC according to the following formulae:

ALL MATRICES OTHER THAN WATER:

$$EMPC_n\ (\mu g/kg) = \frac{Q_{is} \times (A_n^1 + A_n^2) \times D}{W \times (A_{is}^1 + A_{is}^2) \times RF_n}$$

WATER:

$$EMPC_n\ (ng/L) = \frac{Q_{is} \times (A_n^1 + A_n^2) \times D}{V \times (A_{is}^1 + A_{is}^2) \times RF_n}$$

where:

A_x^1 and A_x^2 = Areas of both the quantitation ions.

A_{is}^1, A_{is}^2, Q_{is}, RF, D, W, and V are defined in Secs. 7.13.3.2 and 7.15.1.

7.15.8 Toxic equivalent concentration (TEQ) calculation - The 2,3,7,8-TCDD toxic equivalent concentration of PCDDs/PCDFs present in the sample is calculated according to the method recommended by the Chlorinated Dioxins Workgroup (CDWG) of the EPA and the Centers for Disease Control (CDC). This method assigns a 2,3,7,8-TCDD toxicity equivalency factor (TEF) to each of the seventeen 2,3,7,8-substituted PCDDs/PCDFs shown in Table 11 *("Update of Toxicity Equivalency Factors [TEFs] for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-Dioxins and -Dibenzofurans [CDDs/CDFs]" March 1989 [EPA 625/3-89/016]).*

7.15.8.1 The 2,3,7,8-TCDD TEQ of the PCDDs/PCDFs present in the sample is calculated by summing the product of the concentration for each of the compounds listed in Table 11 and the TEF for each compound. The principal purpose of making this calculation is to provide the data user with a single value, normalized to the toxicity of 2,3,7,8-TCDD, that can more readily be used in decisions related to mixtures of these highly toxic compounds.

7.15.8.1.1 The exclusion of homologues such as mono-, di-, tri- and the non-2,3,7,8-substituted isomers in the higher homologues does not mean that they are not toxic. Their toxicity, as estimated at this time, is much less than the toxicity of the compounds listed in Table 11. Hence, only the 2,3,7,8-substituted isomers are included in the TEF calculations. The

procedure for calculating the 2,3,7,8-TCDD toxic equivalence cited above is not claimed by the CDWG to be based on a thoroughly established scientific foundation. Rather, the procedure represents a "consensus recommendation on science policy."

7.15.8.1.2 When calculating the TEQ of a sample, include only those 2,3,7,8-substituted isomers that were detected in the sample and met all of the qualitative identification criteria in Sec. 7.14.5. Do not include EMPC or EDL values in the TEQ calculation.

7.15.8.2 The TEQ of a sample is also used in this analytical procedure to determine when second column confirmation may be necessary. The need for second column confirmation is based on the known difficulties in separating 2,3,7,8-TCDF from other isomers. Historical problems have been associated with the separation of 2,3,7,8-TCDF from 1,2,4,9-, 1,2,7,9-, 2,3,4,6-, 2,3,4,7- and 2,3,4,8-TCDF. Because of the toxicological concern associated with 2,3,7,8-TCDF, additional analyses may be required for some samples as described below. If project-specific requirements do not include second column confirmation or specify a different approach to confirmation, then this step may be omitted and the project-specific requirements take precedence.

7.15.8.2.1 If the TEQ calculated in Sec. 7.15.8.1 is greater than 0.7 ppb for soil/sediment or fly ash, 7 ppb for chemical waste, or 7 ppt for an aqueous sample, and 2,3,7,8-TCDF is either detected or reported as an EMPC, then better isomer specificity may be required than can be achieved on the DB-5 column. The TEQ values listed here for the various matrices are equivalent to 70% of the historical "Action Level" set by the CDC for soil concentrations of 2,3,7,8-TCDD at Superfund sites. As such, it provides a conservative mechanism for determining when the additional specificity provided by a second column confirmation may be required.

7.15.8.2.2 The sample extract may be reanalyzed on a 60 m SP-2330 or SP-2331 GC column (or equivalent) in order to achieve better GC resolution, and therefore, better identification and quantitation of 2,3,7,8-TCDF. Other columns that provide better specificity for 2,3,7,8-TCDF than the DB-5 column may also be used.

7.15.8.2.3 Regardless of the GC column used, for a gas chromatographic peak to be identified as a 2,3,7,8-substituted PCDD/PCDF isomer during the second column confirmation, it must meet the ion abundance, signal-to-noise, and retention time criteria listed in Sec. 7.14.5.

7.15.8.2.4 The second column confirmation analysis may be optimized for the analysis of 2,3,7,8-TCDF, and need not be used to confirm the results for any other 2,3,7,8-substituted PCDDs/PCDFs identified during the original analysis.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures necessary to evaluate the GC/MS system operation include evaluation of chromatographic resolution, retention time windows, calibration verification and chromatographic analysis of samples. Performance criteria are given in the following sections of this method:

8.2.1 GC resolution criteria for the DB-5 or equivalent column are given in Sec. 7.12.1.

8.2.2 GC resolution criteria for SP-2331 or equivalent column are given in Sec. 7.12.2.

8.2.3 Initial calibration criteria are given in Sec. 7.13.3.1.

8.2.4 Response factor criteria for the initial calibration are given in Sec. 7.13.3.4.

8.2.5 Calibration verification criteria are given in Sec. 7.13.3.6.

8.2.6 Ion abundance criteria are given in Secs. 7.13.3.1, 7.13.3.6, and 7.14.5.4.

8.2.7 Instrument sensitivity criteria are given in Sec. 7.13.3.6.

8.2.8 Response factor criteria for the calibration verification are given in Sec. 7.13.3.6.

8.2.9 Identification criteria are given in Sec. 7.14.5.

8.2.10 Criteria for isotopic ratio measurements for PCDDs/PCDFs are given in Secs. 7.13.3.1, 7.13.3.6, and Table 9.

8.3 Initial Demonstration of Proficiency - Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Method 8000, Sec. 8.0 for information on how to accomplish this demonstration.

8.4 Sample Quality Control for Preparation and Analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, a matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch.

8.4.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair. Consult Sec. 8 of Method 8000 for information on developing acceptance criteria for the MS/MSD.

8.4.2 A laboratory control sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix. Consult Sec. 8 of Method 8000 for information on developing acceptance criteria for the LCS.

8.4.3 The analysis of method blanks is critical to the provision of meaningful sample results.

8.4.3.1 Method blanks should be prepared at a frequency of at least 5%, that is, one method blank for each group of up to 20 samples prepared at the same time, by the same procedures.

8.4.3.2 When sample extracts are subjected to cleanup procedures, the associated method blank must also be subjected to the same cleanup procedures.

8.4.3.4 As described in Chapter One, the results of the method blank should be:

8.4.3.4.1 Less than the MDL for the analyte.

8.4.3.4.2 Less than 5% of the regulatory limit associated with an analyte.

8.4.3.4.3 Or less than 5% of the sample result for the same analyte, whichever is greater.

8.4.3.4.4 If the method blank results do not meet the acceptance criteria above, then the laboratory should take corrective action to locate and reduce the source of the contamination and to re-extract and reanalyze any samples associated with the contaminated method blank.

8.4.4 The laboratory should <u>not</u> subtract the results of the method blank from those of any associated samples. Such "blank subtraction" is inappropriate and often leads to negative sample results. If the method blank results do not meet the acceptance criteria in 8.4.3 and reanalysis is not practical, then the data user should be provided with the sample results, the method blank results, and a discussion of the corrective actions undertaken by the laboratory.

8.5 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

No performance data are available at this time.

10.0 REFERENCES

1. "Update of Toxicity Equivalency Factors (TEFs) for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-*p*-Dioxins and Dibenzofurans (CDDs/CDFs)," March 1989 (EPA 6251/3-89/016).

2. "Method 8290: Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS)," Test Methods for Evaluating Solid Waste, Physical/Chemical Methods (EPA OSW SW-846).

3. "Statement of Work for Analysis of Polychlorinated Dibenzo-*p*-dioxins (PCDD) and Polychlorinated Dibenzofurans, Multi-Media, Multi-Concentration, DFLM01.1," September 1991.

4. "Method 613: 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin," 40 CFR Part 136, Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act, October 26, 1984.

5. "Extraction of Polychlorinated Dibenzo-*p*-Dioxins and Polychlorinated Dibenzofurans from Environmental Samples Using Accelerated Solvent Extraction (ASE)," B. E. Richter, J. L. Ezzell, D. E. Knowles, and F. Hoefler, *Chemosphere*, 34 (5-7), 975-987, 1997.

11.0 RECOMMENDED SAFETY AND HANDLING PROCEDURES FOR PCDDs/PCDFs

11.1 The following safety practices are excerpts from EPA Method 613, Sec. 4 (July 1982 version) and amended for use in conjunction with this method. The 2,3,7,8-TCDD isomer has been found to be acnegenic, carcinogenic, and teratogenic in laboratory animal studies. Other PCDDs and PCDFs containing chlorine atoms in positions 2,3,7,8 are known to have toxicities comparable to that of 2,3,7,8-TCDD. The analyst should note that finely divided dry soils contaminated with PCDDs and PCDFs are particularly hazardous because of the potential for inhalation and ingestion. It is recommended that such samples be processed in a confined environment, such as a hood or a glove box. Laboratory personnel handling these types of samples should wear masks fitted with charcoal filters to prevent inhalation of dust.

11.2 The toxicity or carcinogenicity of each reagent used in this method is not precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be kept to a minimum. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals listed in this method. A reference file of material safety data sheets should be made available to all personnel involved in the chemical analysis of samples suspected to contain PCDDs and/or PCDFs.

11.3 Each laboratory must develop a strict safety program for the handling of PCDDs and PCDFs. The laboratory practices listed below are recommended.

11.3.1 Contamination of the laboratory will be minimized by conducting most of the manipulations in a hood.

11.3.2 The effluents of sample splitters for the gas chromatograph and roughing pumps on the HRGC/HRMS system should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high boiling alcohols.

11.3.3 Liquid waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light at a wavelength less than 290 nm for several days (use F 40 BL lamps, or equivalent). Using this analytical method, analyze the irradiated liquid wastes and dispose of the solutions when 2,3,7,8-TCDD and -TCDF congeners can no longer be detected.

11.4 The following precautions were issued by Dow Chemical U.S.A. for safe handling of 2,3,7,8-TCDD in the laboratory and amended for use in conjunction with this method. The following statements on safe handling are as complete as possible on the basis of available toxicological information. The precautions for safe handling and use are necessarily general in nature since detailed, specific recommendations can be made only for the particular exposure and circumstances of each individual use. Assistance in evaluating the health hazards of particular plant conditions may be obtained from certain consulting laboratories and from State Departments of Health or of Labor, many of which have an industrial health service. The 2,3,7,8-TCDD isomer is extremely toxic to certain kinds of laboratory animals. However, it has been handled for years without injury in analytical and biological laboratories. Many techniques used in handling radioactive and infectious materials are applicable to 2,3,7,8-TCDD.

11.4.1 Protective Equipment - Disposable plastic gloves, apron or lab coat, safety glasses and laboratory hood adequate for radioactive work. However, PVC gloves should not be used.

11.4.2 Training - Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

11.4.3 Personal Hygiene - Thorough washing of hands and forearms after each manipulation and before breaks (coffee, lunch, and shift).

11.4.4 Confinement - Isolated work area, posted with signs, segregated glassware and tools, plastic backed absorbent paper on bench tops.

11.4.5 Waste - Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans.

11.4.6 Disposal of Hazardous Wastes - Refer to the November 7, 1986 issue of the Federal Register on Land Ban Rulings for details concerning the handling of dioxin containing wastes.

11.4.7 Decontamination of Personnel - apply a mild soap with plenty of scrubbing action. Glassware, tools and surfaces - Chlorothene NU Solvent (Trademark of the Dow Chemical Company) is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with Chlorothene, then washing with a detergent and water. Dish water may be disposed to the sewer after percolation through a charcoal bed filter. It is prudent to minimize solvent wastes because they require special disposal through commercial services that are expensive.

11.4.8 Laundry - Clothing known to be contaminated should be disposed with the precautions described under "Disposal of Hazardous Wastes". Laboratory coats or other clothing worn in 2,3,7,8-TCDD work area may be laundered. Clothing should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the launderer knows the problem. The washer should be run through one full cycle before being used again for other clothing.

11.4.9 Wipe Tests - A useful method for determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper, extract the filter paper and analyze the extract.

11.4.10 Inhalation - Any procedure that may generate airborne contamination must be carried out with good ventilation. Gross losses to a ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no significant inhalation hazards except in case of an accident.

11.4.11 Accidents - Remove contaminated clothing immediately, taking precautions not to contaminate skin or other articles. Wash exposed skin vigorously and repeatedly until medical attention is obtained.

11.5 It is recommended that personnel working in laboratories where PCDD/PCDF are handled be given periodic physical examinations (at least annually). Such examinations should include specialized tests, such as those for urinary porphyrins and for certain blood parameters which, based upon published clinical observations, are appropriate for persons who may be exposed to PCDDs/PCDFs. Periodic facial photographs to document the onset of dermatologic problems are also advisable.

TABLE 1

CALIBRATION SOLUTIONS

Analyte	Concentration of Standard in ng/µL				
	CC1	CC2	CC3	CC4	CC5
2,3,7,8-TCDD	0.1	0.25	0.5	1.0	2.0
2,3,7,8-TCDF	0.1	0.25	0.5	1.0	2.0
1,2,3,7,8-PeCDF	0.1	0.25	0.5	1.0	2.0
1,2,3,7,8-PeCDD	0.1	0.25	0.5	1.0	2.0
* 2,3,4,7,8-PeCDF			0.5		
* 1,2,3,4,7,8-HxCDF			1.25		
1,2,3,6,7,8-HxCDF	0.25	0.625	1.25	2.5	5.0
* 1,2,3,4,7,8-HxCDD			1.25		
1,2,3,6,7,8-HxCDD	0.25	0.625	1.25	2.5	5.0
* 1,2,3,7,8,9-HxCDD			1.25		
* 2,3,4,6,7,8-HxCDF			1.25		
* 1,2,3,7,8,9-HxCDF			1.25		
* 1,2,3,4,7,8,9-HpCDF			1.25		
1,2,3,4,6,7,8-HpCDF	0.25	0.625	1.25	2.5	5.0
1,2,3,4,6,7,8-HpCDD	0.25	0.625	1.25	2.5	5.0
OCDD	0.5	1.25	2.5	5.0	10.0
OCDF	0.5	1.25	2.5	5.0	10.0
$^{13}C_{12}$-2,3,7,8-TCDD	0.5	0.5	0.5	0.5	0.5
$^{13}C_{12}$-2,3,7,8-TCDF	0.5	0.5	0.5	0.5	0.5
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	0.5	0.5	0.5	0.5	0.5
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	1.0	1.0	1.0	1.0	1.0
$^{13}C_{12}$-OCDD	1.0	1.0	1.0	1.0	1.0
$^{13}C_{12}$-1234-TCDD	0.5	0.5	0.5	0.5	0.5
$^{13}C_{12}$-123789-HxCDD	0.5	0.5	0.5	0.5	0.5
$^{37}Cl_4$-2378-TCDD			0.25		

* These compounds are only *required* in the CC3 solution. Therefore, do not perform % RSD calculations on these analytes unless they are present in all five solutions.

TABLE 2

QUANTITATION LIMITS FOR TARGET COMPOUNDS

Analyte	CAS Number	Water (ng/L)	Fly Soil (µg/kg)	Chemical Ash (µg/kg)	Waste* (µg/kg)
2,3,7,8-TCDD	1746-01-6	10	1.0	1.0	10
2,3,7,8-TCDF	51207-31-9	10	1.0	1.0	10
1,2,3,7,8-PeCDF	57117-41-6	25	2.5	2.5	25
1,2,3,7,8-PeCDD	40321-76-4	25	2.5	2.5	25
2,3,4,7,8-PeCDF	57117-31-4	25	2.5	2.5	25
1,2,3,4,7,8-HxCDF	70648-26-9	25	2.5	2.5	25
1,2,3,6,7,8-HxCDF	57117-44-9	25	2.5	2.5	25
1,2,3,4,7,8-HxCDD	39227-28-6	25	2.5	2.5	25
1,2,3,6,7,8-HxCDD	57653-85-7	25	2.5	2.5	25
1,2,3,7,8,9-HxCDD	19408-74-3	25	2.5	2.5	25
2,3,4,6,7,8-HxCDF	60851-34-5	25	2.5	2.5	25
1,2,3,7,8,9-HxCDF	72918-21-9	25	2.5	2.5	25
1,2,3,4,6,7,8-HpCDF	67562-39-4	25	2.5	2.5	25
1,2,3,4,6,7,8-HpCDD	35822-46-9	25	2.5	2.5	25
1,2,3,4,7,8,9-HpCDF	55673-89-7	25	2.5	2.5	25
OCDD	3268-87-9	50	5.0	5.0	50
OCDF	39001-02-0	50	5.0	5.0	50

* "Chemical waste" includes the matrices of oils, still bottoms, oily sludge, wet fuel oil, oil-laced soil, and surface water heavily contaminated with these matrices.

TABLE 3

INTERNAL STANDARD, RECOVERY STANDARD, AND CLEANUP STANDARD SOLUTIONS

INTERNAL STANDARD SOLUTION	
Internal Standards	**Concentration**
$^{13}C_{12}$-2,3,7,8-TCDD	5 ng/µL
$^{13}C_{12}$-2,3,7,8-TCDF	5 ng/µL
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	5 ng/µL
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	10 ng/µL
$^{13}C_{12}$-OCDD	10 ng/µL

RECOVERY STANDARD SOLUTION	
Recovery Standards	**Concentration**
$^{13}C_{12}$-1,2,3,4-TCDD	5 ng/µL
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	5 ng/µL

CLEANUP STANDARD SOLUTION	
Cleanup Standard	**Concentration**
$^{37}Cl_4$-2,3,7,8-TCDD	5 ng/µL

TABLE 4

CALIBRATION VERIFICATION SOLUTION

Volume	Solution
500 µL	CC4 (Table 1)
125 µL	Supplemental Calibration solution (below)
50 µL	Internal Standard solution (Table 3)
50 µL	Recovery Standard solution (Table 3)
50 µL	Cleanup Standard solution (Table 3)
225 µL	Tridecane (or nonane)

This solution will yield a final volume of 1.0 mL at the concentrations specified for the CC3 solution in Table 1.

Supplemental Calibration Solution Prepared from Commercially-Available Materials

Analyte	Concentration (ng/µL)
2,3,4,7,8-PeCDF	4
1,2,3,7,8,9-HxCDD	10
1,2,3,4,7,8-HxCDD	10
1,2,3,4,7,8-HxCDF	10
1,2,3,7,8,9-HxCDF	10
2,3,4,6,7,8-HxCDF	10
1,2,3,4,7,8,9-HpCDF	10

TABLE 5

MATRIX SPIKING SOLUTION

Analyte	Concentration (ng/µL)
2,3,7,8-TCDD	2.5
2,3,7,8-TCDF	2.5
1,2,3,7,8-PeCDF	6.25
1,2,3,7,8-PeCDD	6.25
1,2,3,6,7,8-HxCDF	6.25
1,2,3,6,7,8-HxCDD	6.25
1,2,3,4,6,7,8-HpCDF	6.25
1,2,3,4,6,7,8-HpCDD	6.25
OCDD	12.5
OCDF	12.5

This solution is prepared in tridecane (or nonane) and diluted with acetone prior to use (see Sec. 5.16).

TABLE 6

PCDD/PCDF ISOMERS IN THE WINDOW DEFINING MIX FOR A 60 m DB-5 COLUMN

Homologue	First Eluted	Last Eluted	Approximate Concentration (µg/mL)
TCDD	1,3,6,8-	1,2,8,9-	1.0
TCDF	1,3,6,8-	1,2,8,9-	1.0
PeCDD	1,2,4,7,9-	1,2,3,8,9-	1.0
PeCDF	1,3,4,6,8-	1,2,3,8,9-	1.0
HxCDD	1,2,4,6,7,9-	1,2,3,4,6,7-	1.0
HxCDF	1,2,3,4,6,8-	1,2,3,4,8,9-	1.0
HpCDD	1,2,3,4,6,7,9-	1,2,3,4,6,7,8-	1.0
HpCDF	1,2,3,4,6,7,8-	1,2,3,4,7,8,9-	1.0

TABLE 7

RECOMMENDED SELECTED ION MONITORING DESCRIPTORS

Descriptor 1	Descriptor 2	Descriptor 3	Descriptor 4
243	277	311	345
259	293	327	361
277	311	345	379
293	327	361	395
304	338	374	408
306	340	376	410
316	342	390	420
318	354	392	422
320	356	402	424
322	358	404	426
328	374	408	442
332	376	410	444
334	390	420	458
340	392	422	460
342	402	424	470
356	404	426	472
358	410	446	480
376	446	480	514

The ions at m/z 376 (HxCDPE), 410 (HpCDPE), 446 (OCDPE), 480 (NCDPE) and 514 (DCDPE) represent the polychlorinated diphenyl ethers.

The ions in each of the four recommended descriptors are arranged so that there is overlap between the descriptors. The ions for the TCDD, TCDF, PeCDD, and PeCDF isomers are in the first descriptor, the ions for the PeCDD, PeCDF, HxCDD and HxCDF isomers are in the second descriptor, the ions for the HxCDD, HxCDF, HpCDD and HpCDF isomers are in the third, and the ions for the HpCDD, HpCDF, OCDD and OCDF isomers are in the fourth descriptor.

TABLE 8

IONS SPECIFIED FOR SELECTED ION MONITORING FOR PCDDs/PCDFs

Analyte	Quantitation Ions		M-[COCl]$^+$
TCDD	320	322	259
PeCDD	356	358	293
HxCDD	390	392	327
HpCDD	424	426	361
OCDD	458	460	395
TCDF	304	306	243
PeCDF	340	342	277
HxCDF	374	376	311
HpCDF	408	410	345
OCDF	442	444	379
Internal Standards			
$^{13}C_{12}$-2,3,7,8-TCDD	332	334	—
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	402	404	—
$^{13}C_{12}$-OCDD	470	472	—
$^{13}C_{12}$-2,3,7,8-TCDF	316	318	—
$^{13}C_{12}$-1,2,3,4,6,7,8-HPCDF	420	422	—
Recovery Standards			
$^{13}C_{12}$-1,2,3,4-TCDD	332	334	—
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	402	404	—
Cleanup Standard			
$^{37}Cl_4$-2,3,7,8-TCDD	328	(1)	265
Polychlorinated diphenyl ethers			
HxCDPE	376	—	—
HpCDPE	410	—	—
OCDPE	446	—	—
NCDPE	480	—	—
DCDPE	514	—	—

(1) There is only one quantitation ion monitored for the cleanup standard.

TABLE 9

CRITERIA FOR ISOTOPIC RATIO MEASUREMENTS FOR PCDDs/PCDFs

Analyte	Selected Ions	Theoretical Ion Abundance	Control Limits
TCDD	320/322	0.77	0.65 - 0.89
PeCDD	356/358	1.55	1.32 - 1.78
HxCDD	390/392	1.24	1.05 - 1.43
HpCDD	424/426	1.04	0.88 - 1.20
OCDD	458/460	0.89	0.76 - 1.02
TCDF	304/306	0.77	0.65 - 0.89
PeCDF	340/342	1.55	1.32 - 1.78
HxCDF	374/376	1.24	1.05 - 1.43
HpCDF	408/410	1.04	0.88 - 1.20
OCDF	442/444	0.89	0.76 - 1.02
Internal Standards			
$^{13}C_{12}$-1,2,3,4-TCDD	332/334	0.77	0.65 - 0.89
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	402/404	1.24	1.05 - 1.43
$^{13}C_{12}$-OCDD	470/472	0.89	0.76 - 1.01
$^{13}C_{12}$-2,3,7,8-TCDF	316/318	0.77	0.65 - 0.89
$^{13}C_{12}$-1,2,3,4,6,7,8-HPCDF	420/422	1.04	0.88 - 1.20
Recovery Standards			
$^{13}C_{12}$-,1,2,3,4-TCDD	332/334	0.77	0.65 - 0.89
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	402/404	1.24	1.05 - 1.43

TABLE 10

RELATIONSHIP OF INTERNAL STANDARDS TO ANALYTES, AND RECOVERY STANDARDS TO INTERNAL STANDARDS, CLEANUP STANDARD, AND ANALYTES

INTERNAL STANDARDS VS. ANALYTES

Internal Standard	Analyte
$^{13}C_{12}$-TCDD	2,3,7,8-TCDD
	1,2,3,7,8-PeCDD
$^{13}C_{12}$-HxCDD	1,2,3,6,7,8-HxCDD
	1,2,3,7,8,9-HxCDD
	1,2,3,4,7,8-HxCDD
	1,2,3,4,6,7,8-HpCDD
$^{13}C_{12}$-OCDD	1,2,3,4,6,7,8,9-OCDD
	1,2,3,4,6,7,8,9-OCDF
$^{13}C_{12}$-TCDF	2,3,7,8-TCDF
	1,2,3,7,8-PeCDF
	2,3,4,7,8-PeCDF
$^{13}C_{12}$-HpCDF	1,2,3,6,7,8-HxCDF
	1,2,3,7,8,9-HxCDF
	1,2,3,4,7,8-HxCDF
	2,3,4,6,7,8-HxCDF
	1,2,3,4,5,8,9-HpCDF
	1,2,3,4,7,8,9-HpCDF

TABLE 10 (cont.)

RECOVERY STANDARDS VS. ANALYTES, INTERNAL STANDARDS, AND CLEANUP STANDARD

Recovery Standard	Analyte, Internal Standard
$^{13}C_{12}$-1,2,3,4-TCDD	2,3,7,8-TCDD
	1,2,3,7,8-PeCDD
	2,3,7,8-TCDF
	1,2,3,7,8-PeCDF
	2,3,4,7,8-PeCDF
	$^{13}C_{12}$-2,3,7,8-TCDD
	$^{13}C_{12}$-2378-TCDF
	$^{37}Cl_4$-2378-TCDD
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1,2,3,6,7,8-HxCDD
	1,2,3,7,8,9-HxCDD
	1,2,3,4,7,8-HxCDD
	1,2,3,6,7,8-HxCDF
	1,2,3,7,8,9-HxCDF
	1,2,3,4,7,8-HxCDF
	2,3,4,6,7,8-HxCDF
	1,2,3,4,5,8,9-HpCDF
	1,2,3,4,7,8,9-HpCDF
	1,2,3,4,6,7,8,9-OCDD
	1,2,3,4,6,7,8,9-OCDF
	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF
	$^{13}C_{12}$-OCDD

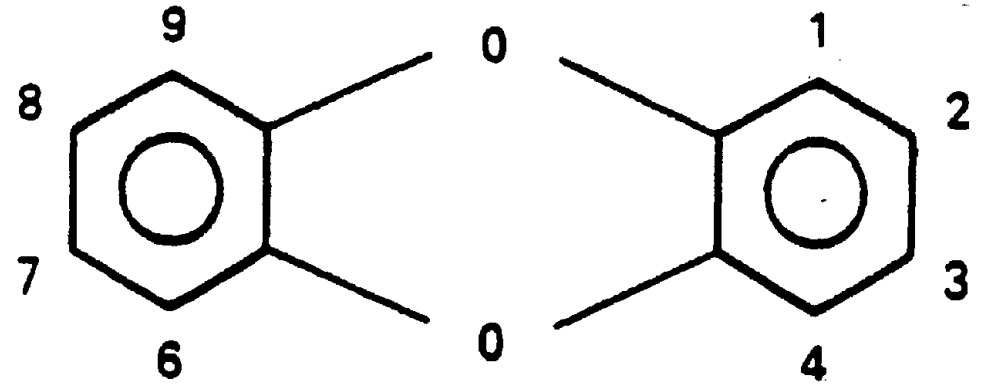
TABLE 11

2,3,7,8-TCDD TOXICITY EQUIVALENCY FACTORS (TEFs) FOR THE PCDDs/PCDFs

Compound	Toxicity Equivalency Factor (TEF)
Mono-, di-, and trichloro dibenzo-*p*-dioxins	0.0
2,3,7,8-tetrachloro-dibenzo-*p*-dioxin	1.0
All other tetrachloro-dibenzo-*p*-dioxins	0.0
1,2,3,7,8-pentachloro-dibenzo-*p*-dioxin	0.5
All other pentachloro-dibenzo-*p*-dioxins	0.0
1,2,3,4,7,8-hexachloro-dibenzo-*p*-dioxin	0.1
1,2,3,6,7,8-hexachloro-dibenzo-*p*-dioxin	0.1
1,2,3,7,8,9-hexachloro-dibenzo-*p*-dioxin	0.1
All other hexachloro-dibenzo-*p*-dioxins	0.0
1,2,3,4,6,7,8-heptachloro-dibenzo-*p*-dioxin	0.01
All other heptachloro-dibenzo-*p*-dioxins	0.0
Octachloro-dibenzo-*p*-dioxin	0.001
All mono-, di-, and trichloro dibenzofurans	0.0
2,3,7,8-tetrachlorodibenzofuran	0.1
All other tetrachlorodibenzofurans	0.0
1,2,3,7,8-pentachlorodibenzofuran	0.05
2,3,4,7,8-pentachlorodibenzofuran	0.5
All other pentachlorodibenzofurans	0.0
1,2,3,4,7,8-hexachlorodibenzofuran	0.1
1,2,3,6,7,8-hexachlorodibenzofuran	0.1
1,2,3,7,8,9-hexachlorodibenzofuran	0.1
2,3,4,6,7,8-hexachlorodibenzofuran	0.1
All other hexachlorodibenzofurans	0.0
1,2,3,4,6,7,8-heptachlorodibenzofuran	0.01
1,2,3,4,7,8,9-heptachlorodibenzofuran	0.01
All other heptachlorodibenzofurans	0.0
Octachlorodibenzofuran	0.001

FIGURE 1

GENERAL STRUCTURES OF PCDDs (top) AND PCDFs (bottom)



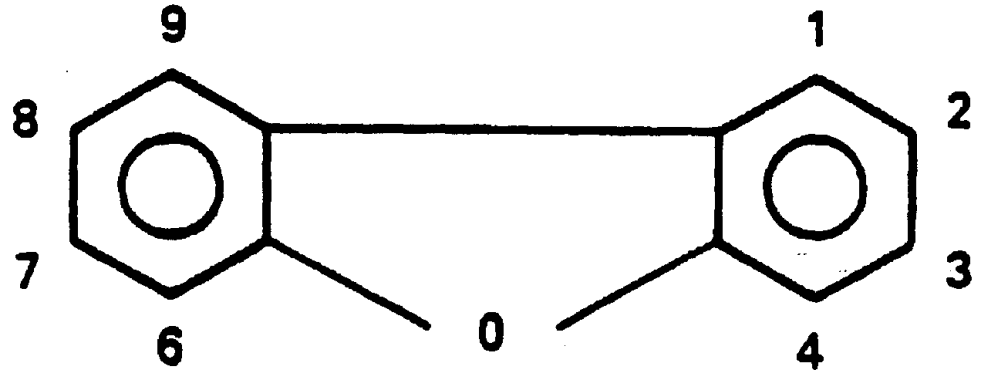
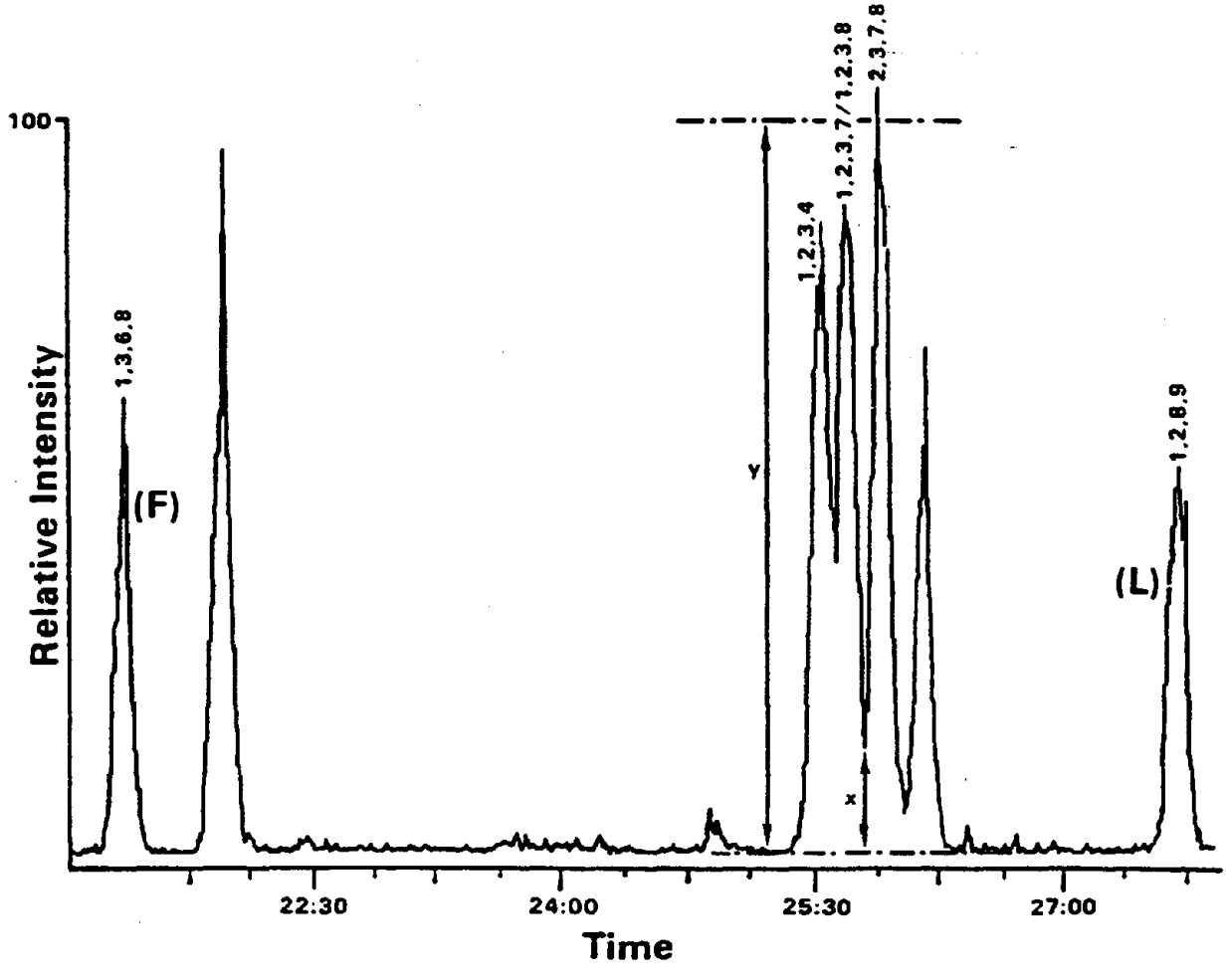


FIGURE 2

VALLEY BETWEEN 2,3,7,8-TCDD AND OTHER CLOSELY ELUTING ISOMERS ON A DB-5 GC COLUMN



Selected ion current profile for m/z 322 (TCDD) produced by MS analysis of GC performance check solution on a 60 m x 0.32 mm DB-5 fused silica capillary column with 0.25 µm film thickness.

Injector temp:	270°C
Starting temp:	200°C for 2 min
	200 to 220°C @ 5°/min and held for 16 min
	220 to 235°C @ 5°/min and held for 7 min
	235 to 330°C @ 5°/min and held for 5 min
Splitless valve time:	45 sec
Total time:	60 min

FIGURE 3

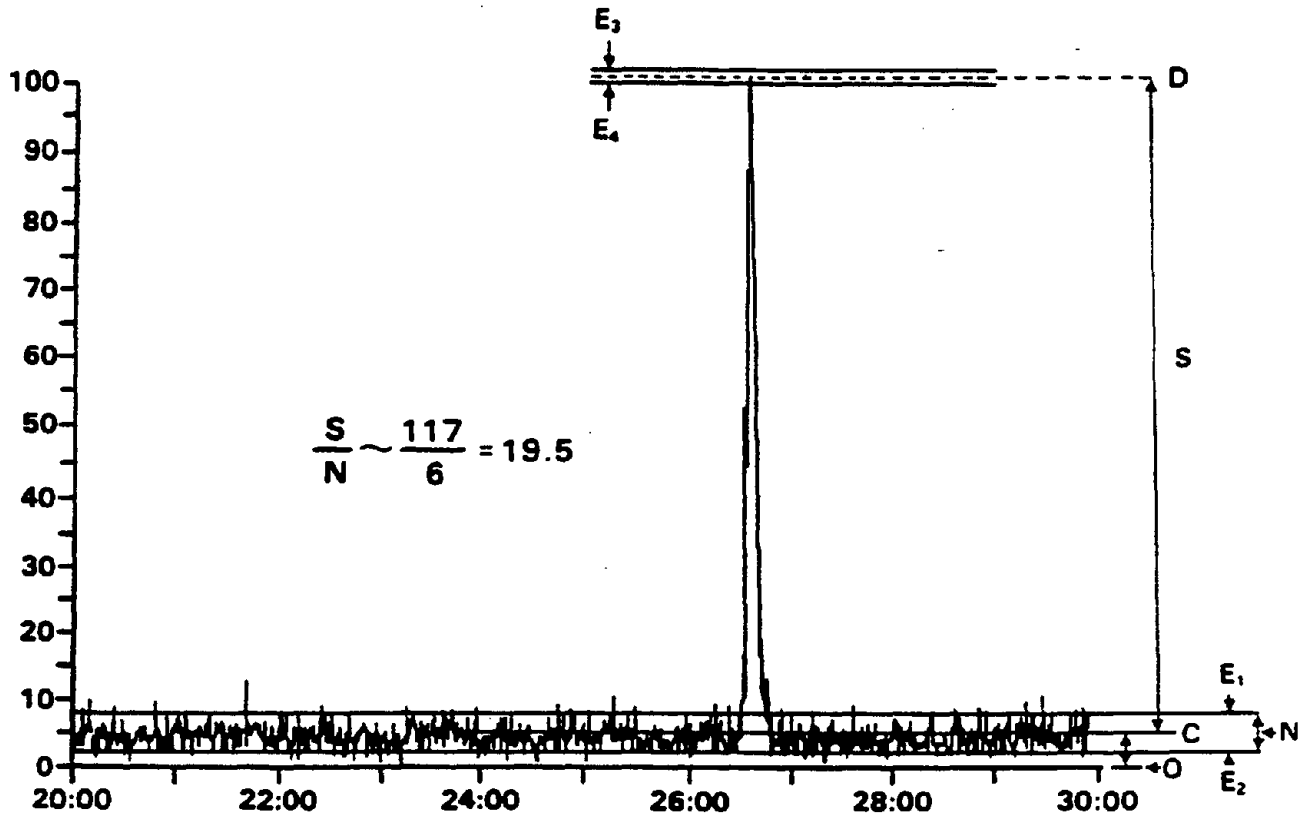
EXAMPLE OF THE ANALYTICAL SEQUENCE FOR CALIBRATING AN SP-2331 COLUMN

Time	Analysis
Hour 0	Window Defining Mix Column Performance Solution (SP-2331) CC3 CC1 (Initial Calibration) CC2 CC4 CC5 Blanks and Samples CC1 (must be injected within the 12-hour period.)
Hour 12	Column Performance Solution (SP-2331) CC3 Blanks and Samples CC1 (must be injected within the 12-hour period.)
Hour 24	Column Performance Solution (SP-2331) CC3 Blanks and Samples CC1 (must be injected within the 12-hour period.)

NOTE: When a column other than SP-2331 is employed, the column performance solution need not be analyzed.

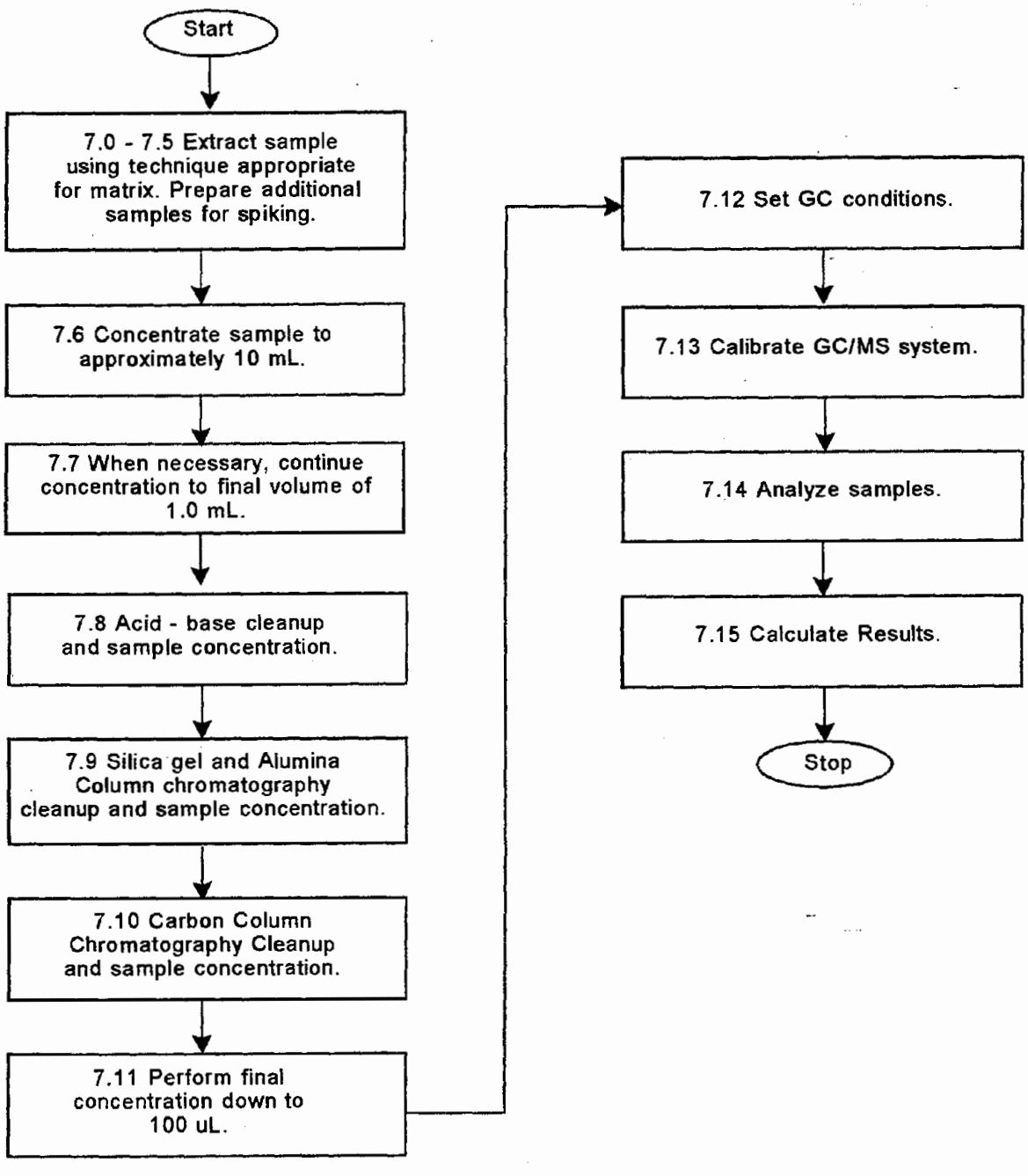
FIGURE 4

MANUAL DETERMINATION OF S/N



The peak height (S) is measured between the mean noise (lines C and D). These mean signal values are obtained by tracing the line between the baseline average noise extremes, E1 and E2, and between the apex average noise extremes, E3 and E4, at the apex of the signal.

NOTE: It is imperative that the instrument interface amplifier electronic zero offset be set high enough so that negative going baseline noise is recorded.


Start
7.0 - 7.5 Extract sample using technique appropriate for matrix. Prepare additional samples for spiking.
7.6 Concentrate sample to approximately 10 mL.
7.7 When necessary, continue concentration to final volume of 1.0 mL.
7.8 Acid - base cleanup and sample concentration.
7.9 Silica gel and Alumina Column chromatography cleanup and sample concentration.
7.10 Carbon Column Chromatography Cleanup and sample concentration.
7.11 Perform final concentration down to 100 uL.
7.12 Set GC conditions.
7.13 Calibrate GC/MS system.
7.14 Analyze samples.
7.15 Calculate Results.
Stop

METHOD 8290A

POLYCHLORINATED DIBENZODIOXINS (PCDDs) AND POLYCHLORINATED DIBENZOFURANS (PCDFs) BY HIGH-RESOLUTION GAS CHROMATOGRAPHY/HIGH-RESOLUTION MASS SPECTROMETRY (HRGC/HRMS)

1.0 SCOPE AND APPLICATION

1.1 This method provides procedures for the detection and quantitative measurement of polychlorinated dibenzo-*p*-dioxins (tetra- through octachlorinated homologues; PCDDs), and polychlorinated dibenzofurans (tetra- through octachlorinated homologues; PCDFs) in a variety of environmental matrices and at part-per-trillion (ppt) to part-per-quadrillion (ppq) concentrations. The following compounds can be determined by this method:

Analyte	CAS Registry No.
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	1746-01-6
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin (PeCDD)	40321-76-4
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)	39227-28-6
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)	57653-85-7
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin (HxCDD)	19408-74-3
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin (HpCDD)	35822-46-9
1,2,3,4,5,6,7,8-Octachlorodibenzo-*p*-dioxin (OCDD)	3268-87-9
2,3,7,8-Tetrachlorodibenzofuran (TCDF)	51207-31-9
1,2,3,7,8-Pentachlorodibenzofuran (PeCDF)	57117-41-6
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)	57117-31-4
1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)	70648-26-9
1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF)	57117-44-9
1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF)	72918-21-9
2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF)	60851-34-5
1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)	67562-39-4
1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF)	55673-89-7
1,2,3,4,5,6,7,8-Octachlorodibenzofuran (OCDF)	39001-02-0
Total Tetrachlorodibenzo-*p*-dioxin (TCDD)	41903-57-5
Total Pentachlorodibenzo-*p*-dioxin (PeCDD)	36088-22-9
Total Hexachlorodibenzo-*p*-dioxin (HxCDD)	34465-46-8
Total Heptachlorodibenzo-*p*-dioxin (HpCDD)	37871-00-4
Total Tetrachlorodibenzofuran (TCDF)	55722-27-5
Total Pentachlorodibenzofuran (PeCDF)	30402-15-4
Total Hexachlorodibenzofuran (HxCDF)	55684-94-1
Total Heptachlorodibenzofuran (HpCDF)	38998-75-3

1.2 The analytical method calls for the use of high-resolution gas chromatography and high-resolution mass spectrometry (HRGC/HRMS) on purified sample extracts. Table 1 lists the various sample types covered by this analytical protocol, the 2,3,7,8-TCDD-based method calibration limits (MCLs), and other pertinent information. Samples containing concentrations of specific congeneric analytes (PCDDs and PCDFs) considered within the scope of this method that are greater than ten times the upper MCLs must be analyzed by a protocol designed for such concentration levels, e.g., Method 8280. An optional method for reporting the analytical results using a 2,3,7,8-TCDD toxicity equivalency factor (TEF) is described.

1.3 The sensitivity of this method is dependent upon the level of interferences within a given matrix. The calibration range of the method for a 1-L water sample is 10 to 2000 ppq for TCDD/TCDF and PeCDD/PeCDF, and 1.0 to 200 ppt for a 10-g soil, sediment, fly ash, or tissue sample for the same analytes (Table 1). Analysis of a one-tenth aliquot of the sample permits measurement of concentrations up to 10 times the upper MCL. The actual limits of detection and quantitation will differ from the lower MCL, depending on the complexity of the matrix.

1.4 This method is designed for use by analysts who are experienced with residue analysis and skilled in HRGC/HRMS.

1.5 Because of the extreme toxicity of many of these compounds, the analyst must take the necessary precautions to prevent exposure to materials known or believed to contain PCDDs or PCDFs. It is the responsibility of the laboratory personnel to ensure that safe handling procedures are employed. Sec. 11 of this method discusses safety procedures.

2.0 SUMMARY OF METHOD

2.1 This procedure uses matrix-specific extraction, analyte-specific cleanup, and HRGC/HRMS analysis techniques.

2.2 If interferences are encountered, the method provides selected cleanup procedures to aid the analyst in their elimination. A simplified analysis flow chart is presented at the end of this method.

2.3 A specified amount (see Table 1) of soil, sediment, fly ash, water, sludge (including paper pulp), still bottom, fuel oil, chemical reactor residue, fish tissue, or human adipose tissue is spiked with a solution containing specified amounts of each of the nine isotopically ($^{13}C_{12}$) labeled PCDDs/PCDFs listed in Column 1 of Table 2. The sample is then extracted according to a matrix-specific extraction procedure. Aqueous samples that are judged to contain 1 percent or more solids, and solid samples that show an aqueous phase, are filtered, the solid phase (including the filter) and the aqueous phase extracted separately, and the extracts combined before extract cleanup. The extraction procedures are:

a) Toluene: Soxhlet extraction for soil, sediment, fly ash, and paper pulp samples;
b) Methylene chloride: liquid-liquid extraction for water samples;
c) Toluene: Dean-Stark extraction for fuel oil, and aqueous sludge samples;
d) Toluene extraction for still bottom samples;
e) Hexane/methylene chloride: Soxhlet extraction or methylene chloride: Soxhlet extraction for fish tissue samples; and
f) Methylene chloride extraction for human adipose tissue samples.

g) As an option, all solid samples (wet or dry) may be extracted with toluene using a Soxhlet/Dean Stark extraction system or using pressurized fluid extraction (PFE) (Method 3545).

The decision for the selection of an extraction procedure for chemical reactor residue samples is based on the appearance (consistency, viscosity) of the samples. Generally, they can be handled according to the procedure used for still bottom (or chemical sludge) samples.

2.4 The extracts are submitted to an acid-base washing treatment and dried. Following a solvent exchange step, the extracts are cleaned up by column chromatography on alumina, silica gel, and activated carbon.

2.4.1 The extracts from adipose tissue samples are treated with silica gel impregnated with sulfuric acid before chromatography on acidic silica gel, neutral alumina, and activated carbon.

2.4.2 Fish tissue and paper pulp extracts are subjected to an acid wash treatment only, prior to chromatography on alumina and activated carbon.

2.5 The preparation of the final extract for HRGC/HRMS analysis is accomplished by adding 10 to 50 µL (depending on the matrix) of a nonane solution containing 50 pg/µL of the recovery standards $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD (Table 2). The former is used to determine the percent recoveries of tetra- and pentachlorinated PCDD/PCDF congeners, while the latter is used to determine the percent recoveries of the hexa-, hepta- and octachlorinated PCDD/PCDF congeners.

2.6 A 2-µL aliquot of the concentrated extract are injected into an HRGC/HRMS system capable of performing selected ion monitoring at resolving power of at least 10,000 (10 percent valley definition).

2.7 The identification of OCDD and nine of the fifteen 2,3,7,8-substituted congeners (Table 3), for which ^{13}C-labeled standards are available in the sample fortification and recovery standard solutions (Table 2), is based on their elution at their exact retention time (within 0.005 retention time units measured in the routine calibration) and the simultaneous detection of the two most abundant ions in the molecular ion region. The remaining six 2,3,7,8-substituted congeners (i.e., 2,3,4,7,8-PeCDF; 1,2,3,4,7,8-HxCDD; 1,2,3,6,7,8-HxCDF; 1,2,3,7,8,9-HxCDF; 2,3,4,6,7,8-HxCDF, and 1,2,3,4,7,8,9-HpCDF), for which no carbon-labeled internal standards are available in the sample fortification solution, and all other PCDD/PCDF congeners are identified when their relative retention times fall within their respective PCDD/PCDF retention time windows, as established from the routine calibration data, and the simultaneous detection of the two most abundant ions in the molecular ion region. The identification of OCDF is based on its retention time relative to $^{13}C_{12}$-OCDD and the simultaneous detection of the two most abundant ions in the molecular ion region. Identification also is based on a comparison of the ratios of the integrated ion abundance of the molecular ion species to their theoretical abundance ratios.

2.8 Quantitation of the individual congeners, total PCDDs, and total PCDFs is achieved in conjunction with the establishment of a multipoint (five points) calibration curve for each homologue, during which each calibration solution is analyzed once.

3.0 INTERFERENCES

3.1 Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts or elevated baselines that may cause misinterpretation of the chromatographic data (see references 1 and 2.) All of these materials must be demonstrated to be free from interferants under the conditions of analysis by performing laboratory method blanks. Analysts should avoid using PVC gloves.

3.2 The use of high purity reagents and solvents helps minimize interference problems. Purification of solvents by distillation in all-glass systems may be necessary.

3.3 Interferants coextracted from the sample will vary considerably from matrix to matrix. PCDDs and PCDFs are often associated with other interfering chlorinated substances such as polychlorinated biphenyls (PCBs), polychlorinated diphenyl ethers (PCDPEs), polychlorinated naphthalenes, and polychlorinated alkyldibenzofurans, that may be found at concentrations several orders of magnitude higher than the analytes of interest. Retention times of target analytes must be verified using reference standards. These values must correspond to the retention time windows established in Sec. 8.1.1.3. While cleanup techniques are provided as part of this method, unique samples may require additional cleanup steps to achieve lower detection limits.

3.4 A high-resolution capillary column (60-m DB-5, J&W Scientific, or equivalent) is used in this method. However, no single column is known to resolve all isomers. The 60-m DB-5 GC column is capable of 2,3,7,8-TCDD isomer specificity (Sec. 8.1.1). In order to determine the concentration of the 2,3,7,8-TCDF (if detected on the DB-5 column), the sample extract must be reanalyzed on a column capable of 2,3,7,8-TCDF isomer specificity (e.g., DB-225, SP-2330, SP-2331, or equivalent).

4.0 APPARATUS AND MATERIALS

4.1 High-resolution gas chromatograph/high-resolution mass spectrometer/data system (HRGC/HRMS/DS) - The GC must be equipped for temperature programming, and all required accessories must be available, such as syringes, gases, and capillary columns.

4.1.1 GC injection port - The GC injection port must be designed for capillary columns. The use of splitless injection techniques is recommended. On-column 1-µL injections can be used on the 60-m DB-5 column. The use of a moving needle injection port is also acceptable. When using the method described in this protocol, a 2-µL injection volume is used consistently (i.e., the injection volumes for all extracts, blanks, calibration solutions and the performance check samples are 2 µL). The use of 1-µL injections is allowed; however, laboratories must remain consistent throughout the analyses by using the same injection volume at all times.

4.1.2 GC/MS interface - The GC/MS interface components should withstand 350°C. The interface must be designed so that the separation of 2,3,7,8-TCDD from the other TCDD isomers achieved in the gas chromatographic column is not appreciably degraded. Cold spots or active surfaces (adsorption sites) in the GC/MS interface can cause peak tailing and peak broadening. It is recommended that the GC column be fitted directly into the mass spectrometer ion source without being exposed to the ionizing electron beam. Graphite ferrules should be avoided in the injection port because they may adsorb the PCDDs and PCDFs. Vespel™, or equivalent, ferrules are recommended.

4.1.3 Mass spectrometer - The static resolving power of the instrument must be maintained at a minimum of 10,000 (10 percent valley).

4.1.4 Data system - A dedicated data system is employed to control the rapid multiple-ion monitoring process and to acquire the data. Quantitation data (peak areas or peak heights) and SIM traces (displays of intensities of each ion signal being monitored including the lock-mass ion as a function of time) must be acquired during the analyses and stored. Quantitations may be reported based upon computer-generated peak areas or upon measured peak heights (chart recording). The data system must be capable of acquiring data at a minimum of 10 ions in a single scan. It is also recommended to have a data system capable of switching to different sets of ions (descriptors) at specified times during an HRGC/HRMS acquisition. The data system should be able to provide hard copies of individual ion chromatograms for selected gas chromatographic time intervals. It should also be able to acquire mass spectral peak profiles (Sec. 8.1.2.3) and provide hard copies of peak profiles to demonstrate the required resolving power. The data system should permit the measurement of noise on the base line.

NOTE: The detector ADC zero setting must allow peak-to-peak measurement of the noise on the base line of every monitored channel and allow for good estimation of the instrument resolving power. The effect of different zero settings on the measured resolving power is shown in Figure 2.

4.2 GC columns

Fused-silica capillary columns are needed. The columns shall demonstrate the required separation of all 2,3,7,8-specific isomers whether a dual-column or a single-column analysis is chosen. Chromatographic performance must be demonstrated and documented (Sec. 8.2.2) at the beginning of each 12-hour period (after mass resolution and GC resolution are demonstrated) during which sample extracts or concentration calibration solutions will be analyzed. Recommended operating conditions for the recommended columns are shown in Sec. 7.6.

4.2.1 60-m DB-5 (J&W Scientific) or equivalent fused-silica capillary column

In order to have an isomer-specific determination of 2,3,7,8-TCDD and to allow the detection of OCDD/OCDF within a reasonable time interval in one HRGC/HRMS analysis, use of this column is recommended. Isomer specificity for all 2,3,7,8-substituted PCDDs/PCDFs cannot be achieved on the 60-m DB-5 column. Problems have been associated with the separation of 2,3,7,8-TCDD from 1,2,3,7-TCDD and 1,2,6,8-TCDD, and separation of 2,3,7,8-TCDF from 1,2,4,9-, 1,2,7,9-, 2,3,4,6-, 2,3,4,7-, and 2,3,4,8-TCDF. Because of the toxicologic concern associated with 2,3,7,8-TCDD and 2,3,7,8-TCDF, additional analyses may be necessary for some samples.

4.2.2 30-m DB-225 (J&W Scientific) or equivalent fused-silica capillary column

For the DB-225 column, problems are associated with the separation of 2,3,7,8-TCDF from 2,3,4,7-TCDF and a combination of 1,2,3,9- and 2,3,4,8-TCDF.

4.3 Miscellaneous equipment and materials - The following list of items does not necessarily constitute an exhaustive compendium of the equipment needed for this analytical method.

NOTE: Reuse of glassware should be minimized to avoid the risk of contamination. All glassware that is reused should be scrupulously cleaned as soon as possible after use, according to the following procedure: Rinse glassware with the last solvent used in it. Wash with hot detergent water, then rinse with copious amounts of tap water and several portions of organic-free reagent water. Rinse with high purity acetone and hexane and store it inverted or capped with solvent rinsed aluminum foil in a clean environment.

4.3.1 Nitrogen evaporation apparatus with variable flow rate.

4.3.2 Balances capable of accurately weighing to 0.01 g and 0.0001 g.

4.3.3 Centrifuge.

4.3.4 Water bath - equipped with concentric ring covers and capable of being temperature controlled within ± 2°C.

4.3.5 Stainless steel or glass container large enough to hold contents of one-pint sample containers.

4.3.6 Glove box.

4.3.7 Drying oven.

4.3.8 Stainless steel spoons and spatulas.

4.3.9 Laboratory hoods.

4.3.10 Pipets - disposable, Pasteur, 150 mm long x 5 mm ID.

4.3.11 Pipets - disposable, serological, 10-mL, for the preparation of the carbon columns specified in Sec. 7.5.3.

4.3.12 Reaction vial - 2-mL, silanized amber glass (Reacti-vial, or equivalent).

4.3.13 Stainless steel meat grinder with a 3 to 5 mm hole size inner plate.

4.3.14 Separatory funnels - 125-mL and 2000-mL.

4.3.15 Kudema-Danish concentrator - 500-mL, fitted with 10-mL concentrator tube and three-ball Snyder column.

4.3.16 PTFE or Carborundum (silicon carbide) boiling chips (or equivalent), washed with hexane before use.

NOTE: PFTE boiling chips may float in methylene chloride, may not work in the presence of any water phase, and may be penetrated by nonpolar organic compounds.

4.3.17 Chromatographic columns - glass, 300 mm x 10.5 mm, fitted with PTFE stopcock.

4.3.18 Adapters for concentrator tubes.

4.3.19 Glass fiber filters - 0.70-µm, Whatman GFF, or equivalent.

4.3.20 Dean-Stark trap - 5- or 10-mL, with T-joints, condenser and 125-mL flask.

4.3.21 Continuous liquid-liquid extractor - 1-L sample capacity, suitable for use with heavier than water solvents.

4.3.22 All glass Soxhlet apparatus with 500-mL flask.

4.3.23 Soxhlet/Dean-Stark extractor (optional) - all glass, 500-mL flask.

4.3.24 Glass funnels - sized to hold 170 mL of liquid.

4.3.25 Desiccator.

4.3.26 Solvent reservoir (125-mL) - compatible with gravity carbon column.

4.3.27 Rotary evaporator with a temperature-controlled water bath.

4.3.28 High speed tissue homogenizer - equipped with an EN-8 probe, or equivalent.

4.3.29 Glass wool - extract with methylene chloride, dry, and store in a glass jar.

4.3.30 Extraction jars - glass, 250-mL, with PTFE-lined screw cap.

4.3.31 Volumetric flasks - Class A, 10-mL to 1000-mL.

4.3.32 Glass vials - 1-dram (or metric equivalent).

5.0 REAGENTS AND STANDARD SOLUTIONS

5.1 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.2 Column chromatography reagents

5.2.1 Alumina - neutral, 80/200 mesh (Super 1, Woelm®, or equivalent). Store in a sealed container at room temperature, in a desiccator, over self-indicating silica gel.

5.2.2 Alumina - acidic AG4, (Bio Rad Laboratories catalog #132-1240, or equivalent). Soxhlet extract with methylene chloride for 24 hours if blanks show contamination, and activate by heating in a foil covered glass container for 24 hours at 190°C. Store in a glass bottle sealed with a PTFE-lined screw cap.

5.2.3 Silica gel - high purity grade, type 60, 70-230 mesh. Soxhlet extract with methylene chloride for 24 hours if blanks show contamination, and activate by heating in a foil

covered glass container for 24 hours at 190°C. Store in a glass bottle sealed with a PTFE-lined screw cap.

5.2.4 Silica gel impregnated with sodium hydroxide. Add one part (by weight) of 1 M NaOH solution to two parts (by weight) silica gel (extracted and activated) in a screw cap bottle and mix with a glass rod until free of lumps. Store in a glass bottle sealed with a PTFE-lined screw cap.

5.2.5 Silica gel impregnated with 40 percent (by weight) sulfuric acid. Add two parts (by weight) concentrated sulfuric acid to three parts (by weight) silica gel (extracted and activated), mix with a glass rod until free of lumps, and store in a screw capped glass bottle. Store in a glass bottle sealed with a PTFE-lined screw cap.

5.2.6 Celite 545® (Supelco), or equivalent.

5.2.7 Charcoal carbon - Activated carbon, Carbopak C (Supelco) or equivalent, prewashed with methanol and dried *in vacuo* at 110°C. Store in a glass bottle sealed with a PTFE-lined screw cap. (Note: AX-21 [Anderson Development Company] carbon is no longer available, but existing stocks may be utilized).

5.3 Reagents

5.3.1 Sulfuric acid, H_2SO_4, concentrated, ACS grade, specific gravity 1.84.

5.3.2 Potassium hydroxide, KOH, ACS grade, 20 percent (w/v) in organic-free reagent water.

5.3.3 Sodium chloride, NaCl, analytical reagent, 5 percent (w/v) in organic-free reagent water.

5.3.4 Potassium carbonate, K_2CO_3, anhydrous, analytical reagent.

5.4 Sodium sulfate (powder, anhydrous), Na_2SO_4 - Purify by heating at 400°C for 4 hours in a shallow tray. If, after heating, the sodium sulfate develops a noticeable grayish cast (due to the presence of carbon in the crystal matrix) that batch of sodium sulfate is not suitable for use and should be discarded. Extraction with methylene chloride may produce sodium sulfate that is suitable for use in such instances, but following extraction, a reagent blank must be analyzed that demonstrates that there is no interference from the sodium sulfate.

5.5 Solvents - all solvents must be (at a minimum) pesticide grade or equivalent, distilled-in-glass.

5.5.1 Methylene chloride, CH_2Cl_2.

5.5.2 Hexane, C_6H_{14}.

5.5.3 Methanol, CH_3OH.

5.5.4 Nonane, C_9H_{20}.

5.5.5 Toluene, $C_6H_5CH_3$.

5.5.6 Cyclohexane, C_6H_{12}.

5.5.7 Acetone, CH_3C0CH_3.

5.6 High-Resolution Concentration Calibration Solutions (Table 5) - Five nonane solutions containing 17 unlabeled and 11 carbon-labeled PCDDs and PCDFs at known concentrations are used to calibrate the instrument. The concentration ranges are homologue-dependent, with the lowest values for the tetrachlorinated dioxin and furan (1.0 pg/µL) and the highest values for the octachlorinated congeners (1000 pg/µL). Standards containing more carbon-labeled PCDDs and PCDFs may also be employed.

5.7 GC Column Performance Check Solution - This solution contains the first and last eluting isomers for each homologous series from tetra- through heptachlorinated congeners. The solution also contains a series of other TCDD isomers for the purpose of documenting the chromatographic resolution. The $^{13}C_{12}$-2,3,7,8-TCDD is also present. The laboratory is required to use nonane as the solvent and adjust the volume so that the final concentration does not exceed 100 pg/µL per congener. Table 7 summarizes the qualitative composition (minimum requirement) of this performance evaluation solution.

5.8 Sample Fortification Solution - This nonane solution contains the nine internal standards at the nominal concentrations that are listed in Table 2. The solution contains at least one carbon-labeled standard for each homologous series, and it is used to measure the concentrations of the native substances. (Note that $^{13}C_{12}$-OCDF is not present in the solution.) Standards containing more carbon-labeled PCDDs and PCDFs may also be employed, provided that the same labeled compounds are contained in the calibration standards in Sec. 5.6.

5.9 Recovery Standard Solution - This nonane solution contains two recovery standards, $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD, at a nominal concentration of 50 pg/µL per compound. 10 to 50 µL of this solution will be spiked into each sample extract before the final concentration step and HRGC/HRMS analysis.

5.10 Matrix Spike Fortification Solution - Solution used to prepare the MS and MSD samples. It contains all unlabeled analytes listed in Table 5 at concentrations corresponding to the HRCC 3.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

6.2 Sample collection

6.2.1 Sample collection personnel should, to the extent possible, homogenize samples in the field before filling the sample containers. This should minimize or eliminate the necessity for sample homogenization in the laboratory. The analyst should make a judgment, based on the appearance of the sample, regarding the necessity for additional mixing. If the sample is clearly not homogeneous, the entire contents should be transferred to a glass or stainless steel pan for mixing with a stainless steel spoon or spatula before removal of a sample portion for analysis.

6.2.2 Grab and composite samples must be collected in glass containers. Conventional sampling practices must be followed. The bottle must not be prewashed with

sample before collection. Sampling equipment must be free of potential sources of contamination.

6.3 Grinding or blending of fish samples - If not otherwise specified in a project plan, the whole fish (frozen) should be blended or ground to provide a homogeneous sample. The use of a stainless steel meat grinder with a 3 to 5 mm hole size inner plate is recommended. In some circumstances, analysis of fillet or specific organs of fish may be requested. If so requested, the above whole fish requirement is superseded.

6.4 Storage and holding times - All samples, except fish and adipose tissue samples, must be stored at 4°C in the dark, and should be extracted within 30 days and completely analyzed within 45 days of extraction. Fish and adipose tissue samples must be stored at -20°C in the dark, and should be extracted within 30 days and completely analyzed within 45 days of collection.

NOTE: The holding times listed in Sec. 6.4 are recommendations. PCDDs and PCDFs are very stable in a variety of matrices, and holding times under the conditions listed in Sec. 6.4 may be as high as a year for certain matrices.

6.5 Phase separation

This is a guideline for phase separation for very wet (>25 percent water) soil, sediment and paper pulp samples. Place a 50-g portion in a suitable centrifuge bottle and centrifuge for 30 minutes at 2,000 rpm. Remove the bottle and mark the interface level on the bottle. Estimate the relative volume of each phase. With a disposable pipet, transfer the liquid layer into a clean bottle. Mix the solid with a stainless steel spatula and remove a portion to be weighed and analyzed (percent dry weight determination, extraction). Return the remaining solid portion to the original sample bottle (empty) or to a clean sample bottle that is properly labeled, and store it as appropriate. Analyze the solid phase by using only the soil, sediment and paper pulp method. Take note of, and report, the estimated volume of liquid before disposing of the liquid as a liquid waste.

6.6 Soil, sediment, or paper sludge (pulp) percent dry weight determination

When results are to be reported on a dry-weight basis, the percent dry weight of soil, sediment or paper pulp samples may be determined according to the following procedure. Weigh a 10-g portion of the soil or sediment sample (± 0.5 g) to three significant figures. Dry it to constant weight at 110°C in an adequately ventilated oven. Allow the sample to cool in a desiccator. Weigh the dried solid to three significant figures. Calculate and report the percent dry weight. Do not use this solid portion of the sample for extraction, but instead dispose of it as hazardous waste.

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

CAUTION: Finely divided soils and sediments contaminated with PCDDs/PCDFs are hazardous because of the potential for inhalation or ingestion of particles containing PCDDs/PCDFs (including 2,3,7,8-TCDD). Such samples should be handled in a confined environment (i.e., a closed hood or a glove box).

6.7 Lipid content determination

6.7.1 Fish tissue - To determine the lipid content of fish tissue, concentrate 125 mL of the fish tissue extract (Sec. 7.2.2), in a tared 200-mL round-bottom flask, on a rotary evaporator until a constant weight (W) is achieved.

$$\text{lipid} = \frac{\text{weight of residue} \times 2}{\text{weight of sample}} \times 10$$

The factor of 2 accounts for the use of half of the extract (e.g., 125 mL of 250 mL total volume) for the lipid determination.

Dispose of the lipid residue as a hazardous waste if the results of the analysis indicate the presence of PCDDs or PCDFs.

Other procedures and other extract volumes may be employed for the lipid determination, provided that they are clearly described and documented. Adjustments to the amount of internal standards spiked in Sec. 7.1 will be required if different volumes are employed.

6.7.2 Adipose tissue - Details for the determination of the adipose tissue lipid content are provided in Sec. 7.3.3.

7.0 PROCEDURE

7.1 Internal standard addition

The sample fortification solution (Sec. 5.8) containing the carbon-labeled internal standards is added to each sample prior to extraction.

7.1.1 Select an appropriate size sample aliquot. Typical sample size requirements for different matrices are given in Sec. 7.4 and in Table 1. Transfer the sample portion to a tared flask and determine its weight.

7.1.2 Except for adipose tissue, add an appropriate quantity of the sample fortification mixture (Sec. 5.8) to the sample. All samples should be spiked with 100 µL of the sample fortification mixture to give internal standard concentrations as indicated in Table 1. As an example, for $^{13}C_{12}$-2,3,7,8-TCDD, a 10-g soil sample requires the addition of 1000 pg of $^{13}C_{12}$-2,3,7,8-TCDD to give the required 100 ppt fortification level. The fish tissue sample (20 g) must be spiked with 200 µL of the internal standard solution, because half of the extract will be used to determine the lipid content (Sec. 6.7.1).

7.1.2.1 For the fortification of soil, sediment, fly ash, water, fish tissue, paper pulp and wet sludge samples, mix the sample fortification solution with 1.0 mL acetone.

7.1.2.2 Do not dilute the nonane solution for the other matrices.

7.1.2.3 The fortification of adipose tissue is carried out at the time of homogenization (Sec. 7.3.2.3).

7.2 Extraction and purification of fish and paper pulp samples

7.2.1 Add 60 g of anhydrous sodium sulfate to a 20-g portion of a homogeneous fish sample (Sec. 6.3) and mix thoroughly with a stainless steel spatula. After breaking up any lumps, place the fish/sodium sulfate mixture in the Soxhlet apparatus on top of a glass wool plug. Add 250 mL methylene chloride or hexane/methylene chloride (1:1) to the Soxhlet apparatus and reflux for 16 hours. The solvent must cycle completely through the system five times per hour. Follow the same procedure for the partially dewatered paper pulp sample (using a 10-g sample, 30 g of anhydrous sodium sulfate and 200 mL of toluene).

NOTE: As an option, a Soxhlet/Dean-Stark extractor system may be used, with toluene as the solvent. No sodium sulfate is added when using this option.

7.2.2 Transfer the fish extract from Sec. 7.2.1 to a 250-mL volumetric flask and fill to the mark with methylene chloride. Mix well, then remove 125 mL for the determination of the lipid content (Sec. 6.7.1). Transfer the remaining 125 mL of the extract, plus two 15-mL hexane/methylene chloride rinses of the volumetric flask, to a K-D apparatus equipped with a Snyder column. Quantitatively transfer all of the paper pulp extract to a K-D apparatus equipped with a Snyder column.

NOTE: As an option, a rotary evaporator may be used in place of the K-D apparatus for the concentration of the extracts.

7.2.3 Add a PTFE (or equivalent) boiling chip. Concentrate the extract in a water bath to an apparent volume of 10 mL. Remove the apparatus from the water bath and allow to cool for 5 minutes.

7.2.4 Add 50 mL hexane and a new boiling chip to the K-D flask. Concentrate in a water bath to an apparent volume of 5 mL. Remove the apparatus from the water bath and allow to cool for 5 minutes.

NOTE: The methylene chloride must have been completely removed before proceeding with the next step.

7.2.5 Remove and invert the Snyder column and rinse it into the K-D apparatus with two 1-mL portions of hexane. Decant the contents of the K-D apparatus and concentrator tube into a 125-mL separatory funnel. Rinse the K-D apparatus with two additional 5-mL portions of hexane and add the rinses to the funnel. Proceed with the cleanup according to the instructions in Sec. 7.5.1.1, but omit the procedures described in Secs. 7.5.1.2 and 7.5.1.3.

7.3 Extraction and purification of human adipose tissue

7.3.1 Human adipose tissue samples must be stored at a temperature of -20°C or lower from the time of collection until the time of analysis. The use of chlorinated materials during the collection of the samples must be avoided. Samples are handled with stainless steel forceps, spatulas, or scissors. All sample bottles (glass) are cleaned as specified in the note at the end of Sec. 4.3. PTFE-lined caps should be used.

NOTE: The specified storage temperature of -20°C is the maximum storage temperature permissible for adipose tissue samples. Lower storage temperatures are recommended.

7.3.2 Adipose tissue extraction

7.3.2.1 Weigh a 10-g portion of a frozen adipose tissue sample to the nearest 0.01 g, into a culture tube (2.2 x 15 cm).

NOTE: The sample size may be smaller, depending on availability. In such situations, the analyst is required to adjust the volume of the internal standard solution added to the sample to meet the fortification level stipulated in Table 1.

7.3.2.2 Allow the adipose tissue specimen to reach room temperature (up to 2 hours).

7.3.2.3 Add 10 mL of methylene chloride and 100 µL of the sample fortification solution. Homogenize the mixture for approximately 1 minute with a tissue homogenizer.

7.3.2.4 Allow the mixture to separate, then remove the methylene chloride extract from the residual solid material with a disposable pipet. Percolate the methylene chloride through a filter funnel containing a clean glass wool plug and 10 g of anhydrous sodium sulfate. Collect the dried extract in a graduated 100-mL volumetric flask.

7.3.2.5 Add a second 10 mL portion of methylene chloride to the sample and homogenize for 1 minute. Decant the solvent, dry it, and transfer it to the 100-mL volumetric flask (Sec. 7.3.2.4).

7.3.2.6 Rinse the culture tube with at least two additional portions of methylene chloride (10-mL each), and transfer the entire contents to the filter funnel containing the anhydrous sodium sulfate. Rinse the filter funnel and the anhydrous sodium sulfate contents with additional methylene chloride (20 to 40 mL) into the 100-mL flask. Discard the sodium sulfate.

7.3.2.7 Adjust the volume to the 100-mL mark with methylene chloride.

7.3.3 Adipose tissue lipid content determination

7.3.3.1 Preweigh a clean 1-dram (or metric equivalent) glass vial to the nearest 0.0001 g on an analytical balance tared to zero.

7.3.3.2 Accurately transfer 1.0 mL of the final extract (100 mL) from Sec. 7.3.2.7 to the vial. Reduce the volume of the extract on a water bath (50-60°C) by a gentle stream of purified nitrogen until an oily residue remains. Nitrogen evaporation is continued until a constant weight is achieved.

NOTE: When the sample size of the adipose tissue is smaller than 10 g, then the analyst may use a larger portion (up to 10 percent) of the extract defined in Sec. 7.3.2.7 for the lipid determination.

7.3.3.3 Accurately weigh the vial with the residue to the nearest 0.0001 g and calculate the weight of the lipid present in the vial based on the difference of the weights.

7.3.3.4 Calculate the percent lipid content of the original sample to the nearest 0.1 percent as shown below:

$$\% \text{ Lipid} = \frac{W_{lr} \times V_{ext}}{W_{at} \times V_{al}} \times 100$$

where:

W_{lr} = weight of the lipid residue to the nearest 0.0001 g calculated from Sec. 7.3.3.3,

V_{ext} = total volume (100 mL) of the extract in mL from Sec. 7.3.2.7,

W_{at} = weight of the original adipose tissue sample to the nearest 0.01 g from Sec. 7.3.2.1, and

V_{al} = volume of the aliquot of the final extract in mL used for the quantitative measure of the lipid residue (1.0 mL) from Sec. 7.3.3.2.

7.3.3.5 Record the weight of the lipid residue measured in Sec. 7.3.3.3 and the percent lipid content from Sec. 7.3.3.4.

7.3.4 Adipose tissue extract concentration

7.3.4.1 Quantitatively transfer the remaining extract from Sec. 7.3.3.2 (99.0 mL) to a 500-mL Erlenmeyer flask. Rinse the volumetric flask with 20 to 30 mL of additional methylene chloride to ensure quantitative transfer.

7.3.4.2 Concentrate the extract on a rotary evaporator and a water bath at 40°C until an oily residue remains.

7.3.5 Adipose tissue extract cleanup

7.3.5.1 Add 200 mL of hexane to the lipid residue in the 500-mL Erlenmeyer flask and swirl the flask to dissolve the residue.

7.3.5.2 Slowly add, with stirring, 100 g of 40 percent (w/w) sulfuric acid-impregnated silica gel. Stir with a magnetic stirrer for two hours at room temperature.

7.3.5.3 Allow the solid phase to settle, and decant the liquid through a filter funnel containing 10 g of anhydrous sodium sulfate on a glass wool plug, into another 500-mL Erlenmeyer flask.

7.3.5.4 Rinse the solid phase with two 50-mL portions of hexane. Stir each rinse for 15 minutes, decant, and dry as described under Sec. 7.3.5.3. Combine the hexane extracts from Sec. 7.3.5.3 with the rinses.

7.3.5.5 Rinse the sodium sulfate in the filter funnel with an additional 25 mL of hexane and combine this rinse with the hexane extracts from Sec. 7.3.5.4.

7.3.5.6 Prepare an acidic silica column as follows: Pack a 2 cm x 10 cm chromatographic column with a glass wool plug, add approximately 20 mL of hexane, add 1 g of silica gel and allow to settle, then add 4 g of 40 percent (w/w) sulfuric acid-impregnated silica gel and allow to settle. Elute the excess hexane from the column until the solvent level reaches the top of the chromatographic packing. Verify that the column does not have any air bubbles and channels.

7.3.5.7 Quantitatively transfer the hexane extract from the Erlenmeyer flask (Secs. 7.3.5.3 through 7.3.5.5) to the silica gel column reservoir. Allow the hexane extract to percolate through the column and collect the eluate in a 500-mL K-D apparatus.

7.3.5.8 Complete the elution by percolating 50 mL of hexane through the column into the K-D apparatus. Concentrate the eluate on a steam bath to about 5 mL. Use nitrogen evaporation to bring the final volume to about 100 µL.

NOTE: If the silica gel impregnated with 40 percent sulfuric acid is highly discolored throughout the length of the adsorbent bed, the cleaning procedure must be repeated beginning with Sec. 7.3.5.1.

7.3.5.9 The extract is ready for the column cleanups described in Secs. 7.5.2 through 7.5.3.6.

7.4 Extraction and purification of environmental and waste samples

7.4.1 Sludge/wet fuel oil

7.4.1.1 Extract aqueous sludge or wet fuel oil samples by refluxing a sample (e.g., 2 g) with 50 mL of toluene in a 125-mL flask fitted with a Dean-Stark water separator. Continue refluxing the sample until all the water is removed.

NOTE: If the sludge or fuel oil sample dissolves in toluene, treat it according to the instructions in Sec. 7.4.2 below. If the sludge sample originates from pulp (paper mills), treat it according to the instructions starting in Sec. 7.2, but without the addition of sodium sulfate.

7.4.1.2 Cool the sample, filter the toluene extract through a glass fiber filter, or equivalent, into a 100-mL round-bottom flask.

7.4.1.3 Rinse the filter with 10 mL of toluene and combine the extract with the rinse.

7.4.1.4 Concentrate the combined solutions to near dryness on a rotary evaporator at 50°C or using nitrogen evaporation. Proceed with Sec. 7.4.4.

7.4.2 Still bottom/oil

7.4.2.1 Extract still bottom or oil samples by mixing a sample portion (e.g., 1.0 g) with 10 mL of toluene in a small beaker and filtering the solution through a glass

fiber filter (or equivalent) into a 50-mL round-bottom flask. Rinse the beaker and filter with 10 mL of toluene.

7.4.2.2 Concentrate the combined toluene solutions to near dryness on a rotary evaporator at 50°C or using nitrogen evaporation. Proceed with Sec. 7.4.4.

7.4.3 Fly ash

NOTE: Because of the tendency of fly ash to "fly", all handling steps should be performed in a hood in order to minimize contamination.

7.4.3.1 Weigh about 10 g of fly ash to two decimal places and transfer to an extraction jar. Add 100 µL of the sample fortification solution (Sec. 5.8), diluted to 1 mL with acetone, to the sample. Add 150 mL of 1 M HCl to the fly ash sample. Seal the jar with the PTFE-lined screw cap and shake for 3 hours at room temperature.

7.4.3.2 Rinse a glass fiber filter with toluene, and filter the sample through the filter paper, placed in a Buchner funnel, into a 1-L flask. Wash the fly ash cake with approximately 500 mL of organic-free reagent water and dry the filter cake overnight at room temperature in a desiccator.

7.4.3.3 Add 10 g of anhydrous powdered sodium sulfate, mix thoroughly, let sit in a closed container for one hour, mix again, let sit for another hour, and mix again.

7.4.3.4 Place the sample and the filter paper into an extraction thimble, and extract in a Soxhlet extraction apparatus charged with 200 mL of toluene for 16 hours using a five cycle/hour schedule.

NOTE: As an option, a Soxhlet/Dean-Stark extractor system may be used, with toluene as the solvent. No sodium sulfate is added when using this option.

7.4.3.5 Cool and filter the toluene extract through a glass fiber filter into a 500-mL round-bottom flask. Rinse the filter with 10 mL of toluene. Add the rinse to the extract and concentrate the combined toluene solutions to near dryness on a rotary evaporator at 50°C or using nitrogen evaporation. Proceed with Sec. 7.4.4.

7.4.3.6 Alternatively, fly ash samples may be extracted with a toluene/acetic acid mixture using pressurized fluid extraction (PFE), as described in Method 3545. When using PFE, the HCl pretreatment in Sec. 7.4.3.1 may be omitted.

7.4.4 Transfer the concentrate to a 125-mL separatory funnel using 15 mL of hexane. Rinse the flask with two 5-mL portions of hexane and add the rinses to the funnel. Shake the combined solutions in the separatory funnel for two minutes with 50 mL of 5 percent sodium chloride solution, discard the aqueous layer, and proceed with Sec. 7.5.

7.4.5 Aqueous samples

7.4.5.1 Allow the sample to come to ambient temperature, then mark the water meniscus on the side of the 1-L sample bottle for later determination of the exact sample volume. Add the required acetone diluted sample fortification solution (Sec. 5.8).

7.4.5.2 When the sample is judged to contain 1 percent or more solids, the sample must be filtered through a glass fiber filter that has been rinsed with toluene. If the suspended solids content is too great to filter through the 0.45-µm filter, centrifuge the sample, decant, and then filter the aqueous phase.

NOTE: Paper mill effluent samples normally contain 0.02%-0.2% solids, and would not require filtration. However, for optimum analytical results, all paper mill effluent samples should be filtered, the isolated solids and filtrate extracted separately, and the extracts recombined.

7.4.5.3 Combine the solids from the centrifuge bottle(s) with the particulates on the filter and with the filter itself and proceed with the Soxhlet extraction as specified in Secs. 7.4.6.1 through 7.4.6.4.

NOTE: Pressurized fluid extraction has *not* been evaluated for the extraction of the particulate fraction.

Remove and invert the Snyder column and rinse it down into the K-D apparatus with two 1-mL portions of hexane.

7.4.5.4 Pour the aqueous filtrate into a 2-L separatory funnel. Add 60 mL of methylene chloride to the sample bottle, seal and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting.

7.4.5.5 Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation (e.g., glass stirring rod).

7.4.5.6 Collect the methylene chloride in a K-D apparatus (mounted with a 10-mL concentrator tube) by passing the sample extracts through a filter funnel packed with a glass wool plug and 5 g of anhydrous sodium sulfate.

NOTE: As an option, a rotary evaporator may be used in place of the K-D apparatus for the concentration of the extracts.

7.4.5.7 Repeat the extraction twice with fresh 60-mL portions of methylene chloride. After the third extraction, rinse the sodium sulfate with an additional 30 mL of methylene chloride to ensure quantitative transfer. Combine all extracts and the rinse in the K-D apparatus.

NOTE: A continuous liquid-liquid extractor may be used in place of a separatory funnel when experience with a sample from a given source indicates that a serious emulsion problem will result or an emulsion is encountered when using a separatory funnel. Add 60 mL of methylene chloride to the sample bottle, seal, and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the extractor. Repeat the rinse of the sample bottle with an additional 50- to 100-mL portion of methylene chloride and add the rinse to the extractor. Add 200 to 500 mL of methylene chloride to the distilling flask, add sufficient organic-free reagent water (Sec. 5.1) to ensure proper operation, and extract for 24 hours. Allow to cool, then detach the distilling

flask. Dry and concentrate the extract as described in Secs. 7.4.5.6 and 7.4.5.8 through 7.4.5.10. Proceed with Sec. 7.4.5.11.

7.4.5.8 Attach a Snyder column and concentrate the extract on a water bath until the apparent volume of the liquid is 5 mL. Remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

7.4.5.9 Remove the Snyder column, add 50 mL of hexane, add the concentrate obtained from the Soxhlet extraction of the suspended solids (Sec. 7.4.5.3), if applicable, re-attach the Snyder column, and concentrate to approximately 5 mL. Add a new boiling chip to the K-D apparatus before proceeding with the second concentration step.

7.4.5.10 Rinse the flask and the lower joint with two 5-mL portions of hexane and combine the rinses with the extract to give a final volume of about 15 mL.

7.4.5.11 Determine the original sample volume by filling the sample bottle to the mark with water and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL. Proceed with Sec. 7.5.

7.4.6 Soil/sediment

7.4.6.1 Add 10 g of anhydrous powdered sodium sulfate to the sample aliquot (10 g or less) and mix thoroughly with a stainless steel spatula. After breaking up any lumps, place the soil/sodium sulfate mixture in the Soxhlet apparatus on top of a glass wool plug (the use of an extraction thimble is optional).

NOTE: As an option, a Soxhlet/Dean-Stark extractor system may be used, with toluene as the solvent. No sodium sulfate is added when using this option.

7.4.6.2 Add 200 to 250 mL of toluene to the Soxhlet apparatus and reflux for 16 hours. The solvent must cycle completely through the system five times per hour.

NOTE: If the dried sample is not of free flowing consistency, more sodium sulfate must be added.

7.4.6.3 Cool and filter the extract through a glass fiber filter into a 500-mL round-bottom flask for evaporation of the toluene. Rinse the filter with 10 mL of toluene, and concentrate the combined fractions to near dryness on a rotary evaporator at 50°C. Remove the flask from the water bath and allow to cool for 5 minutes.

7.4.6.4 Transfer the residue to a 125-mL separatory funnel, using 15 mL of hexane. Rinse the flask with two additional portions of hexane, and add the rinses to the funnel. Proceed with Sec. 7.5.

7.4.6.5 Alternatively, soil/sediment samples may be extracted with toluene using pressurized fluid extraction (PFE), as described in Method 3545.

7.5 Cleanup

7.5.1 Acid-base washing

7.5.1.1 Partition the hexane extract against 40 mL of concentrated sulfuric acid. Shake for two minutes. Remove and discard the sulfuric acid layer (bottom). Repeat the acid washing until no color is visible in the acid layer (perform a maximum of four acid washings).

7.5.1.2 Omit this step for the fish sample extract. Partition the extract against 40 mL of 5 percent (w/v) aqueous sodium chloride. Shake for two minutes. Remove and discard the aqueous layer (bottom).

7.5.1.3 Omit this step for the fish sample extract. Partition the extract against 40 mL of 20 percent (w/v) aqueous potassium hydroxide (KOH). Shake for two minutes. Remove and discard the aqueous layer (bottom). Repeat the base washing until no color is visible in the bottom layer (perform a maximum of four base washings). Strong base (KOH) is known to degrade certain PCDDs/PCDFs, so contact time must be minimized.

7.5.1.4 Partition the extract against 40 mL of 5 percent (w/v) aqueous sodium chloride. Shake for two minutes. Remove and discard the aqueous layer (bottom). Dry the extract by pouring it through a filter funnel containing anhydrous sodium sulfate on a glass wool plug, and collect it in a 50-mL round-bottom flask. Rinse the funnel with the sodium sulfate with two 15-mL portions of hexane, add the rinses to the 50-mL flask, and concentrate the hexane solution to near dryness on a rotary evaporator (35°C water bath) or nitrogen evaporation, making sure all traces of toluene (when applicable) are removed.

7.5.2 Silica/alumina column cleanup

7.5.2.1 Pack a gravity column (glass, 30 cm x 10.5 mm), fitted with a PTFE stopcock, with of silica gel as follows: Insert a glass wool plug into the bottom of the column. Place 1 g of silica gel in the column and tap the column gently to settle the silica gel. Add 2 g of sodium hydroxide-impregnated silica gel, 4 g of sulfuric acid-impregnated silica gel, and 2 g of silica gel. Tap the column gently after each addition. A small positive pressure (5 psi) of clean nitrogen may be used if needed. Elute with 10 mL of hexane and close the stopcock just before exposure of the top layer of silica gel to air. Discard the eluate. Check the column for channeling. If channeling is observed, discard the column. Do not tap the wetted column.

7.5.2.2 Pack a gravity column (glass, 300 mm x 10.5 mm), fitted with a PTFE stopcock, with alumina as follows: Insert a glass wool plug into the bottom of the column. Add a 4 g layer of sodium sulfate. Add a 4 g layer of Woelm® Super 1 neutral alumina. Tap the top of the column gently. Woelm® Super 1 neutral alumina need not be activated or cleaned before use, but it should be stored in a sealed desiccator. Add a 4 g layer of anhydrous sodium sulfate to cover the alumina. Elute with 10 mL hexane and close the stopcock just before exposure of the sodium sulfate layer to air. Discard the eluate. Check the column for channeling. If channeling is observed, discard the column. Do not tap a wetted column.

NOTE: Alternatively, acidic alumina (Sec. 5.2.2) may be used in place of neutral alumina.

7.5.2.3 Dissolve the residue from Sec. 7.5.1.4 in 2 mL of hexane and apply the hexane solution to the top of the silica gel column. Rinse the flask with enough hexane (3-4 mL) to quantitatively transfer of the sample to the surface of the silica gel.

7.5.2.4 Elute the silica gel column with 90 mL of hexane, concentrate the eluate on a rotary evaporator (35°C water bath) to approximately 1 mL, and apply the concentrate to the top of the alumina column (Sec. 7.5.2.2). Rinse the rotary evaporator flask twice with 2 mL of hexane, and add the rinses to the top of the alumina column.

7.5.2.5 Add 20 mL of hexane to the alumina column and elute until the hexane level is just below the top of the sodium sulfate. Do not discard the eluted hexane, but collect it in a separate flask and store it for later use, as it may be useful in determining where the labeled analytes are being lost if recoveries are not satisfactory.

7.5.2.6 Add 15 mL of 60 percent methylene chloride in hexane (v/v) to the alumina column and collect the eluate in a conical-shaped (15-mL) concentration tube. With a carefully regulated stream of nitrogen, concentrate the 60 percent methylene chloride/hexane fraction to about 2 mL.

7.5.3 Carbon column cleanup

7.5.3.1 Thoroughly mix 9.0 g of activated carbon (Sec. 5.2.7) and 41.0 g of Celite 545® to produce an 18% w/w mixture. Activate the mixture at 130°C for 6 hours, and store in a desiccator.

NOTE: Check each new batch of the carbon/Celite mixture by adding 50 µL of the calibration verification solution to 950 µL of hexane. Take this solution through the carbon column cleanup step, concentrate to 50 µL and analyze. If the recovery of any of the analytes is less than 80%, this batch of carbon/Celite mixture may not be used.

7.5.3.2 Prepare a 4-inch long glass column by cutting off each end of a 10-mL disposable serological pipet. Fire polish both ends and flare if desired. Insert a glass wool plug at one end of the column, and pack it with 1 g of the carbon/Celite mixture. Insert an additional glass wool plug in the other end.

CAUTION: It is very important that the column be packed properly to ensure that carbon fines are not carried into the eluate. PCDDs/PCDFs will adhere to the carbon fines and greatly reduce recovery. If carbon fines are carried into the eluate, filter the eluate, using a 0.7-µm filter (pre-rinsed with toluene), then proceed to Sec. 7.5.3.6.

7.5.3.3 Rinse the column with:

4 mL of toluene
2 mL of methylene chloride/methanol/toluene (75:20:5 v/v)
4 mL of cyclohexane/methylene chloride (50:50 v/v)

The flow rate should be less than 0.5 mL/min. Discard all the column rinsates.

7.5.3.4 While the column is still wet, transfer the concentrated eluate from Sec. 7.5.2.6 to the prepared carbon column. Rinse the eluate container with two 0.5-mL portions of hexane and transfer the rinses to the carbon column. Elute the column with the following sequence of solvents.

10 mL of cyclohexane/methylene chloride (50:50 v/v).
5 mL of methylene chloride/methanol/toluene (75:20:5 v/v).

NOTE: The above two eluates may be collected and combined, and used as a check on column efficiency.

7.5.3.5 Once the solvents have eluted through the column, turn the column over, and elute the PCDD/PCDF fraction with 20 mL of toluene, and collect the eluate.

7.5.3.6 Concentrate the toluene fraction to about 1 mL on a rotary evaporator by using a water bath at 50°C or with nitrogen evaporation. Carefully transfer the concentrate into a 1-mL minivial and, again at elevated temperature (50°C), reduce the volume to about 100 µL using a stream of nitrogen and a sand bath. Rinse the rotary evaporator flask three times with 300 µL of a solution of 1 percent toluene in methylene chloride, and add the rinses to the concentrate. Add 10 µL of the nonane recovery standard solution (Sec. 5.9) for soil, sediment, water, fish, paper pulp and adipose tissue samples, or 50 µL of the recovery standard solution for sludge, still bottom and fly ash samples. Store the sample at room temperature in the dark.

7.6 Chromatographic/mass spectrometric conditions and data acquisition parameters

7.6.1 Gas chromatograph operating conditions

Column coating:	DB-5
Film thickness:	0.25 µm
Column dimension:	60-m x 0.32 mm
Injector temperature:	270°C
Splitless valve time:	45 s
Interface temperature:	Function of the final temperature
Temperature program	
Initial temperature:	200°C
Initial hold time:	2 min
1st temp. ramp:	5 °C/min to 220°C, hold for 16 minutes
2nd temp. ramp:	5 °C/min to 235°C, hold for 7 minutes
3rd temp. ramp:	5 °C/min to 330°C, hold for 5 minutes

Total time: 60 min

7.6.2 Mass spectrometer

7.6.2.1 The mass spectrometer must be operated in a selected ion monitoring (SIM) mode with a total cycle time (including the voltage reset time) of one second or less (Sec. 7.6.3.1). At a minimum, the ions listed in Table 6 for each of the five SIM descriptors must be monitored. Note that with the exception of the last descriptor (OCDD/OCDF), all descriptors contain 10 ions. The selection (Table 6) of the molecular ions M and M+2 for ^{13}C-HxCDF and ^{13}C-HpCDF rather than M+2 and M+4 (for consistency) was made to eliminate, even under high-resolution mass spectrometric conditions, interferences occurring in these two ion channels for samples containing high levels of native HxCDDs and HpCDDs. It is important to maintain the same set of ions for both calibration and sample extract analyses. The selection of the lock-mass ion is left to the performing laboratory.

NOTE: At the option of the analyst, the tetra- and pentachlorinated dioxins and furans may be combined into a single descriptor.

7.6.2.2 The recommended mass spectrometer tuning conditions are based on the groups of monitored ions shown in Table 6. By using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10,000 (10 percent valley) at m/z 304.9824 (PFK) or any other reference signal close to m/z 303.9016 (from TCDF). By using peak matching conditions and the aforementioned PFK reference peak, verify that the exact mass of m/z 380.9760 (PFK) is within 5 ppm of the required value. Note that the selection of the low- and high-mass ions must be such that they provide the largest voltage jump performed in any of the five mass descriptors (Table 6).

7.6.3 Data acquisition

7.6.3.1 The total cycle time for data acquisition must be $\leq$ 1 second. The total cycle time includes the sum of all the dwell times and voltage reset times.

7.6.3.2 Acquire SIM data for all of the ions in the descriptors in Table 6.

7.7 Calibration

7.7.1 Initial calibration

Initial calibration is required before any samples are analyzed for PCDDs and PCDFs and must meet the acceptance criteria in Sec. 7.7.2. Initial calibration is also required if any routine calibration (Sec. 7.7.3) does not meet the required criteria listed in Sec. 7.7.2.

7.7.1.1 All five high-resolution concentration calibration solutions listed in Table 5 must be used for the initial calibration.

7.7.1.2 Tune the instrument with PFK, as described in Sec. 7.6.2.2.

7.7.1.3 Inject 2 µL of the GC column performance check solution (Sec. 5.7) and acquire SIM mass spectral data as described earlier in Sec. 7.6.2. The total cycle time must be $\leq$ 1 second. The laboratory must not perform any further analysis until it is demonstrated and documented that the criteria listed in Sec. 8.2.1 were met.

7.7.1.4 By using the same GC (Sec. 7.6.1) and MS (Sec. 7.6.2) conditions that produced acceptable results with the column performance check solution, analyze a 2-µL portion of each of the five concentration calibration solutions once with the following mass spectrometer operating parameters.

7.7.1.4.1 The ratio of integrated ion current for the ions appearing in Table 8 (homologous series quantitation ions) must be within the indicated control limits (set for each homologous series) for all unlabeled calibration standards in Table 5.

7.7.1.4.2 The ratio of integrated ion current for the ions belonging to the carbon-labeled internal and recovery standards (Table 5) must be within the control limits stipulated in Table 8.

NOTE: Secs. 7.7.1.4.1 and 7.7.1.4.2 require that 17 ion ratios from Sec. 7.7.1.4.1 and 11 ion ratios from Sec. 7.7.1.4.2 be within the specified control limits simultaneously in one run. It is the laboratory's responsibility to take corrective action if the ion abundance ratios are outside the limits.

7.7.1.4.3 For each selected ion current profile (SICP) and for each GC signal corresponding to the elution of a target analyte and of its labeled standards, the signal-to-noise ratio (S/N) must be better than or equal to 10. Measurement of S/N is required for any GC peak that has an apparent S/N of less than 5:1. The result of the calculation must appear on the SICP above the GC peak in question.

7.7.1.4.4 Referring to Table 9, calculate the 17 relative response factors (RF) for unlabeled target analytes [RF(n); n = 1 to 17] relative to their appropriate internal standards (Table 5) and the nine RFs for the $^{13}C_{12}$-labeled internal standards [RF(is); is = 18 to 26)] relative to the two recovery standards (Table 5) according to the following formulae:

$$RF_n = \frac{(A_n^1 + A_n^2) \times Q_{is}}{(A_{is}^1 + A_{is}^2) \times Q_n}$$

$$RF_{is} = \frac{(A_{is}^1 + A_{is}^2) \times Q_{rs}}{(A_{rs}^1 + A_{rs}^2) \times Q_{is}}$$

where:

A_n^1 and A_n^2 = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 9) for unlabeled PCDDs/PCDFs,

A_{is}^1 and A_{is}^2 = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 9) for the labeled internal standards,

A_{rs}^1 and A_{rs}^2 = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 9) for the labeled recovery standards,

Q_{is} = quantity of the internal standard injected (pg),

Q_{rs} = quantity of the recovery standard injected (pg), and

Q_n = quantity of the unlabeled PCDD/PCDF analyte injected (pg).

The RF_n and RF_{is} are dimensionless quantities; the units used to express Q_{is}, Q_{rs} and Q_n must be the same.

7.7.1.4.5 Calculate the $\overline{RF}$ values and their respective percent relative standard deviations (%RSD) for the five calibration solutions:

$$\overline{RF}_n = \frac{\sum_{j=1}^{5} RF_{n(j)}}{5}$$

where n represents a particular PCDD/PCDF (2,3,7,8-substituted) congener (n = 1 to 17; Table 9), and j is the injection number (or calibration solution number; j = 1 to 5).

7.7.1.4.6 The relative response factors to be used for the determination of the concentration of total isomers in a homologous series (Table 9) are calculated as follows:

7.7.1.4.6.1 For congeners that belong to a homologous series containing only one isomer (e.g., OCDD and OCDF) or only one 2,3,7,8-substituted isomer (Table 4; TCDD, PeCDD, HpCDD, and TCDF), the $\overline{RF}$ used will be the same as the $\overline{RF}$ determined in Sec. 7.7.1.4.5.

NOTE: The calibration solutions do not contain $^{13}C_{12}$-OCDF as an internal standard. This is because a minimum resolving power of 12,000 is required to resolve the $[M+6]^+$ ion of $^{13}C_{12}$-OCDF from the $[M+2]^+$ ion of OCDD (and $[M+4]^+$ from $^{13}C_{12}$-OCDF with $[M]^+$ of OCDD). Therefore, the $\overline{RF}$ or OCDF is calculated relative to $^{13}C_{12}$-OCDD.

7.7.1.4.6.2 For congeners that belong to a homologous series containing more than one 2,3,7,8-substituted isomer (Table 4), the $\overline{RF}$ used for those homologous series will be the mean of the RFs calculated for all individual 2,3,7,8-substituted congeners using the equation below:

$$\overline{RF}_k = \frac{\sum_{n=1}^{t} RF_n}{t}$$

where:

k = 27 to 30 (Table 9), with 27 = PeCDF; 28 = HxCDF; 29 = HxCDD; and 30 = HpCDF,

t = total number of 2,3,7,8-substituted isomers present in the calibration solutions (Table 5) for each homologous series (e.g., two for PeCDF, four for HxCDF, three for HxCDD, two for HpCDF).

NOTE: Presumably, the HRGC/HRMS response factors of different isomers within a homologous series are different. However, this analytical protocol will make the assumption that the HRGC/HRMS responses of all isomers in a homologous series that do not have the 2,3,7,8-substitution pattern are the same as the responses of one or more of the 2,3,7,8-substituted isomer(s) in that homologous series.

7.7.1.4.7 Relative response factors (RF_m) to be used for the determination of the percent recoveries for the nine internal standards are calculated as follows:

$$RF_m = \frac{A_{is}^m \times Q_{rs}}{Q_{is}^m \times A_{rs}}$$

$$\overline{RF}_m = \frac{\sum_{j=1}^{5} RF_{mj}}{5}$$

where:

m = 18 to 26 (congener type) and j = 1 to 5 (injection number),

A_{is}^m = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 9) for a given internal standard (m = 18 to 26),

A_{rs} = sum of the integrated ion abundances of the quantitation ions (Tables 6 and 9) for the appropriate recovery standard (see Table 5, footnotes),

Q_{rs}, Q_{is}^m = quantities of, respectively, the recovery standard (rs) and a particular internal standard (is = m) injected (pg),

RF_m = relative response factor of a particular internal standard (m) relative to an appropriate recovery standard, as determined from one injection, and

$\overline{RF}_m$ = calculated mean relative response factor of a particular internal standard (m) relative to an appropriate recovery standard, as determined from the five initial calibration injections (j).

7.7.2 Criteria for acceptable calibration - The criteria listed below for acceptable calibration must be met before sample analyses are performed.

7.7.2.1 The percent relative standard deviations for the mean response factors ($\overline{RF}_n$ and $\overline{RF}_m$) from the 17 unlabeled standards must not exceed ± 20 percent, and those for the nine labeled reference compounds must not exceed ± 30 percent.

7.7.2.2 The S/N for the GC signals present in every SICP (including the ones for the labeled standards) must be ≥ 10.

7.7.2.3 The ion abundance ratios (Table 8) must be within the specified control limits.

NOTE: If the criterion for acceptable calibration listed in Sec. 7.7.2.1 is met, the analyte-specific RF can then be considered independent of the analyte quantity for the calibration concentration range. The mean RFs will be used for all calculations until the routine calibration criteria (Sec. 7.7.4) are no longer met. At such time, new $\overline{RF}$ values will be calculated from a new set of injections of the calibration solutions.

7.7.3 Routine calibration (continuing calibration check) - Routine calibrations must be performed at the beginning of a 12-hour period, after successful mass resolution and GC resolution performance checks. A routine calibration is also required at the end of a 12-hour shift. Inject 2 µL of the concentration calibration solution HRCC-3 standard (Table 5). By using the same HRGC/HRMS conditions as used in Secs. 7.6.1 and 7.6.2, determine and document an acceptable calibration as provided in Sec. 7.7.4.

7.7.4 Criteria for acceptable routine calibration - The following criteria must be met before further analysis is performed.

7.7.4.1 The measured RFs [RF_n for the unlabeled standards] obtained during the routine calibration runs must be within ± 20 percent of the mean values established during the initial calibration (Sec. 7.7.1.4.5).

7.7.4.2 The measured RFs [RF_m for the labeled standards] obtained during the routine calibration runs must be within ± 30 percent of the mean values established during the initial calibration (Sec. 7.7.1.4.7).

7.7.4.3 The ion abundance ratios (Table 8) must be within the allowed control limits.

7.7.4.4 If either one of the criteria in Secs. 7.7.4.1 and 7.7.4.2 is not satisfied, repeat one more time. If these criteria are still not satisfied, the entire routine calibration process (Sec. 7.7.1) must be reviewed. If the ion abundance ratio criterion (Sec. 7.7.4.3) is not satisfied, refer to the note in Sec. 7.7.1.4.2 for resolution.

NOTE: An initial calibration must be carried out whenever the HRCC-3, the sample fortification, or the recovery standard solution is replaced by a new solution from a different lot.

7.8 Analysis

7.8.1 Remove the sample or blank extract (from Sec. 7.5.3.6) from storage. With a stream of dry, purified nitrogen, reduce the extract volume to 10 µL to 50 µL.

NOTE: A final volume of 20 µL or more should be used whenever possible. A 10-µL final volume is difficult to handle, and injection of 2 µL out of 10 µL leaves little sample for confirmations and repeat injections, and for archiving.

7.8.2 Inject a 2-µL aliquot of the extract into the GC, operated under the conditions that have been established to produce acceptable results with the performance check solution (Secs. 7.6.1 and 7.6.2).

7.8.3 Acquire SIM data according to Secs. 7.6.2 and 7.6.3. Use the same acquisition and mass spectrometer operating conditions previously used to determine the relative response factors (Secs. 7.7.1.4.4 through 7.7.1.4.7). Ions characteristic of polychlorinated diphenyl ethers are included in the descriptors listed in Table 6.

NOTE: The acquisition period must at least encompass the PCDD/PCDF overall retention time window previously determined (Sec. 8.2.1.3). Selected ion current profiles (SICP) for the lock-mass ions (one per mass descriptor) must also be recorded and included in the data package. These SICPs must be true representations of the evolution of the lock-mass ions amplitudes during the HRGC/HRMS run (see Sec. 8.2.2 for the proper level of reference compound to be metered into the ion chamber.) The analyst may be required to monitor a PFK ion, not as a lock-mass, but as a regular ion, in order to meet this requirement. It is recommended to examine the lock-mass ion SICP for obvious basic sensitivity and stability changes of the instrument during the GC/MS run that could affect the measurements.

7.8.4 Identification criteria - For a gas chromatographic peak to be identified as a PCDD or PCDF, it must meet all of the following criteria:

7.8.4.1 Retention times

7.8.4.1.1 For 2,3,7,8-substituted congeners, which have an isotopically-labeled internal or recovery standard present in the sample extract (this represents a total of 10 congeners including OCDD; Tables 2 and 3), the retention time (RRT; at maximum peak height) of the sample components (i.e., the two ions used for quantitation purposes listed in Table 6) must be within -1 to +3 seconds of the isotopically-labeled standard.

7.8.4.1.2 For 2,3,7,8-substituted compounds that do not have an isotopically-labeled internal standard present in the sample extract (this represents a total of six congeners; Table 3), the retention time must fall within 0.005 retention time units of the relative retention times measured in the routine calibration. Identification of OCDF is based on its retention time relative to $^{13}C_{12}$-OCDD as determined from the daily routine calibration results.

7.8.4.1.3 For non-2,3,7,8-substituted compounds (tetra through octa; totaling 119 congeners), the retention time must be within the corresponding homologous retention time windows established by analyzing the column performance check solution (Sec. 8.1.3).

7.8.4.1.4 The ion current responses for both ions used for quantitative purposes (e.g., for TCDDs: m/z 319.8965 and 321.8936) must reach maximum simultaneously (± 2 seconds).

7.8.4.1.5 The ion current responses for both ions used for the labeled standards (e.g., for $^{13}C_{12}$-TCDD: m/z 331.9368 and m/z 333.9339) must reach maximum simultaneously (± 2 seconds).

NOTE: The analyst is required to verify the presence of 1,2,8,9-TCDD and 1,3,4,6,8-PeCDF (Sec. 8.1.3) in the SICPs of the daily performance checks. Should either one compound be missing, the analyst is required to take corrective action as it may indicate a potential problem with the ability to detect all the PCDDs/PCDFs.

7.8.4.2 Ion abundance ratios

The integrated ion currents for the two ions used for quantitation purposes must have a ratio between the lower and upper limits established for the homologous series to which the peak is assigned. See Secs. 7.7.1.4.1 and 7.7.1.4.2 and Table 8 for details.

7.8.4.3 Signal-to-noise ratio

All ion current intensities must be $\geq$ 2.5 times noise level for positive identification of an unlabeled PCDD/PCDF compound or a group of coeluting isomers. Figure 6 describes the procedure to be followed for the determination of the S/N. Labeled analytes must have a S/N $\geq$ 10.

7.8.4.4 Polychlorinated diphenyl ether interferences

In addition to the above criteria, the identification of a GC peak as a PCDF can only be made if no signal having a S/N $\geq$ 2.5 is detected at the same retention time (± 2 seconds) in the corresponding polychlorinated diphenyl ether (PCDPE, Table 6) channel.

7.9 Calculations

7.9.1 For gas chromatographic peaks that have met the criteria outlined in Sec. 7.8.4, calculate the concentration of the PCDD or PCDF compounds using the formula:

$$C_x = \frac{A_x \times Q_{is}}{A_{is} \times W \times \overline{RF}_n}$$

where:

C_x = concentration of unlabeled PCDD/PCDF congeners (or group of coeluting isomers within an homologous series) in pg/g,

A_x = sum of the integrated ion abundances of the quantitation ions (Table 6) for unlabeled PCDDs/PCDFs,

A_{is} = sum of the integrated ion abundances of the quantitation ions (Table 6) for the labeled internal standards,

Q_{is} = quantity, in pg, of the internal standard added to the sample before extraction,

W = weight, in g, of the sample (solid or organic liquid), or volume in mL of an aqueous sample, and

$\overline{RF}_n$ = calculated mean relative response factor for the analyte ($\overline{RF}_n$ with n = 1 to 17; Sec. 7.7.1.4.5).

If the analyte is identified as one of the 2,3,7,8-substituted PCDDs or PCDFs, $\overline{RF}_n$ is the value calculated using the equation in Sec. 7.7.1.4.5. However, if it is a non-2,3,7,8-substituted congener, the $\overline{RF}(k)$ value is the one calculated using the equation in Sec. 7.7.1.4.6.2. (RF_k, for k = 27 to 30).

7.9.2 Calculate the percent recovery of the nine internal standards measured in the sample extract, using the formula:

$$\text{percent recovery} = \frac{A_{is} \times Q_{rs}}{Q_{is} \times A_{rs} \times \overline{RF}_n} \times 100$$

where:

A_{is} = sum of the integrated ion abundances of the quantitation ions (Table 6) for the labeled internal standard,

A_{rs} = sum of the integrated ion abundances of the quantitation ions (Table 6) for the labeled recovery standard; the selection of the recovery standard depends on the type of congeners (see Table 5, footnotes),

Q_{is} = quantity, in pg, of the internal standard added to the sample before extraction,

Q_{rs} = quantity, in pg, of the recovery standard added to the cleaned-up sample residue before HRGC/HRMS analysis, and

$\overline{RF}_m$ = calculated mean relative response factor for the labeled internal standard relative to the appropriate (see Table 5, footnotes) recovery standard. This represents the mean obtained in Sec. 7.7.1.4.7 ($\overline{RF}_m$ with m = 18 to 26).

NOTE: For human adipose tissue, adjust the percent recoveries by adding 1 percent to the calculated value to compensate for the 1 percent of the extract diverted for the lipid determination.

7.9.3 If the concentration in the final extract of any of the fifteen 2,3,7,8-substituted PCDD/PCDF compounds (Table 3) exceeds the upper method calibration limits (MCL) listed in Table 1 (e.g., 200 pg/µL for TCDD in soil), the linear range of response versus concentration may have been exceeded, and a second analysis of the sample (using a one-tenth aliquot) should be undertaken. The volumes of the internal and recovery standard solutions should remain the same as described for the sample preparation (Secs. 7.1 to 7.9.3).

If a smaller sample size would not be representative of the entire sample, one of the following options is recommended:

(1) Re-extract an additional aliquot of sufficient size to insure that it is representative of the entire sample. Spike it with a higher concentration of internal standard. Prior to GC/MS analysis, dilute the sample so that it has a concentration of internal standard equivalent to that present in the calibration standard. Then, analyze the diluted extract.

(2) Re-extract an additional aliquot of sufficient size to insure that it is representative of the entire sample. Spike it with a higher concentration of internal standard. Immediately following extraction, transfer the sample to a volumetric flask and dilute to known volume. Remove an appropriate aliquot and proceed with cleanup and analysis.

(3) Use the original analysis data to quantitate the internal standard recoveries. Respike the original extract (note that no additional cleanup is necessary) with 100 times the usual quantity of internal standards. Dilute the re-spiked extract by a factor of 100. Reanalyze the diluted sample using the internal standard recoveries calculated from the initial analysis to correct the results for losses during isolation and cleanup.

7.9.4 The total concentration for each homologous series of PCDD and PCDF is calculated by summing up the concentrations of all positively identified isomers of each homologous series. Therefore, the total should also include the 2,3,7,8-substituted congeners. The total number of GC signals included in the homologous total concentration value must be specified in the report. If an isomer is not detected, use zero (0) in this calculation.

7.9.5 Sample specific estimated detection limit - The sample specific estimated detection limit (EDL) is the concentration of a given analyte required to produce a signal with a peak height of at least 2.5 times the background signal level. An EDL is calculated for each 2,3,7,8-substituted congener that is not identified, regardless of whether or not other non-2,3,7,8-substituted isomers are present. Two methods of calculation can be used, as follows, depending on the type of response produced during the analysis of a particular sample.

7.9.5.1 Samples giving a response for both quantitation ions (Tables 6 and 9) that is less than 2.5 times the background level.

Use the expression below to calculate an EDL for each 2,3,7,8-substituted PCDD/PCDF that does <u>not</u> have a response with S/N $\geq$ 2.5). The background level is determined by measuring the range of the noise (peak to peak) for the two quantitation ions (Table 6) of a particular 2,3,7,8-substituted isomer within an homologous series, in the region of the SICP trace corresponding to the elution of the internal standard (if the congener possesses an internal standard) or in the region of the SICP where the congener is expected to elute by comparison with the routine calibration data (for those

congeners that do not have a ^{13}C-labeled standard), multiplying that noise height by 2.5, and relating the product to an estimated concentration that would produce that peak height. Use the formula:

$$EDL = \frac{2.5 \times H_x \times Q_{is}}{H_{is} \times W \times \overline{RF}_n}$$

where:

EDL = estimated detection limit for homologous 2,3,7,8-substituted PCDDs/PCDFs.

H_x = sum of the height of the noise level for each quantitation ion (Table 6) for the unlabeled PCDDs/PCDFs, measured as shown in Figure 6.

H_{is} = sum of the height of the noise level for each quantitation ion (Table 6) for the labeled internal standard, measured as shown in Figure 6.

W, $\overline{RF}_n$, and Q_{is} retain the same meanings as defined in Sec. 7.9.1.

7.9.5.2 Estimated maximum possible concentration - An estimated maximum possible concentration (EMPC) is calculated for 2,3,7,8-substituted isomers that are characterized by a response with an S/N of at least 2.5 for both the quantitation ions, and meet all of the identification criteria in Sec. 7.8.4 except the ion abundance ratio criteria or when a peak representing a PCDPE has been detected. An EMPC is a worst-case estimate of the concentration. Calculate the EMPC according to the expression shown in Sec. 7.9.1.

7.9.6 The relative percent difference (RPD) of any duplicate sample results are calculated as follows:

$$RPD = \frac{|S_1 - S_2|}{\frac{S_1 + S_2}{2}} \times 100$$

where S_1 and S_2 represent sample and duplicate sample results.

7.9.7 The 2,3,7,8-TCDD toxicity equivalents (TE) of PCDDs and PCDFs present in the sample are calculated, if requested by the data user, according to the method recommended by the Chlorinated Dioxins Workgroup (CDWG) of the EPA and the Center for Disease Control (CDC). This method assigns a 2,3,7,8-TCDD toxicity equivalency factor (TEF) to each of the fifteen 2,3,7,8-substituted PCDDs and PCDFs (Table 3) and to OCDD and OCDF, as shown in Table 10. The 2,3,7,8-TCDD equivalent of the PCDDs and PCDFs present in the sample is calculated by summing the TEF times their concentration for each of the compounds or groups of compounds listed in Table 10. The exclusion of other homologous series such as mono-, di-, and tri- chlorinated dibenzodioxins and dibenzofurans does not mean that they are non-toxic. However, their toxicity, as known at this time, is much lower than the toxicity of the compounds listed in Table 10. The above procedure for calculating the 2,3,7,8-TCDD toxicity equivalents is not claimed by the CDWG to be based on a thoroughly

established scientific foundation. The procedure, rather, represents a "consensus recommendation on science policy." Since the procedure may be changed in the future, reporting requirements for PCDD and PCDF data would still include the reporting of the analyte concentrations of the PCDD/PCDF congener as calculated in Secs. 7.9.1 and 7.9.4.

7.9.8 Two GC column TEF determination

7.9.8.1 The concentration of 2,3,7,8-TCDD (see note below), is calculated from the analysis of the sample extract on the 60-m DB-5 (or equivalent) fused-silica capillary column. The experimental conditions remain the same as the conditions described previously in Sec. 7.8, and the calculations are performed as outlined in Sec. 7.9. The chromatographic separation between the 2,3,7,8-TCDD and its close eluters (1,2,3,7/1,2,3,8-TCDD and 1,2,3,9-TCDD) must be equal or less than 25 percent valley.

7.9.8.2 The concentration of the 2,3,7,8-TCDF is obtained from the analysis of the sample extract on the 30-m DB-225 (or equivalent) fused-silica capillary column. However, the GC/MS conditions must be altered so that: (1) only the first three descriptors (i.e., tetra-, penta-, and hexachlorinated congeners) of Table 6 are used; and (2) the switching time between descriptor 2 (pentachlorinated congeners) and descriptor 3 (hexachlorinated congeners) takes place following the elution of $^{13}C_{12}$-1,2,3,7,8-PeCDD. The concentration calculations are performed as outlined in Sec. 7.9. The chromatographic separation between the 2,3,7,8-TCDF and its close eluters (2,3,4,7-TCDF and 1,2,3,9-TCDF) must be equal or less than 25 percent valley.

NOTE: The confirmation and quantitation of 2,3,7,8-TCDD (Sec. 7.9.7.1.1) may be accomplished on the SP-2330 GC column instead of the DB-5 column, provided the criteria listed in Sec. 8.2.1 are met and the requirements described in Sec. 8.3.2 are followed.

7.9.8.3 For a gas chromatographic peak to be identified as a 2,3,7,8-substituted PCDD/PCDF congener, it must meet the ion abundance and signal-to-noise ratio criteria listed in Secs. 7.8.4.2 and 7.8.4.3, respectively. In addition, the retention time identification criterion described in Sec. 7.8.4.1.1 applies here for congeners for which a carbon-labeled analogue is available in the sample extract. However, the relative retention time (RRT) of the 2,3,7,8-substituted congeners for which no carbon-labeled analogues are available must fall within 0.006 units of the carbon-labeled standard RRT. Experimentally, this is accomplished by using the attributions described in Table 11 and the results from the routine calibration run on the SP-2330 column.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One for specific quality control (QC) procedures. Quality control to validate sample extraction is covered in Method 3500. If extract cleanup was performed, follow the QC in Method 3600 and in the specific cleanup method.

8.2 System performance criteria - System performance criteria are presented below. The laboratory may use the recommended GC column described in Sec. 4.2. It must be documented that all applicable system performance criteria (specified in Secs. 8.2.1 and 8.2.2) were met before analysis of any sample is performed. Sec. 7.6.1 provides recommended GC conditions that can be used to satisfy the required criteria. Figure 3 provides a typical 12-hour analysis sequence, whereby the response factors and mass spectrometer resolving power checks must be performed at the

beginning and the end of each 12-hour period of operation. A GC column performance check is only required at the beginning of each 12-hour period during which samples are analyzed. An HRGC/HRMS method blank run is required between a calibration run and the first sample run. The same method blank extract may thus be analyzed more than once if the number of samples within a batch requires more than 12 hours of analyses.

8.2.1 GC column performance

8.2.1.1 Inject 2 µL (Sec. 4.1.1) of the column performance check solution (Sec. 5.7) and acquire selected ion monitoring (SIM) data as described in Sec. 7.6.2 within a total cycle time of $\leq$ 1 second (Sec. 7.6.3.1).

8.2.1.2 The chromatographic separation between 2,3,7,8-TCDD and the peaks representing any other unlabeled TCDD isomers must be resolved with a valley of $\leq$ 25 percent (Figure 4), where:

$$\text{Valley percent} = (x/y) \times (100)$$

x = measured as in Figure 4 from the 2,3,7,8-closest TCDD eluting isomer
y = the peak height of 2,3,7,8-TCDD

It is the responsibility of the laboratory to verify the conditions suitable for the appropriate resolution of 2,3,7,8-TCDD from all other TCDD isomers. The GC column performance check solution also contains the known first and last PCDD/PCDF eluters under the conditions specified in this protocol. Their retention times are used to determine the eight homologue retention time windows that are used for qualitative (Sec. 7.8.4.1) and quantitative purposes. All peaks (that includes $^{13}C_{12}$-2,3,7,8-TCDD) should be labeled and identified on the chromatograms. Furthermore, all first eluters of a homologous series should be labeled with the letter F, and all last eluters of a homologous series should be labeled with the letter L (Figure 4 shows an example of peak labeling for TCDD isomers). Any individual selected ion current profile (SICP) (for the tetras, this would be the SICP for m/z 322 and m/z 304) or the reconstructed homologue ion current (for the tetras, this would correspond to m/z 320 + m/z 322 + m/z 304 + m/z 306) constitutes an acceptable form of data presentation. An SICP for the labeled compounds (e.g., m/z 334 for labeled TCDD) is also required.

8.2.1.3 The retention times for the switching of SIM ions characteristic of one homologous series to the next higher homologous series must be indicated in the SICP. Accurate switching at the appropriate times is absolutely necessary for accurate monitoring of these compounds. Allowable tolerance on the daily verification with the GC performance check solution should be better than 10 seconds for the absolute retention times of all the components of the mixture. Particular caution should be exercised for the switching time between the last tetrachlorinated congener (i.e., 1,2,8,9-TCDD) and the first pentachlorinated congener (i.e., 1,3,4,6,8-PeCDF), as these two compounds elute within 15 seconds of each other on the 60-m DB-5 column. A laboratory with a GC/MS system that is not capable of detecting both congeners (1,2,8,9-TCDD and 1,3,4,6,8-PeCDF) within one analysis must take corrective action. If the recommended column is not used, then the first-and last-eluting isomer of each homologue must be determined experimentally on the column which is used, and the appropriate isomers must then be used for window definition and switching times.

8.2.2 Mass spectrometer performance

8.2.2.1 The mass spectrometer must be operated in the electron ionization mode. A static resolving power of at least 10,000 (10 percent valley definition) must be demonstrated at appropriate masses before any analysis is performed (Sec. 7.8). Static resolving power checks must be performed at the beginning and at the end of each 12-hour period of operation. However, it is recommended that a check of the static resolution be made and documented before and after each analysis. Corrective action must be implemented whenever the resolving power does not meet the requirement.

8.2.2.2 Chromatography time for PCDDs and PCDFs exceeds the long term mass stability of the mass spectrometer. Because the instrument is operated in the high-resolution mode, mass drifts of a few ppm (e.g., 5 ppm in mass) can have serious adverse effects on instrument performance. Therefore, a mass drift correction is mandatory. To that effect, it is recommended to select a lock-mass ion from the reference compound (PFK is recommended) used for tuning the mass spectrometer. The selection of the lock-mass ion is dependent on the masses of the ions monitored within each descriptor. Table 6 offers some suggestions for the lock-mass ions. However, an acceptable lock-mass ion at any mass between the lightest and heaviest ion in each descriptor can be used to monitor and correct mass drifts. The level of the reference compound (PFK) metered into the ion chamber during HRGC/HRMS analyses should be adjusted so that the amplitude of the most intense selected lock-mass ion signal (regardless of the descriptor number) does not exceed 10 percent of the full scale deflection for a given set of detector parameters. Under those conditions, sensitivity changes that might occur during the analysis can be more effectively monitored.

NOTE: Excessive PFK (or any other reference substance) may cause noise problems and contamination of the ion source resulting in an increase in downtime for source cleaning.

8.2.2.3 Documentation of the instrument resolving power must then be accomplished by recording the peak profile of the high-mass reference signal (m/z 380.9760) obtained during the above peak matching experiment by using the low-mass PFK ion at m/z 304.9824 as a reference. The minimum resolving power of 10,000 must be demonstrated on the high-mass ion while it is transmitted at a lower accelerating voltage than the low-mass reference ion, which is transmitted at full sensitivity. The format of the peak profile representation (Figure 5) must allow manual determination of the resolution, i.e., the horizontal axis must be a calibrated mass scale (amu or ppm per division). The result of the peak width measurement (performed at 5 percent of the maximum, which corresponds to the 10 percent valley definition) must appear on the hard copy and cannot exceed 100 ppm at m/z 380.9760 (or 0.038 amu at that particular mass).

8.3 Quality control samples

8.3.1 Performance evaluation samples - When available, performance evaluation (PE) samples containing known amounts of unlabeled 2,3,7,8-substituted PCDDs/PCDFs or other PCDD/PCDF congeners should be analyzed alongside routine field samples.

8.3.2 Performance check solutions

8.3.2.1 At the beginning of each 12-hour period during which samples are to be analyzed, an aliquot of the 1) GC column performance check solution and 2) high-resolution concentration calibration solution No. 3 (HRCC-3; see Table 5) shall be analyzed to demonstrate adequate GC resolution and sensitivity, response factor reproducibility, and mass range calibration, and to establish the PCDD/PCDF retention time windows. A mass resolution check shall also be performed to demonstrate adequate mass resolution using an appropriate reference compound (PFK is recommended). If the required criteria are not met, remedial action must be taken before any samples are analyzed.

8.3.2.2 To validate positive sample data, the routine or continuing calibration (HRCC-3; Table 5) and the mass resolution check must be performed also at the end of each 12-hour period during which samples are analyzed. Furthermore, an HRGC/HRMS method blank run must be recorded following a calibration run and the first sample run.

8.3.2.2.1 If the laboratory operates only during one period (shift) each day of 12 hours or less, the GC performance check solution must be analyzed only once (at the beginning of the period) to validate the data acquired during the period. However, the mass resolution and continuing calibration checks must be performed at the beginning as well as at the end of the period.

8.3.2.2.2 If the laboratory operates during consecutive 12-hour periods (shifts), analysis of the GC performance check solution must be performed at the beginning of each 12-hour period. The mass resolution and continuing calibration checks from the previous period can be used for the beginning of the next period.

8.3.2.3 Results of at least one analysis of the GC column performance check solution and of two mass resolution and continuing calibration checks must be reported with the sample data collected during a 12-hour period.

8.3.2.4 Deviations from criteria specified for the GC performance check or for the mass resolution check invalidate all positive sample data collected between analyses of the performance check solution, and the extracts from those positive samples shall be reanalyzed.

If the routine calibration run fails at the beginning of a 12-hour shift, the instructions in Sec. 7.7.4.4 must be followed. If the continuing calibration check performed at the end of a 12 hour period fails by no more than 25 percent RPD for the 17 unlabeled compounds and 35 percent RPD for the 9 labeled reference compounds, use the mean to the two "daily" RF values from the two daily routine calibration runs to compute the analyte concentrations, instead of the $\overline{RF}$ values obtained from the initial calibration. A new initial calibration (new $\overline{RF}$s) is required immediately (within two hours) following the analysis of the samples, whenever the RPD from the end-of-shift routine calibration exceeds 25 percent or 35 percent, respectively. Failure to perform a new initial calibration immediately following the analysis of the samples will automatically require reanalysis of all positive sample extracts analyzed before the failed end-of-shift continuing calibration check.

8.3.3 The GC column performance check mixture, high-resolution concentration calibration solutions, and the sample fortification solutions may be obtained from commercial sources.

8.3.4 Field blanks - Batches of field samples may contain a field blank sample of uncontaminated soil, sediment or water that is to be fortified before analysis according to Sec. 8.3.4.1. In addition to this field blank, a batch of samples may include a rinsate, which is a portion of the solvent that was used to rinse sampling equipment. The rinsate is analyzed to assure that the samples were not contaminated by the sampling equipment.

8.3.4.1 Fortified field blank

8.3.4.1.1 Weigh a 10-g portion or use 1-L (for aqueous samples) of the specified field blank sample and add 100 µL of the solution containing the nine internal standards (Table 2) diluted with 1.0 mL acetone (Sec. 7.1).

8.3.4.1.2 Extract by using the procedures beginning in Secs. 7.4.5 or 7.4.6, as applicable, add 10 µL of the recovery standard solution (Sec. 7.5.3.6) and analyze a 2-µL aliquot of the concentrated extract.

8.3.4.1.3 Calculate the concentration (Sec. 7.9.1) of 2,3,7,8-substituted PCDDs/PCDFs and the percent recovery of the internal standards (Sec. 7.9.2).

8.3.4.1.4 Extract and analyze a new simulated fortified field blank whenever new lots of solvents or reagents are used for sample extraction or for column chromatographic procedures.

8.3.4.2 Rinsate sample

8.3.4.2.1 Take a 100-mL (± 0.5 mL) portion of the sampling equipment rinse solvent (rinsate sample), filter, if necessary, and add 100 µL of the solution containing the nine internal standards (Table 2).

8.3.4.2.2 Using a K-D apparatus, concentrate to about 5 mL.

NOTE: As an option, a rotary evaporator may be used in place of the K-D apparatus for the concentration of the rinsate.

8.3.4.2.3 Transfer the 5 mL concentrate from the K-D concentrator tube in 1-mL portions to a 1-mL minivial, reducing the volume in the minivial as necessary with a gentle stream of dry nitrogen.

8.3.4.2.4 Rinse the K-D concentrator tube with two 0.5 mL portions of hexane and transfer the rinses to the 1 mL minivial. Concentrate with dry nitrogen, as necessary.

8.3.4.2.5 Just before analysis, add 10 µL recovery standard solution (Table 2) and reduce the volume to its final volume, as necessary (Sec. 7.8.1). No column chromatography is required.

8.3.4.2.6 Analyze an aliquot of the solution following the same procedures used to analyze samples.

8.3.4.2.7 Report percent recovery of the internal standard and the presence of any PCDD/PCDF compounds in µg/L of rinsate solvent.

8.3.5 Duplicate analyses

In each batch of samples, locate the sample specified for duplicate analysis, and analyze a second 10-g soil or sediment sample portion or 1-L water sample, or an appropriate amount of the type of matrix under consideration.

8.3.5.1 The results of the laboratory duplicates (percent recovery and concentrations of 2,3,7,8-substituted PCDD/PCDF compounds) should agree within 25 percent relative difference (difference expressed as percentage of the mean). Report all results.

8.3.5.2 Recommended actions to help locate problems

Verify satisfactory instrument performance (Secs. 8.2 and 8.3).

If possible, verify that no error was made while weighing the sample portions.

Review the analytical procedures with the performing laboratory personnel.

8.3.6 Matrix spike and matrix spike duplicate

8.3.6.1 Locate the sample for the MS and MSD analyses (the sample may be labeled "double volume").

8.3.6.2 Add an appropriate volume of the matrix spike fortification solution (Sec. 5.10) and of the sample fortification solution (Sec. 5.8), adjusting the fortification level as specified in Table 1 under IS Spiking Levels.

8.3.6.3 Analyze the MS and MSD samples as described in Sec. 7.

8.3.6.4 The results obtained from the MS and MSD samples (concentrations of 2,3,7,8-substituted PCDDs/PCDFs) should agree within 20 percent relative difference.

8.4 Percent recovery of the internal standards - For each sample, method blank and rinsate, calculate the percent recovery (Sec. 7.9.2). The percent recovery should be between 40 percent and 135 percent for all 2,3,7,8-substituted internal standards.

NOTE: A low or high percent recovery for a blank does not require discarding the analytical data but it may indicate a potential problem with future analytical data.

8.5 Identification criteria

8.5.1 If either one of the identification criteria appearing in Secs. 7.8.4.1.1 through 7.8.4.1.4 is not met for an homologous series, it is reported that the sample does not contain

unlabeled 2,3,7,8-substituted PCDD/PCDF isomers for that homologous series at the calculated detection limit (Sec. 7.9.5)

8.5.2 If the first initial identification criteria (Secs. 7.8.4.1.1 through 7.8.4.1.4) are met, but the criteria appearing in Secs. 7.8.4.1.5 and 7.8.4.2 are not met, that sample is presumed to contain interfering contaminants. This must be noted on the analytical report form, and the sample should be rerun or the extract reanalyzed.

8.6 Unused portions of samples and sample extracts should be preserved for six months after sample receipt to allow further analyses.

8.7 Reuse of glassware is to be minimized to avoid the risk of contamination.

9.0 METHOD PERFORMANCE

9.1 Table 12 provides data from a comparison of Soxhlet extraction and pressurized fluid extraction of samples of ground chimney brick. The data are taken from Reference 8.

9.2 Table 13 provides data from a comparison of Soxhlet extraction and pressurized fluid extraction of samples of urban dust. The data are taken from Reference 8.

9.3 Table 14 provides data from a comparison of Soxhlet extraction and pressurized fluid extraction of samples of fly ash. PFE data are provided for samples that were pretreated with an HCl wash and for samples that were not pretreated, but were extracted with a mixture of toluene and acetic acid. The data are taken from Reference 8.

9.4 Table 15 provides data from a comparison of Soxhlet extraction and pressurized fluid extraction of a soil sample (EC-2) from the National Water Research Institute (Burlington, Ontario, Canada) that contains high levels of PCDDs and PCDFs. The data are taken from Reference 8.

9.5 Table 16 provides data from a comparison of Soxhlet extraction and pressurized fluid extraction of a sediment sample (HS-2) from the National Research Council Institute for Marine Biosciences (Halifax, Nova Scotia, Canada) that contains low levels of PCDDs and PCDFs. The data are taken from Reference 8.

9.6 Table 17 provides data from a comparison of Soxhlet extraction and pressurized fluid extraction for two field-contaminated sediment samples. The data are taken from Reference 8.

10.0 REFERENCES

1. "Control of Interferences in the Analysis of Human Adipose Tissue for 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin". D. G. Patterson, J.S. Holler, D.F. Grote, L.R. Alexander, C.R. Lapeza, R.C. O'Connor and J.A. Liddle. *Environ. Toxicol. Chem.* 5, 355-360 (1986).

2. "Method 8290: Analytical Procedures and Quality Assurance for Multimedia Analysis of Polychlorinated Dibenzo-*p*-Dioxins and Dibenzofurans by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry". Y. Tondeur and W.F. Beckert. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Las Vegas, NV.

3. "Carcinogens - Working with Carcinogens", Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control. National Institute for Occupational Safety and Health. Publication No. 77-206, August 1977.

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

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

6. "Hybrid HRGC/MS/MS Method for the Characterization of Tetrachlorinated Dibenzo-p-dioxins in Environmental Samples." Y. Tondeur, W.J. Niederhut, S.R. Missler, and J.E. Campana, *Mass Spectrom.* 14, 449-456 (1987).

7. USEPA National Dioxin Study - Phase II, "Analytical Procedures and Quality Assurance Plan for the Determination of PCDD/PCDF in Fish", EPA-Duluth, October 26, 1987.

8. "Extraction of Polychlorinated Dibenzo-p-Dioxins and Polychlorinated Dibenzofurans from Environmental Samples Using Accelerated Solvent Extraction (ASE)," B. E. Richter, J. L. Ezzell, D. E. Knowles, and F. Hoefler, *Chemosphere*, 34 (5-7), 975-987, 1997.

11.0 SAFETY

11.1 The following safety practices are excerpts from EPA Method 613, Sec. 4 (July 1982 version) and amended for use in conjunction with this method. The 2,3,7,8-TCDD isomer has been found to be acnegenic, carcinogenic, and teratogenic in laboratory animal studies. Other PCDDs and PCDFs containing chlorine atoms in positions 2,3,7,8 are known to have toxicities comparable to that of 2,3,7,8-TCDD. The analyst should note that finely divided dry soils contaminated with PCDDs and PCDFs are particularly hazardous because of the potential for inhalation and ingestion. It is recommended that such samples be processed in a confined environment, such as a hood or a glove box. Laboratory personnel handling these types of samples should wear masks fitted with charcoal filters to prevent inhalation of dust.

11.2 The toxicity or carcinogenicity of each reagent used in this method is not precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be kept to a minimum. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should be made available to all personnel involved in the chemical analysis of samples suspected to contain PCDDs/PCDFs. Additional references to laboratory safety are given in references 3, 4 and 5.

11.3 Each laboratory must develop a strict safety program for the handling of PCDDs and PCDFs. The laboratory practices listed below are recommended.

11.3.1 Contamination of the laboratory will be minimized by conducting most of the manipulations in a hood.

11.3.2 The effluents of sample splitters for the gas chromatograph and roughing pumps on the HRGC/HRMS system should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high boiling alcohols.

11.3.3 Liquid waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light at a wavelength less than 290 nm for several days (use F 40 BL lamps, or equivalent). Using this analytical method, analyze the irradiated liquid wastes and dispose of the solutions when 2,3,7,8-TCDD and 2,3,7,8-TCDF congeners can no longer be detected.

11.4 The following precautions for safe handling of 2,3,7,8-TCDD in the laboratory were issued by Dow Chemical U.S.A. (revised 11/78) and amended for use in conjunction with this method. They are as complete as possible on the basis of available toxicological information. The precautions for safe handling and use are necessarily general in nature since detailed, specific recommendations can be made only for the particular exposure and circumstances of each individual use. Assistance in evaluating the health hazards of particular plant conditions may be obtained from certain consulting laboratories and from State Departments of Health or of Labor, many of which have an industrial health service. The 2,3,7,8-TCDD isomer is extremely toxic to certain kinds of laboratory animals. However, it has been handled for years without injury in analytical and biological laboratories. Many techniques used in handling radioactive and infectious materials are applicable to 2,3,7,8-TCDD.

11.4.1 Protective equipment: Throw away plastic gloves, apron or lab coat, safety glasses and laboratory hood adequate for radioactive work. However, PVC gloves should not be used.

11.4.2 Training: Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

11.4.3 Personal hygiene: Thorough washing of hands and forearms after each manipulation and before breaks (coffee, lunch, and shift).

11.4.4 Confinement: Isolated work area, posted with signs, segregated glassware and tools, plastic backed absorbent paper on benchtops.

11.4.5 Waste: Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans.

11.4.6 Disposal of hazardous wastes: Refer to the November 7, 1986 issue of the Federal Register on Land Ban Rulings for details concerning the handling of dioxin-containing wastes.

11.4.7 Personnel decontamination: Apply a mild soap with plenty of scrubbing action. Glassware, tools and surfaces - Chlorothene NU Solvent™ (Dow Chemical Company) is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with Chlorothene, then washing with a detergent and water. Dishwater may be disposed to the sewer after percolation through a charcoal bed filter. It is prudent to minimize solvent wastes because they require costly special disposal through commercial services.

11.4.8 Laundry: Clothing known to be contaminated should be disposed with the precautions described under "Disposal of Hazardous Wastes". Laboratory coats or other clothing worn in 2,3,7,8-TCDD work area may be laundered. Clothing should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the launderer knows the problem. The washer should be run through one full cycle before being used again for other clothing.

11.4.9 Wipe tests: A useful method for determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper, extract the filter paper and analyze the extract.

NOTE: A procedure for the collection, handling, analysis, and reporting requirements of wipe tests performed within the laboratory is described in Appendix A. The results and decision making processes are based on the presence of 2,3,7,8-substituted PCDDs/PCDFs.

11.4.10 Inhalation: Any procedure that may generate airborne contamination must be carried out with good ventilation. Gross losses to a ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no significant inhalation hazards except in case of an accident.

11.4.11 Accidents: Remove contaminated clothing immediately, taking precautions not to contaminate skin or other articles. Wash exposed skin vigorously and repeatedly until medical attention is obtained.

APPENDIX A

PROCEDURES FOR THE COLLECTION, HANDLING, ANALYSIS, AND REPORTING OF WIPE TESTS PERFORMED WITHIN THE LABORATORY

This procedure is designed for the periodic evaluation of potential contamination by 2,3,7,8-substituted PCDD/PCDF congeners of the working areas inside the laboratory.

A.1 Perform the wipe tests on surface areas of two inches by one foot with glass fiber paper saturated with distilled in glass acetone using a pair of clean stainless steel forceps. Use one wiper for each of the designated areas. Combine the wipers to one composite sample in an extraction jar containing 200 mL of distilled-in-glass acetone. Place an equal number of unused wipers in 200 mL acetone and use this as a control. Add 100 µL of the sample fortification solution (Sec. 5.8) to each jar containing used or unused wipers.

A.1.1 Close the jar containing the wipers and the acetone and extract for 20 minutes using a wrist action shaker. Transfer the extract into a K-D apparatus fitted with a concentration tube and a three-ball Snyder column. Add two PTFE or Carborundum ™ boiling chips and concentrate the extract to an apparent volume of 1.0 mL on a steam bath. Rinse the Snyder column and the K-D assembly with two 1-mL portions of hexane into the concentrator tube, and concentrate its contents to near dryness with a gentle stream of nitrogen. Add 1.0 mL of hexane to the concentrator tube and swirl the solvent on the walls.

A.1.2 Prepare a neutral alumina column as described in Sec. 7.5.2.2 and follow the steps outlined in Secs. 7.5.2.3 through 7.5.2.5.

A.1.3 Add 10 µL of the recovery standard solution as described in Sec. 7.5.3.6.

A.2 Concentrate the contents of the vial to a final volume of 10 µL (either in a minivial or in a capillary tube). Inject 2 µL of each extract (wipe and control) onto a capillary column and analyze for 2,3,7,8-substituted PCDDs/PCDFs as specified in the analytical method in Sec. 7.8. Perform calculations according to Sec. 7.9.

A.3 Report the presence of 2,3,7,8-substituted PCDDs and PCDFs as a quantity (pg or ng) per wipe test experiment (WTE). Under the conditions outlined in this analytical protocol, a lower limit of calibration of 10 pg/WTE is expected for 2,3,7,8-TCDD. A positive response for the blank (control) is defined as a signal in the TCDD retention time window at any of the masses monitored which is equivalent to or above 3 pg of 2,3,7,8-TCDD per WTE. For other congeners, use the multiplication factors listed in Table 1, footnote (a) (e.g., for OCDD, the lower MCL is 10 x 5 = 50 pg/WTE and the positive response for the blank would be 3 x 5 = 15 pg). Also, report the recoveries of the internal standards during the simplified cleanup procedure.

A.4 At a minimum, wipe tests should be performed when there is evidence of contamination in the method blanks.

A.5 An upper limit of 25 pg per TCDD isomer and per wipe test experiment is allowed (use multiplication factors listed in footnote (a) from Table 1 for other congeners). This value corresponds to 2½ times the lower calibration limit of the analytical method. Steps to correct the contamination must be taken whenever these levels are exceeded. To that effect, first vacuum the working places (hoods, benches, sink) using a vacuum cleaner equipped with a high efficiency particulate absorbent (HEPA) filter and then wash with a detergent. A new set of wipes should be analyzed before anyone is allowed to work in the dioxin area of the laboratory after corrective action has been taken.

TABLE 1

TYPES OF MATRICES, SAMPLE SIZES, AND 2,3,7,8-TCDD-BASED METHOD CALIBRATION LIMITS (PARTS PER TRILLION)

	Water	Soil Sediment Paper Pulp[b]	Fly Ash	Fish Tissue[c]	Human Adipose Tissue	Sludge Fuel Oil	Still Bottom
Lower MCL[a]	0.01	1.0	1.0	1.0	1.0	5.0	10
Upper MCL[a]	2	200	200	200	200	1000	2000
Sample Weight (g)	1000	10	10	20	10	2	1
IS Spiking Level (ppt)	1	100	100	100	100	500	1000
Final Ext. Vol. (µL)[d]	10-50	10-50	50	10-50	10-50	50	50

[a] For other congeners multiply the values by 1 for TCDF/PeCDD/PeCDF, by 2.5 for HxCDD/HxCDF/HpCDD/HpCDF, and by 5 for OCDD/OCDF.

[b] Sample dewatered according to Sec. 6.5.

[c] One half of the extract from the 20 g sample is used for determination of lipid content (Sec. 7.2.2).

[d] See Sec. 7.8.1.

NOTE: Chemical reactor residues are treated as still bottoms, if their appearances so suggest.

TABLE 2

COMPOSITION OF THE SAMPLE FORTIFICATION AND RECOVERY STANDARD SOLUTIONS[a]

Analyte	Sample Fortification Solution Concentration (pg/µL)	Recovery Standard Solution Concentration (pg/µL)
$^{13}C_{12}$-2,3,7,8-TCDD	10	--
$^{13}C_{12}$-2,3,7,8-TCDF	10	--
$^{13}C_{12}$-1,2,3,4-TCDD	--	50
$^{13}C_{12}$-1,2,3,7,8-PeCDD	10	--
$^{13}C_{12}$-1,2,3,7,8-PeCDF	10	--
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	25	--
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	25	--
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	--	50
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	25	--
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	25	--
$^{13}C_{12}$-OCDD	50	--

[a]These solutions should be made freshly every day in nonane or other appropriate solvent because of the possibility of adsorptive losses to glassware. If these solutions are to be kept for more than one day, then the sample fortification solution concentrations should be increased ten fold, and the recovery standard solution concentrations should be doubled. Corresponding adjustments of the spiking volumes must then be made.

TABLE 3

THE FIFTEEN 2,3,7,8-SUBSTITUTED PCDD AND PCDF CONGENERS

PCDD	PCDF
2,3,7,8-TCDD(*)	2,3,7,8-TCDF(*)
1,2,3,7,8-PeCDD(*)	1,2,3,7,8-PeCDF(*)
1,2,3,6,7,8-HxCDD(*)	2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDD	1,2,3,6,7,8-HxCDF
1,2,3,7,8,9-HxCDD(+)	1,2,3,7,8,9-HxCDF
1,2,3,4,6,7,8-HpCDD(*)	1,2,3,4,7,8-HxCDF(*)
	2,3,4,6,7,8-HxCDF
	1,2,3,4,6,7,8-HpCDF(*)
	1,2,3,4,7,8,9-HpCDF

* The 13C-labeled analogue is used as an internal standard.

+ The 13C-labeled analogue is used as a recovery standard.

TABLE 4

ISOMERS OF CHLORINATED DIOXINS AND FURANS
AS A FUNCTION OF THE NUMBER OF CHLORINE ATOMS

Number of Chlorine Atoms	Number of Dioxin Isomers	Number of 2,3,7,8-Dioxins	Number of Furan Isomers	Number of 2,3,7,8-Furans
1	2	—	4	—
2	10	—	16	—
3	14	—	28	—
4	22	1	38	1
5	14	1	28	2
6	10	3	16	4
7	2	1	4	2
8	1	1	1	1
Total	75	7	135	10

TABLE 5

HIGH-RESOLUTION CONCENTRATION CALIBRATION SOLUTIONS

Analyte	Concentration (pg/µL)				
Unlabeled Analytes	5	4	3	2	1
2,3,7,8-TCDD	200	50	10	2.5	1
2,3,7,8-TCDF	200	50	10	2.5	1
1,2,3,7,8-PeCDD	500	125	25	6.25	2.5
1,2,3,7,8-PeCDF	500	125	25	6.25	2.5
2,3,4,7,8-PeCDF	500	125	25	6.25	2.5
1,2,3,4,7,8-HxCDD	500	125	25	6.25	2.5
1,2,3,6,7,8-HxCDD	500	125	25	6.25	2.5
1,2,3,7,8,9-HxCDD	500	125	25	6.25	2.5
1,2,3,4,7,8-HxCDF	500	125	25	6.25	2.5
1,2,3,6,7,8-HxCDF	500	125	25	6.25	2.5
1,2,3,7,8,9-HxCDF	500	125	25	6.25	2.5
2,3,4,6,7,8-HxCDF	500	125	25	6.25	2.5
1,2,3,4,6,7,8-HpCDD	500	125	25	6.25	2.5
1,2,3,4,6,7,8-HpCDF	500	125	25	6.25	2.5
1,2,3,4,7,8,9-HpCDF	500	125	25	6.25	2.5
OCDD	1,000	250	50	12.5	5
OCDF	1,000	250	50	12.5	5
Internal Standards					
$^{13}C_{12}$-2,3,7,8-TCDD	50	50	50	50	50
$^{13}C_{12}$-2,3,7,8-TCDF	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8-PeCDD	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8-PeCDF	50	50	50	50	50
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	125	125	125	125	125
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	125	125	125	125	125
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	125	125	125	125	125
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	125	125	125	125	125
$^{13}C_{12}$-OCDD	250	250	250	250	250
Recovery Standards					
$^{13}C_{12}$-1,2,3,4-TCDD	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	125	125	125	125	125

TABLE 6

IONS MONITORED FOR HRGC/HRMS ANALYSIS OF PCDDS/PCDFS

Descriptor	Accurate Mass[a]	Ion ID	Elemental Composition	Analyte
1	303.9016	M	$C_{12}H_4{}^{35}Cl_4O$	TCDF
	305.8987	M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF
	315.9419	M	$^{13}C_{12}H_4{}^{35}Cl_4O$	TCDF (S)
	317.9389	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF (S)
	319.8965	M	$C_{12}H_4{}^{35}Cl_4O_2$	TCDD
	321.8936	M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD
	331.9368	M	$^{13}C_{12}H_4{}^{35}Cl_4O_2$	TCDD (S)
	333.9338	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD (S)
	375.8364	M+2	$C_{12}H_4{}^{35}Cl_5{}^{37}ClO$	HxCDPE
	[354.9792]	LOCK	C_9F_{13}	PFK
2	339.8597	M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF
	341.8567	M+4	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$	PeCDF
	351.9000	M+2	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF (S)
	353.8970	M+4	$^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$	PeCDF (S)
	355.8546	M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD
	357.8516	M+4	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD
	367.8949	M+2	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD (S)
	369.8919	M+4	$^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD (S)
	409.7974	M+2	$C_{12}H_3{}^{35}Cl_6{}^{37}ClO$	HpCDPE
	[354.9792]	LOCK	C_9F_{13}	PFK
3	373.8208	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF
	375.8178	M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O$	HxCDF
	383.8639	M	$^{13}C_{12}H_2{}^{35}Cl_6O$	HxCDF (S)
	385.8610	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF (S)
	389.8156	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD
	391.8127	M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD

TABLE 6
(continued)

Descriptor	Accurate Mass[a]	Ion ID	Elemental Composition	Analyte
	401.8559	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD (S)
	403.8529	M+4	$^{13}C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD (S)
	445.7555	M+4	$C_{12}H_2{}^{35}Cl_6{}^{37}Cl_2O$	OCDPE
	[430.9728]	LOCK	C_9F_{17}	PFK
4	407.7818	M+2	$C_{12}H^{35}Cl_6{}^{37}ClO$	HpCDF
	409.7788	M+4	$C_{12}H^{35}Cl_5{}^{37}Cl_2O$	HpCDF
	417.8250	M	$^{13}C_{12}H^{35}Cl_7O$	HpCDF (S)
	419.8220	M+2	$^{13}C_{12}H^{35}Cl_6{}^{37}ClO$	HpCDF
	423.7767	M+2	$C_{12}H^{35}Cl_8{}^{37}ClO_2$	HpCDD
	425.7737	M+4	$C_{12}H^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD
	435.8169	M+2	$^{13}C_{12}H^{35}Cl_6{}^{37}ClO_2$	HpCDD (S)
	437.8140	M+4	$^{13}C_{12}H^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD (S)
	479.7165	M+4	$C_{12}H^{35}Cl_7{}^{37}Cl_2O$	NCDPE
	[430.9728]	LOCK	C_9F_{17}	PFK
5	441.7428	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO$	OCDF
	443.7399	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O$	OCDF
	457.7377	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO_2$	OCDD
	459.7348	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$	OCDD
	469.7780	M+2	$^{13}C_{12}{}^{35}Cl_7{}^{37}ClO_2$	OCDD (S)
	471.7750	M+4	$^{13}C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$	OCDD (S)
	513.6775	M+4	$C_{12}{}^{35}Cl_8{}^{37}Cl_2O$	DCDPE
	[442.9728]	LOCK	$C_{10}F_{17}$	PFK

S = internal/recovery standard

[a] The following nuclidic masses were used:

H	=	1.007825	O	=	15.994915
C	=	12.000000	^{35}Cl	=	34.968853
^{13}C	=	13.003355	^{37}Cl	=	36.965903
F	=	18.9984			

TABLE 7

PCDD AND PCDF CONGENERS PRESENT IN THE GC
PERFORMANCE EVALUATION SOLUTION AND USED FOR DEFINING
THE HOMOLOGUE GC RETENTION TIME WINDOWS ON A 60-M DB-5 COLUMN

	PCDD Positional Isomer		PCDF Positional Isomer	
# Chlorine Atoms	First Eluter	Last Eluter	First Eluter	Last Eluter
4[a]	1,3,6,8	1,2,8,9	1,3,6,8	1,2,8,9
5	1,2,4,6,8/1,2,4,7,9	1,2,3,8,9	1,3,4,6,8	1,2,3,8,9
6	1,2,4,6,7,9/1,2,4,6,8,9	1,2,3,4,6,7	1,2,3,4,6,8	1,2,3,4,8,9
7	1,2,3,4,6,7,9	1,2,3,4,6,7,8	1,2,3,4,6,7,8	1,2,3,4,7,8,9
8	1,2,3,4,6,7,8,9		1,2,3,4,6,7,8,9	

[a]In addition to these two TCDD isomers, the 1,2,3,4-, 1,2,3,7-, 1,2,3,8-, 2,3,7,8-, $^{13}C_{12}$-2,3,7,8-, and 1,2,3,9-TCDD isomers must also be present as a check of column resolution.

TABLE 8

THEORETICAL ION ABUNDANCE RATIOS AND THEIR CONTROL LIMITS
FOR PCDDS AND PCDFS

# Chlorine Atoms	Ion Type	Theoretical Abundance Ratio	Control Limits	
			Lower	Upper
4	M/M+2	0.77	0.65	0.89
5	M+2/M+4	1.55	1.32	1.78
6	M+2/M+4	1.24	1.05	1.43
6[a]	M/M+2	0.51	0.43	0.59
7[b]	M/M+2	0.44	0.37	0.51
7	M+2/M+4	1.04	0.88	1.20
8	M+2/M+4	0.89	0.76	1.02

[a]Used only for ^{13}C-HxCDF (IS).

[b]Used only for ^{13}C-HpCDF (IS).

TABLE 9

RELATIVE RESPONSE FACTOR [RF (NUMBER)] ATTRIBUTIONS

Number	Specific Congener Name
1	2,3,7,8-TCDD (and total TCDDs)
2	2,3,7,8-TCDF (and total TCDFs)
3	1,2,3,7,8-PeCDD (and total PeCDDs)
4	1,2,3,7,8-PeCDF
5	2,3,4,7,8-PeCDF
6	1,2,3,4,7,8-HxCDD
7	1,2,3,6,7,8-HxCDD
8	1,2,3,7,8,9-HxCDD
9	1,2,3,4,7,8-HxCDF
10	1,2,3,6,7,8-HxCDF
11	1,2,3,7,8,9-HxCDF
12	2,3,4,6,7,8-HxCDF
13	1,2,3,4,6,7,8-HpCDD (and total HpCDDs)
14	1,2,3,4,6,7,8-HpCDF
15	1,2,3,4,7,8,9-HpCDF
16	OCDD
17	OCDF
18	$^{13}C_{12}$-2,3,7,8-TCDD
19	$^{13}C_{12}$-2,3,7,8-TCDF
20	$^{13}C_{12}$-1,2,3,7,8-PeCDD
21	$^{13}C_{12}$-1,2,3,7,8-PeCDF
22	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
23	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF
24	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD
25	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF
26	$^{13}C_{12}$-OCDD
27	Total PeCDFs
28	Total HxCDFs
29	Total HxCDDs
30	Total HpCDFs

TABLE 10

2,3,7,8-TCDD TOXICITY EQUIVALENCY FACTORS (TEFS)
FOR THE POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS

Analyte	TEF[a]
2,3,7,8-TCDD	1.00
1,2,3,7,8-PeCDD	0.50
1,2,3,6,7,8-HxCDD	0.10
1,2,3,7,8,9-HxCDD	0.10
1,2,3,4,7,8-HxCDD	0.10
1,2,3,4,6,7,8-HpCDD	0.01
1,2,3,4,6,7,8,9-OCDD	0.001
2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDF	0.05
2,3,4,7,8-PeCDF	0.5
1,2,3,6,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDF	0.1
1,2,3,4,7,8-HxCDF	0.1
2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,4,7,8,9-HpCDF	0.01
1,2,3,4,6,7,8,9-OCDF	0.001

[a]Taken from "Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-*p*-Dioxin and -Dibenzofurans (CDDs and CDFs) and 1989 Update", (EPA/625/3-89/016, March 1989).

TABLE 11

ANALYTE RELATIVE RETENTION TIME REFERENCE ATTRIBUTIONS

Analyte	Analyte RRT Reference[a]
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF

[a]The retention time of 2,3,4,7,8-PeCDF on the DB-5 column is measured relative to $^{13}C_{12}$-1,2,3,7,8-PeCDF and the retention time of 1,2,3,4,7,8,9-HpCDF relative to $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF.

TABLE 12

COMPARISON OF SOXHLET AND PRESSURIZED FLUID EXTRACTION (PFE) FOR EXTRACTION OF GROUND CHIMNEY BRICK

Analyte	Soxhlet (n=1) (ng/kg)	PFE (n=2)* (ng/kg)
2,3,7,8-TCDD	6	6
1,2,3,7,8-PeCDD	52	57
1,2,3,4,7,8-HxCDD	46	52
1,2,3,6,7,8-HxCDD	120	130
1,2,3,7,9,9-HxCDD	97	1000
1,2,3,4,6,7,8-HpCDD	1000	820
OCDD	2900	2600
2,3,7,8-TCDF	160	180
1,2,3,7,8 (+ 1,2,3,4,8)-PeCDF	430	470
2,3,4,7,9-PeCDF	390	390
1,2,3,4,7,8 (+ 1,2,3,4,7,9)-HxCDF	1100	1100
1,2,3,6,7,8-HxCDF	540	570
2,3,4,6,7,8-HxCDF	400	360
1,2,3,7,8,9-HxCDF	42	42
1,2,3,4,6,7,8-HpCDF	2100	2000
1,2,3,4,7,8,9-HpCDF	140	120
OCDF	2000	2000
Total TCDD	440	530
Total PeCDD	900	940
Total HxCDD	1800	2000
Total HpCDD	2000	2100
Total TCDF	2300	2600
Total PeCDF	4100	4300
Total HxCDF	4700	4700
Total HpCDF	2800	2600

* Sum of two extractions of each sample
Data from Reference 8

TABLE 13

COMPARISON OF SOXHLET AND PFE FOR EXTRACTION OF URBAN DUST

Analyte	Soxhlet (n=1) (ng/kg)	PFE (n=2)* (ng/kg)
2,3,7,8-TCDD	3.3	3.2
1,2,3,7,8-PeCDD	11.8	13.1
1,2,3,4,7,8-HxCDD	9.8	8.0
1,2,3,6,7,8-HxCDD	11.5	9.5
1,2,3,7,9,9-HxCDD	ND (8)	ND (8)
1,2,3,4,6,7,8-HpCDD	113	107
OCDD	445	314
2,3,7,8-TCDF	12.5	18.6
1,2,3,7,8 (+ 1,2,3,4,8)-PeCDF	9.9	12.0
2,3,4,7,9-PeCDF	13.9	18.1
1,2,3,4,7,8 (+ 1,2,3,4,7,9)-HxCDF	18.7	23.7
1,2,3,6,7,8-HxCDF	10.7	15.8
2,3,4,6,7,8-HxCDF	3.3	8.7
1,2,3,7,8,9-HxCDF	ND (2)	ND (2)
1,2,3,4,6,7,8-HpCDF	13.2	29.4
1,2,3,4,7,8,9-HpCDF	ND (3)	ND (3)
OCDF	ND (10)	ND (10)
Total TCDD	182	325
Total PeCDD	175	281
Total HxCDD	86.7	81.7
Total HpCDD	221	217
Total TCDF	333	419
Total PeCDF	146	179
Total HxCDF	65.9	122
Total HpCDF	13.2	29.4

ND = Not detected, with detection limit given in parentheses
* Sum of two extractions of each sample
Data from Reference 8

TABLE 14

COMPARISON OF SOXHLET AND PFE FOR EXTRACTION OF FLY ASH
(with and without HCl pretreatment for PFE)

Analyte	Soxhlet (n=1) with HCl (µg/kg)†	PFE (n=2)* with HCl (µg/kg)†	PFE (n=2)* w/o HCl (µg/kg)‡
2,3,7,8-TCDD	0.32	0.36	0.28
1,2,3,7,8-PeCDD	1.6	2.1	1.7
1,2,3,4,7,8-HxCDD	1.2	1.4	1.2
1,2,3,6,7,8-HxCDD	2.4	2.7	2.4
1,2,3,7,9,9-HxCDD	2.4	2.3	2.2
1,2,3,4,6,7,8-HpCDD	8.2	9.6	8.1
OCDD	11.4	12.8	10.6
2,3,7,8-TCDF	3.7	4.3	3.4
1,2,3,7,8 (+ 1,2,3,4,8)-PeCDF	4.2	4.6	3.9
2,3,4,7,9-PeCDF	5.6	6.6	5.8
1,2,3,4,7,8 (+ 1,2,3,4,7,9)-HxCDF	7.8	8.7	5.4
1,2,3,6,7,8-HxCDF	7.2	8.5	5.3
2,3,4,6,7,8-HxCDF	6.6	7.2	4.5
1,2,3,7,8,9-HxCDF	0.43	0.56	0.30
1,2,3,4,6,7,8-HpCDF	18.0	17.6	16.8
1,2,3,4,7,8,9-HpCDF	2.3	2.4	2.0
OCDF	13.5	15.8	13.9
Total TCDD	12.0	12.4	10.5
Total PeCDD	16.6	20.5	16.2
Total HxCDD	38.2	42.4	36.7
Total HpCDD	15.0	19.8	16.0
Total TCDF	60.5	67.5	56.1
Total PeCDF	83.5	87.3	77.4
Total HxCDF	65.2	73.5	46.1
Total HpCDF	28.1	32.2	26.5

† Fly ash was pretreated with HCl, followed by a water rinse, and extracted with toluene.
‡ These samples received no HCl pretreatment, and were extracted with a mixture of toluene and acetic acid.
* Sum of two extractions of each sample
Data from Reference 8

TABLE 15

COMPARISON OF SOXHLET AND PFE FOR EXTRACTION OF SOIL (EC-2)

	Soxhlet Results (n=10)		PFE Results (n=2)	
Analyte	ng/kg	% RSD	ng/kg	% RSD
2,3,7,8-TCDD	270	9.1	270	0.0
1,2,3,7,8-PeCDD	24	12	22	3.3
1,2,3,4,7,8-HxCDD	23	8.3	24	3.0
1,2,3,6,7,8-HxCDD	83	3.6	87	0.8
1,2,3,7,9,9-HxCDD	60	6.2	57	7.4
1,2,3,4,6,7,8-HpCDD	720	6.7	720	1.0
OCDD	4000	6.2	4200	0.0
2,3,7,8-TCDF *	100	7.3	82	2.6
1,2,3,7,8 (+ 1,2,3,4,8)-PeCDF	39	14	36	3.9
2,3,4,7,9-PeCDF	62	5.5	60	0.0
1,2,3,4,7,8 (+ 1,2,3,4,7,9)-HxCDF	740	5.3	690	0.0
1,2,3,6,7,8-HxCDF	120	6.2	120	0.0
2,3,4,6,7,8-HxCDF	45	9.0	60	1.2
1,2,3,7,8,9-HxCDF	4.9	31	5.3	15
1,2,3,4,6,7,8-HpCDF	2600	6.7	2500	0.0
1,2,3,4,7,8,9-HpCDF	160	5.5	160	0.0
OCDF	7800	8.3	7000	3.1
Total TCDD	430	9.7	370	1.9
Total PeCDD	300	3.7	280	7.7
Total HxCDD	720	5.8	690	2.0
Total HpCDD	1300	7.0	1300	0.0
Total TCDF	620	12	380	19
Total PeCDF	820	9.4	710	7.0
Total HxCDF	1900	5.7	1900	0.0
Total HpCDF	3800	8.2	3900	3.6

* Single-column analysis only, may include contributions from other isomers that may co-elute. Data from Reference 8

TABLE 16

COMPARISON OF SOXHLET AND PFE FOR EXTRACTION OF SEDIMENT (HS-2)

	Soxhlet Results (n=10)		PFE Results (n=2)	
Analyte	ng/kg	% RSD	ng/kg	% RSD
2,3,7,8-TCDD	ND (1)	--	ND (1)	--
1,2,3,7,8-PeCDD	1.6	4.6	ND (1)	--
1,2,3,4,7,8-HxCDD	4.5	4.8	5.2	11
1,2,3,6,7,8-HxCDD	19	4.3	21	0.0
1,2,3,7,9,9-HxCDD	24	4.3	28	2.6
1,2,3,4,6,7,8-HpCDD	1200	8.1	1300	0.0
OCDD	6500	4.2	7100	0.0
2,3,7,8-TCDF *	8.5	11	6.6	5.4
1,2,3,7,8 (+ 1,2,3,4,8)-PeCDF	1.9	17	2.0	0.0
2,3,4,7,9-PeCDF	3.7	7.9	3.7	3.8
1,2,3,4,7,8 (+ 1,2,3,4,7,9)-HxCDF	17	7.3	17	4.3
1,2,3,6,7,8-HxCDF	3.7	5.6	4.0	5.4
2,3,4,6,7,8-HxCDF	3.7	18	4.4	3.2
1,2,3,7,8,9-HxCDF	ND (1)	--	ND (1)	--
1,2,3,4,6,7,8-HpCDF	91	1.6	96	3.7
1,2,3,4,7,8,9-HpCDF	5.2	6.7	5.3	6.7
OCDF	300	3.8	280	2.6
Total TCDD	3.9	14	2.5	34
Total PeCDD	17	7.8	10	10
Total HxCDD	510	5.6	570	1.3
Total HpCDD	4700	8.3	5100	11
Total TCDF	39	11	24	3.0
Total PeCDF	33	13	28	0.0
Total HxCDF	89	3.2	87	12
Total HpCDF	293	3.3	310	0.0

* Single-column analysis only, may include contributions from other isomers that may co-elute.
. ND = Not detected, with detection limit given in parentheses
. Data from Reference 8

TABLE 17

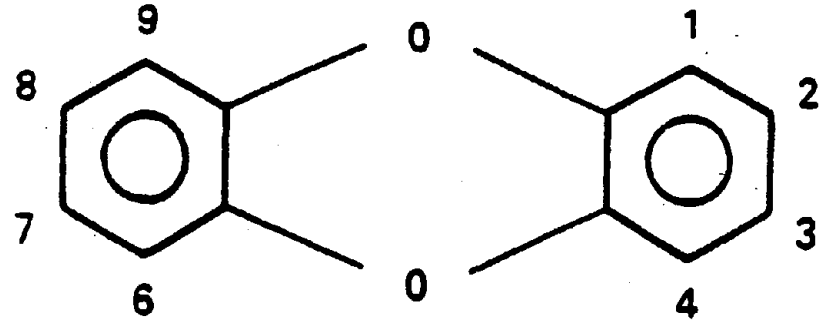
COMPARISON OF SOXHLET AND PFE FOR EXTRACTION OF CONTAMINATED SEDIMENTS

	Hamilton Harbor		Parrots Bay	
Analyte	Soxhlet	PFE	Soxhlet	PFE
2,3,7,8-TCDD	3.7	3.1	19	19
1,2,3,7,8-PeCDD	5.1	5.4	8.3	6.0
1,2,3,4,7,8-HxCDD	6.4	7.2	8.6	6.7
1,2,3,6,7,8-HxCDD	27	26	26	17
1,2,3,7,9,9-HxCDD	20	28	24	18
1,2,3,4,6,7,8-HpCDD	460	430	280	250
OCDD	3100	3100	1900	1600
2,3,7,8-TCDF *	61	44	80	48
1,2,3,7,8 (+ 1,2,3,4,8)-PeCDF	14	14	ND (20)	9.8
2,3,4,7,9-PeCDF	26	25	22	14
1,2,3,4,7,8 (+ 1,2,3,4,7,9)-HxCDF	27	37	79	59
1,2,3,6,7,8-HxCDF	17	16	ND (20)	15
2,3,4,6,7,8-HxCDF	14	14	21	11
1,2,3,7,8,9-HxCDF	ND (2)	1.6	4.9	ND (1)
1,2,3,4,6,7,8-HpCDF	130	130	270	220
1,2,3,4,7,8,9-HpCDF	14	13	17	12
OCDF	270	210	510	370
Total TCDD	50	14	39	48
Total PeCDD	63	15	87	66
Total HxCDD	220	180	230	200
Total HpCDD	850	810	580	530
Total TCDF	370	130	400	270
Total PeCDF	290	110	180	170
Total HxCDF	240	160	230	230
Total HpCDF	350	290	400	360

* Single-column analysis only, may include contributions from other isomers that may co-elute.
. ND = Not detected, with detection limit given in parentheses
. Data from Reference 8

FIGURE 1

GENERAL STRUCTURES OF DIBENZO-*p*-DIOXIN (TOP) AND DIBENZOFURAN (BOTTOM)



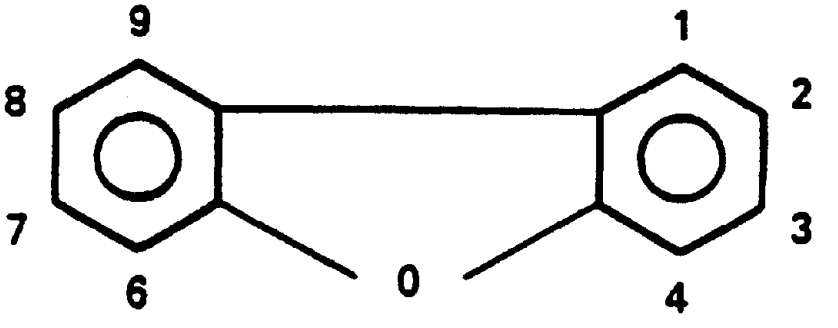
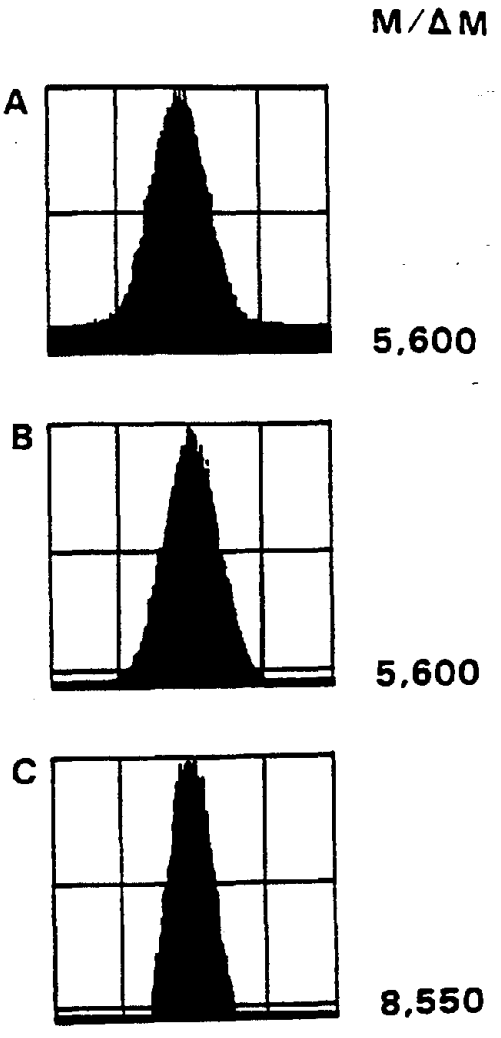


FIGURE 2



Peak profile displays demonstrating the effect of the detector zero on the measured resolving power. In this example, the true resolving power is 5,600.

A) The zero was set too high; no effect is observed upon the measurement of the resolving power.

B) The zero was adjusted properly.

C) The zero was set too low; this results in overestimating the actual resolving power because the peak-to-peak noise cannot be measured accurately.

FIGURE 3

TYPICAL 12-HOUR ANALYSIS SEQUENCE OF EVENTS.

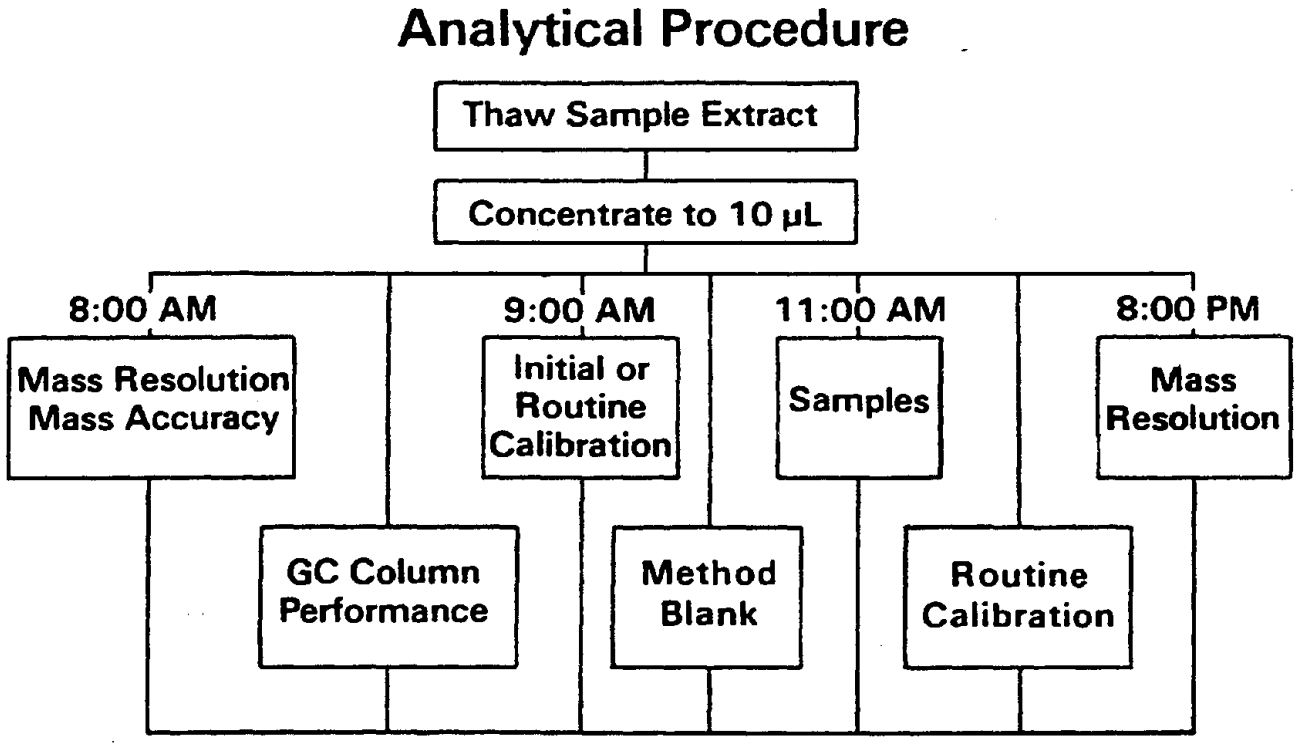
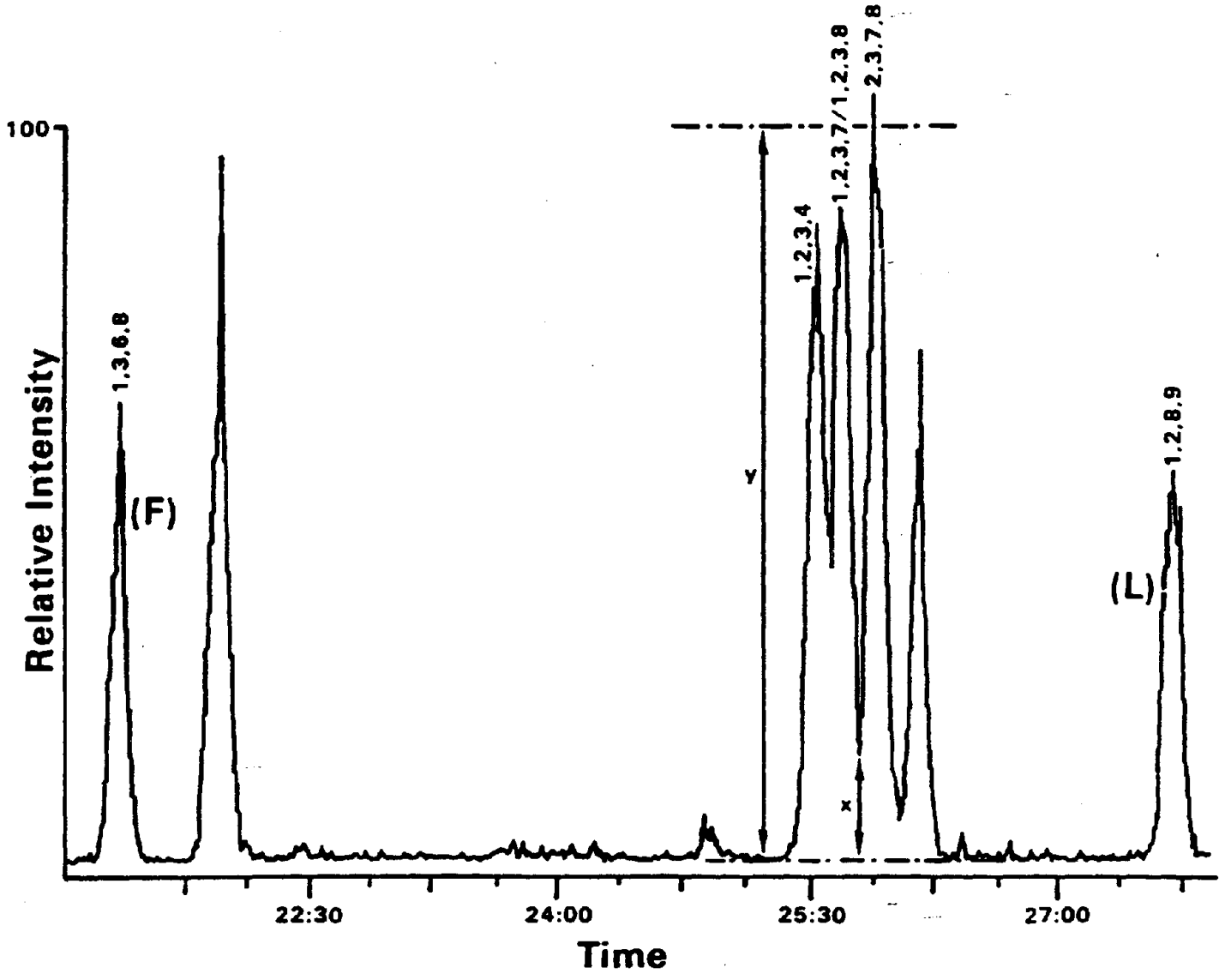
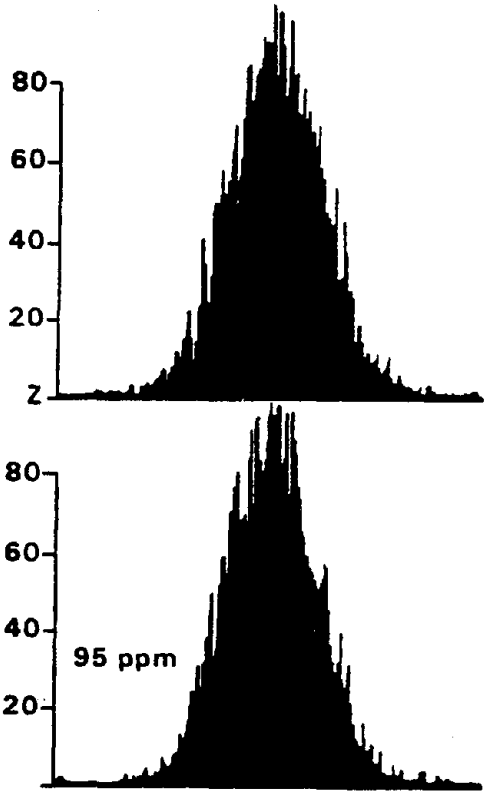


FIGURE 4



Selected ion current profile for m/z 322 (TCDDs) produced by MS analysis of the GC performance check solution on a 60 m DB-5 fused-silica capillary column under the conditions listed in Sec. 7.6.

FIGURE 5



Ref. mass 304.9824 Peak top
Span. 200 ppm

System file name	YVES150
Data file name	A:85Z567
Resolution	10000
Group number	1
Ionization mode	EI+
Switching	VOLTAGE
Ref. masses	304.9824
	380.9260

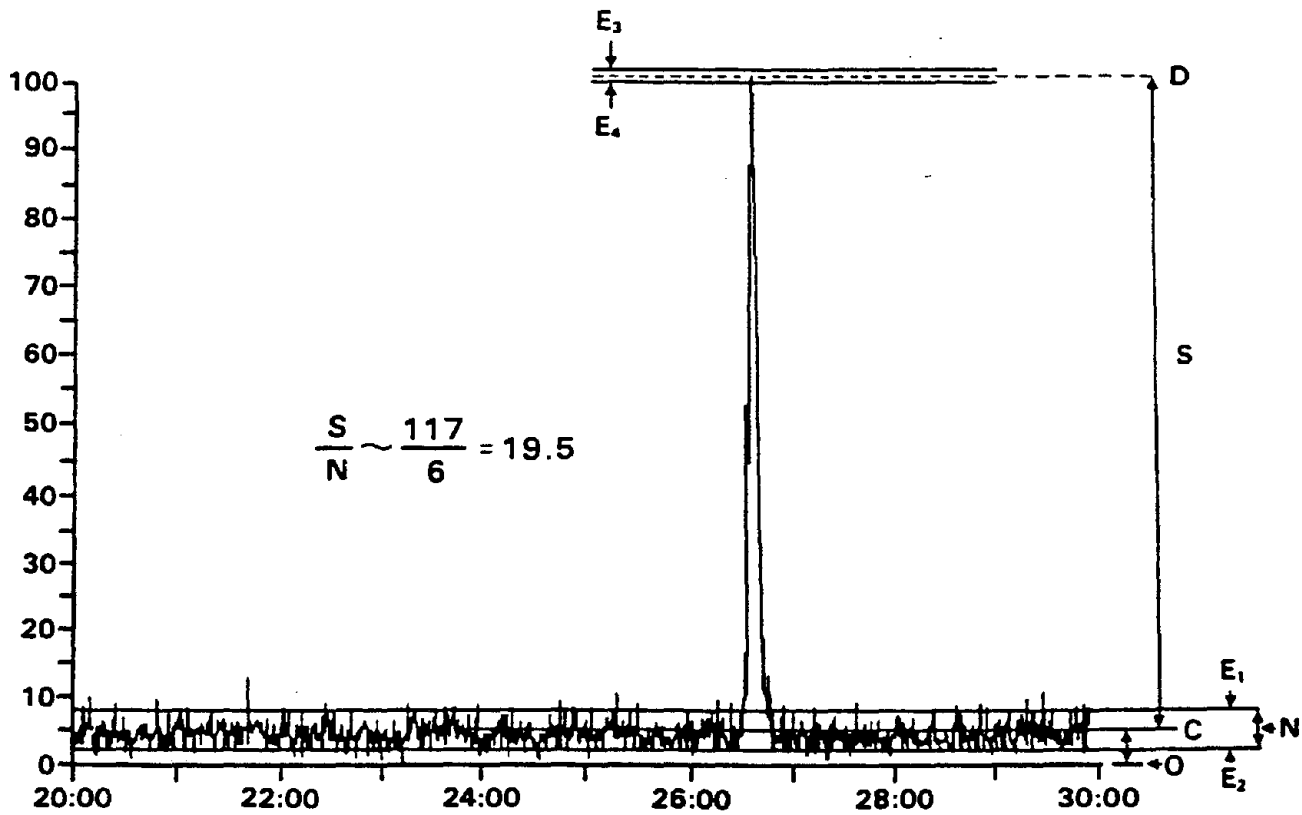
M/ΔM~10,500

Channel B 380.9260 Lock mass
Span 200 ppm

Peak profiles representing two PFK reference ions at m/z 305 and 381. The resolution of the high-mass signal is 95 ppm at 5 percent of the peak height; this corresponds to a resolving power M/ΔM of 10,500 (10 percent valley definition).

FIGURE 6

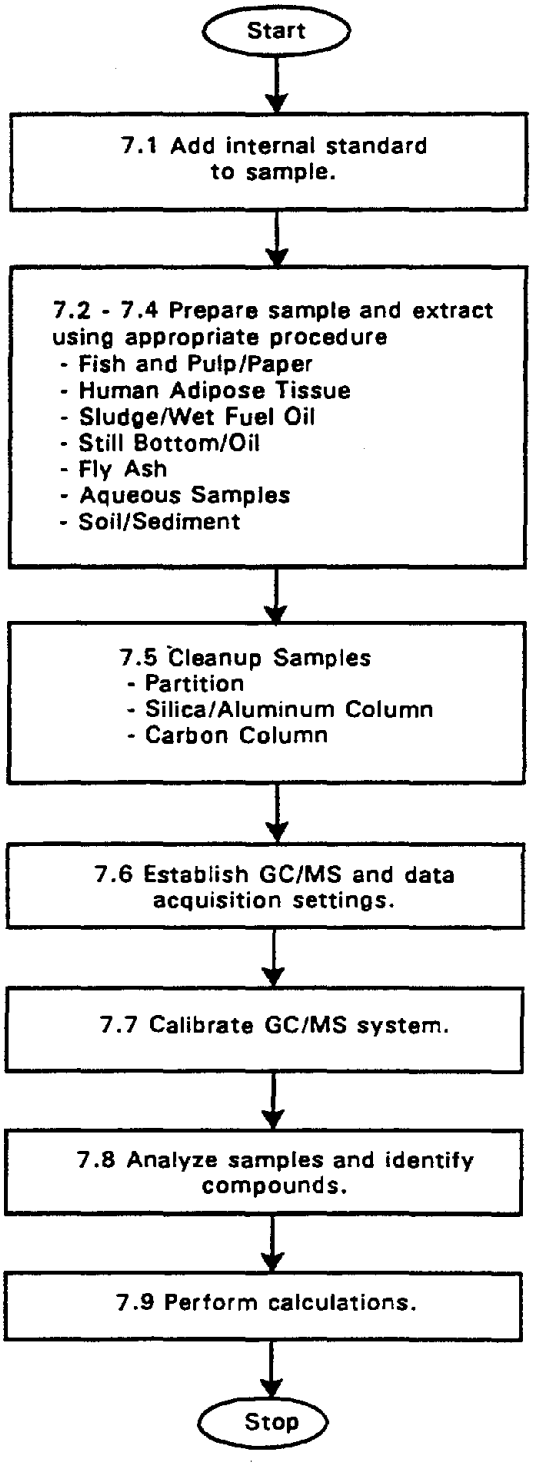
MANUAL DETERMINATION OF S/N.



The peak height (S) is measured between the mean noise (lines C and D). These mean signal values are obtained by tracing the line between the baseline average noise extremes, E1 and E2, and between the apex average noise extremes, E3 and E4, at the apex of the signal.

NOTE: It is imperative that the instrument interface amplifier electronic zero offset be set high enough so that negative going baseline noise is recorded.

POLYCHLORINATED DIBENZODIOXINS (PCDDs) AND POLYCHLORINATED DIBENZOFURANS (PCDFs) BY HIGH-RESOLUTION GAS CHROMATOGRAPHY/HIGH-RESOLUTION MASS SPECTROMETRY (HRGC/HRMS)



4.3 DETERMINATION OF ORGANIC ANALYTES

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgements necessary to meet the data quality objectives or needs for the intended use of the data.

4.3.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS

The following methods are included in this section:

Method 8310:	Polynuclear Aromatic Hydrocarbons
Method 8315A:	Determination of Carbonyl Compounds by High Performance Liquid Chromatography (HPLC)
Appendix A:	Recrystallization of 2,4-Dinitrophenylhydrazine (DNPH)
Method 8316:	Acrylamide, Acrylonitrile and Acrolein by High Performance Liquid Chromatography (HPLC)
Method 8318:	N-Methylcarbamates by High Performance Liquid Chromatography (HPLC)
Method 8321B:	Solvent-Extractable Nonvolatile Compounds by High Performance Liquid Chromatography/Thermospray/Mass Spectrometry (HPLC/TS/MS) or Ultraviolet (UV) Detection
Method 8325:	Solvent Extractable Nonvolatile Compounds by High Performance Liquid Chromatography/Particle Beam/Mass Spectrometry (HPLC/PB/MS)
Method 8330A:	Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC)
Method 8331:	Tetrazene by Reverse Phase High Performance Liquid Chromatography (HPLC)
Method 8332:	Nitroglycerine by High Performance Liquid Chromatography

METHOD 8321B

SOLVENT-EXTRACTABLE NONVOLATILE COMPOUNDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/THERMOSPRAY/MASS SPECTROMETRY (HPLC/TS/MS) OR ULTRAVIOLET (UV) DETECTION

1.0 SCOPE AND APPLICATION

1.1 This method covers the use of high performance liquid chromatography (HPLC), coupled with both thermospray-mass spectrometry (TS-MS) and an ultraviolet (UV) detector, for the determination of disperse azo dyes, organophosphorus compounds, tris(2,3-dibromopropyl) phosphate, chlorinated phenoxyacid compounds and their esters, and carbamates in wastewater, ground water, and soil/sediment matrices. Data are also provided for the determination of chlorophenoxy acid herbicides in fly ash (Table 12), however, recoveries for most compounds are very low, indicating poor extraction efficiency for these analytes using the extraction procedure included in this method. The following compounds may be determined by this method, although not all of the compounds are amenable to UV detection:

Analyte	CAS No.
Azo Dyes	
Disperse Red 1	2872-52-8
Disperse Red 5	3769-57-1
Disperse Red 13	126038-78-6
Disperse Yellow 5	6439-53-8
Disperse Orange 3	730-40-5
Disperse Orange 30	5261-31-4
Disperse Brown 1	17464-91-4
Solvent Red 3	6535-42-8
Solvent Red 23	85-86-9
Anthraquinone Dyes	
Disperse Blue 3	2475-46-9
Disperse Blue 14	2475-44-7
Disperse Red 60	17418-58-5
Coumarin Dyes	
Fluorescent Brighteners	
Fluorescent Brightener 61	8066-05-5
Fluorescent Brightener 236	3333-62-8
Alkaloids	
Caffeine	58-08-2
Strychnine	57-24-9

Analyte	CAS No.
Organophosphorus Compounds	
Methomyl	16752-77-5
Thiofanox	39196-18-4
Famphur	52-85-7
Asulam	3337-71-1
Dichlorvos	62-73-7
Dimethoate	60-51-5
Disulfoton	298-04-4
Fensulfothion	115-90-2
Merphos	150-50-5
Parathion methyl	298-00-0
Monocrotophos	6923-22-4
Naled	300-76-5
Phorate	298-02-2
Trichlorfon	52-68-6
Tris(2,3-dibromopropyl) phosphate (Tris-BP)	126-72-7
Chlorinated Phenoxyacid Compounds	
Dalapon	75-99-0
Dicamba	1918-00-9
2,4-D	94-75-7
MCPA	94-74-6
MCPP	7085-19-0
Dichlorprop	120-36-5
2,4,5-T	93-76-5
Silvex (2,4,5-TP)	93-72-1
Dinoseb	88-85-7
2,4-DB	94-82-6
2,4-D, butoxyethanol ester	1929-73-3
2,4-D, ethylhexyl ester	1928-43-4
2,4,5-T, butyl ester	93-79-8
2,4,5-T, butoxyethanol ester	2545-59-7
Carbamates	
Aldicarb*	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Aminocarb	2032-59-9

Analyte	CAS No.
Barban	101-27-9
Benomyl	17804-35-2
Bromacil	314-40-9
Bendiocarb*	22781-23-3
Carbaryl*	63-25-2
Carbendazim*	10605-21-7
3-Hydroxycarbofuran	16655-82-6
Carbofuran*	1563-66-2
Chloroxuron	1982-47-4
Chloropropham	101-21-3
Diuron*	330-54-1
Fenuron	101-42-8
Fluometuron	2164-17-2
Linuron*	330-55-2
Methiocarb	2032-65-7
Methomyl*	16752-77-5
Mexacarbate	315-18-4
Monuron	150-68-5
Neburon	555-37-3
Oxamyl*	23135-22-0
Propachlor	1918-16-7
Propham	122-42-9
Propoxur	114-26-1
Siduron	1982-49-6
Tebuthiuron	34014-18-1

[a] Chemical Abstract Service Registry Number.
* These carbamates were tested in a multi-laboratory evaluation. All others were tested in a single-laboratory evaluation.

1.2 This method may be applicable to the analysis of other non-volatile or semivolatile compounds that are solvent-extractable, are amenable to HPLC, and can be ionized under thermospray introduction for mass spectrometric detection or can be determined by a UV detector.

1.3 Method 8321 is designed to detect the chlorinated phenoxyacid compounds (free acid form) and their esters without the use of hydrolysis and esterification in the extraction procedure, although hydrolysis to the acid form will simplify quantitation.

1.4 The compounds listed in this method were chosen for analysis by HPLC/MS because they have been designated as problem compounds that are hard to analyze by gas chromatographic

methods. The sensitivity of this method is dependent upon the level of interferants within a given matrix, and varies with compound class and even by compound within a class. Additionally, the sensitivity is dependent upon the mode of operation of the mass spectrometer, with the selected reaction monitoring (SRM) mode providing greater sensitivity than single quadrupole scanning.

1.5 For further compound identification, MS/MS (CAD - Collision Activated Dissociation) can be used as an optional extension of this method.

1.6 Tris-BP has been classified as a carcinogen. Purified standard material and stock standard solutions should be handled in a hood.

1.7 This method is restricted to use by, or under the supervision of, analysts experienced in the use of high performance liquid chromatography using mass spectrometers or ultraviolet detectors. Analysts should also be skilled in the interpretation of liquid chromatograms and mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 This method provides reversed-phase high performance liquid chromatographic (RP/HPLC) and thermospray (TS) mass spectrometric (MS) conditions and ultraviolet (UV) conditions for the detection of the target analytes.

2.1.1 Sample extracts can be analyzed by direct injection into the thermospray or onto a liquid chromatographic-thermospray interface

2.1.2 A gradient elution program is used on the chromatograph to separate the compounds.

2.1.3 Quantitative analysis may be performed by either TS/MS or UV detection, using either an external or internal standard approach. TS/MS detection may be performed in either a negative ionization (discharge electrode) mode or a positive ionization mode, with a single quadrupole mass spectrometer.

2.1.4 In some cases, the thermospray interface may introduce variability that leads to less precise quantitation. In such instances, the MS response may be used to identify the analytes of interest while the quantitative results are derived from the response of the UV detector.

2.2 Prior to analysis, appropriate sample preparation techniques must be used.

2.2.1 Samples for analysis of chlorinated phenoxyacid compounds may be prepared by a modification of Method 8151 (see Sec. 7.3) or other appropriate extraction technique. In general, the pH of a 1-L aqueous sample or 50-g solid sample is adjusted and the sample is extracted with diethyl ether, concentrated, and the solvent exchanged to acetonitrile. Samples for these analytes may also be extracted using solid-phase extraction after a pH adjustment, as described in Method 3535.

2.2.2 For carbamates, 1-L aqueous samples or 40-g solid samples are extracted with methylene chloride (refer to appropriate 3500 series method), concentrated (preferably using a rotary evaporator with adapter) and the solvent exchanged to methanol.

2.2.3 Samples for analysis of the other target analytes are prepared by established extraction techniques. In general, water samples are extracted at a neutral pH with methylene chloride, using an appropriate 3500 series method. Solid samples are extracted with a mixture of methylene chloride/acetone (1:1), using an appropriate 3500 series method. Extract may require concentration and solvent exchange prior to analysis.

2.2.4 A micro-extraction technique for the extraction of Tris-BP from aqueous and non-aqueous matrices is included in this method (see Sec. 7.2).

2.3 An optional thermospray-mass spectrometry/mass spectrometry (TS-MS/MS) confirmatory procedure is provided in this method (see Sec. 7.11). That procedure employs MS/MS Collision Activated Dissociation (CAD) or wire-repeller CAD.

3.0 INTERFERENCES

3.1 Refer to Methods 3500, 3600, 8000 and 8151.

3.2 The use of Florisil Column Cleanup (Method 3620) has been demonstrated to yield recoveries less than 85% for some of the compounds in this method, and is therefore not recommended for all compounds. Refer to Table 2 of Method 3620 for recoveries of organophosphorus compounds as a function of Florisil fractions.

3.3 Compounds with high proton affinity may mask the MS response of some of the target analytes. Therefore, except when the thermospray MS/MS system is used for rapid screening of samples (see Sec. 7.11.1), an HPLC must be used to perform the chromatographic separations necessary for quantitative analyses.

3.4 Analytical difficulties encountered with specific organophosphorus compounds, as applied in this method, may include, but are not limited to, the following:

3.4.1 Methyl parathion shows some minor degradation during analysis.

3.4.2 Naled can undergo debromination to form dichlorvos. This reaction may occur during sample preparation and extraction, and the extent may depend of the nature of the sample matrix. The analyst should consider the potential for debromination of Naled when this compound is to be determined.

3.4.3 Merphos often contains contamination from merphos oxide. Oxidation of merphos can occur during storage, and possibly upon introduction into the mass spectrometer.

3.4.4 The water solubility of dichlorvos (DDVP) is 10 g/L at 20°C, and as a result, recovery of the this compound by solvent extraction from aqueous solutions is poor.

3.4.5 Trichloron rearranges and undergoes dehydrochlorination (loss of HCl) in acidic, neutral, or basic media, forming dichlorvos (DDVP). When either of these compounds are to be determined, the analyst should be aware of the possibility of this rearrangement in order to prevent misidentifications.

3.5 The chlorinated phenoxy acid compounds, being strong organic acids, react readily with alkaline substances and may be lost during analysis. Therefore, glassware and glass wool must be

acid-rinsed, and sodium sulfate must be acidified with sulfuric acid prior to use, to avoid this possibility.

3.6 Due to the reactivity of the chlorinated herbicides, the standards must be prepared in acetonitrile. Methylation will occur slowly, if prepared in methanol.

3.7 Benomyl quickly degrades to carbendazim in the environment (Reference 21).

3.8 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts or elevated baselines, or both, causing misinterpretation of chromatograms or spectra. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running reagent blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

3.9 Interferants co-extracted from the sample will vary considerably from source to source. Retention times of target analytes must be verified by using reference standards.

3.10 The optional use of HPLC/MS/MS methods aids in the confirmation of specific analytes. These methods are less subject to chemical noise than other mass spectrometric methods.

4.0 APPARATUS AND MATERIALS

4.1 HPLC/MS

The following apparatus and materials are necessary for the use of the HPLC/MS portions of this method.

4.1.1 High performance liquid chromatograph (HPLC) - An analytical system with programmable solvent delivery system and all required accessories, including injection loop (with a minimum 10-µL loop volume), analytical columns, purging gases, etc. At a minimum, the solvent delivery system must be capable of delivering a binary solvent system. The chromatographic system must be capable of being interfaced with a mass spectrometer (MS).

4.1.2 HPLC post-column addition pump - If post-column addition of reagents is employed, a pump is required. Ideally, this pump should be a syringe pump, and does not have to be capable of solvent programming. It is also possible to add the ionization reagents to the solvents and not perform post-column addition (see Sec. 7.6).

4.1.3 HPLC/MS interface

4.1.3.1 Interface - Thermospray ionization interface and source that will give acceptable calibration response for each analyte of interest at the concentration required. The source must be capable of generating both positive and negative ions, and have a discharge electrode or filament.

4.1.3.2 Micromixer - 10-µL, connects HPLC column system with HPLC post-column addition solvent system, if post-column addition is used.

4.1.4 Mass spectrometer system

4.1.4.1 A single quadrupole mass spectrometer capable of scanning from 1 to 1000 amu. The spectrometer must also be capable of scanning from 150 to 450 amu in 1.5 sec. or less, using 70 volts (nominal) electron energy in the positive or negative electron impact modes. In addition, the mass spectrometer must be capable of producing a calibrated mass spectrum for PEG 400, 600, or 800 (see Sec. 5.14) or other compounds used for mass calibration.

4.1.4.2 Optional triple quadrupole mass spectrometer - capable of generating daughter ion spectra with a collision gas in the second quadrupole and operation in the single quadrupole mode.

4.1.5 Data system - A computer system that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program must be connected to the mass spectrometer. The computer must have software that allows any MS data file to be searched for ions of a specified mass, and such ion abundances to be plotted versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software must also be available that allows integration of the abundances in any EICP between specified time or scan-number limits. There must be computer software available to operate the specific modes of the mass spectrometer.

4.2 HPLC with UV detector

An analytical system with solvent programmable pumping system for at least a binary solvent system, and all required accessories including syringes, 10-µL injection loop, analytical columns, purging gases, etc. An automatic injector is optional, but is useful for multiple samples. The columns specified in Sec. 4.3 are also used with this system.

If the UV detector is to be used in tandem with the thermospray interface, then the detector cell must be capable of withstanding high pressures (up to 6000 psi). However, the UV detector may be attached to an HPLC independent of the HPLC/TS/MS and, in that case, standard HPLC pressures are acceptable.

4.3 HPLC columns - A guard column and an analytical column are necessary.

The columns listed in this section were those used to develop the method. The mention of these columns is not intended to exclude the use of other columns that are available or that may be developed. Laboratories may use columns of other dimensions and/or packed with different stationary phases, provided that they document method performance data (e.g., chromatographic resolution, analyte breakdown, and quantitation limits) that provide analytical performance that is appropriate for the intended application.

4.3.1 Guard Column - C_{18} reversed-phase guard column, 10 mm x 2.6 mm ID, 0.5-µm frit, or equivalent. The guard column should be packed with the same or similar stationary phase as the analytical column.

4.3.2 Analytical Column - C_{18} reversed-phase column, 100 mm x 2 mm ID, 5-µm particle size of ODS-Hypersil; or C_8 reversed phase column, 100 mm x 2 mm ID, 3-µm particle size of MOS2-Hypersil, or equivalent.

4.4 Purification equipment for azo dye standards

4.4.1 Soxhlet extraction apparatus

4.4.2 Extraction thimbles - single thickness, 43 x 123 mm

4.4.3 Filter paper, 9.0 cm (Whatman qualitative No. 1 or equivalent).

4.4.4 Silica-gel column - 3 in. x 8 in., packed with silica gel (Type 60, EM reagent 70/230 mesh).

4.5 Extraction equipment for chlorinated phenoxyacid compounds

4.5.1 Erlenmeyer flasks - 500-mL wide-mouth glass, 500-mL glass, with 24/40 ground-glass joint, 1000-mL glass.

4.5.2 Separatory funnel - 2000-mL.

4.5.3 Graduated cylinder - 1000-mL.

4.5.4 Funnel - 75-mm diameter.

4.5.5 Wrist shaker - Burrell Model 75 or equivalent.

4.5.6 pH meter.

4.6 Kudema-Danish (K-D) apparatus (optional).

4.6.1 Concentrator tube - 10-mL graduated. A ground-glass stopper is used to prevent evaporation of extracts.

4.6.2 Evaporation flask - 500-mL. Attach to concentrator tube with springs, clamps, or equivalent.

4.6.3 Two-ball micro-Snyder column

4.6.4 Springs - ½ in.

4.6.5 Solvent vapor recovery system (Kontes K-545000-1006 or K-547300-0000, Ace Glass 6614-30, or equivalent).

NOTE: This glassware is recommended for the purpose of solvent recovery during the concentration procedures requiring the use of Kudema-Danish evaporative concentrators. Incorporation of this apparatus may be required by State or local municipality regulations that govern air emissions of volatile organics. EPA recommends the incorporation of this type of reclamation system as a method to implement an emissions reduction program. Solvent recovery is a means to conform with waste minimization and pollution prevention initiatives.

4.7 Disposable serological pipets

4.8 Collection tube - 15-mL conical, graduated.

4.9 Vials - 5-mL conical, glass, with PTFE-lined screw-caps or crimp tops.

4.10 Glass wool

4.11 Microsyringes - 100-µL, 50-µL, 10-µL (Hamilton 701 N or equivalent), and 50 µL (Blunted, Hamilton 705SNR or equivalent).

4.12 Rotary evaporator - Equipped with 1000-mL receiving flask.

4.13 Balances - Analytical, 0.0001 g, top-loading, 0.01 g.

4.14 Volumetric flasks, Class A - 10-mL to 1000-mL.

4.15 Graduated cylinder - 100-mL.

4.16 Separatory funnel - 250-mL.

4.17 Separatory funnel - 2-L, with PTFE stopcock.

4.18 Concentrator adaptor (optional) - for carbamate extraction.

4.19 Nitrogen evaporation apparatus - N-Evap Analytical Evaporator Model 111, Organomation Association Inc., Northborough, MA, or equivalent.

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Organic-free reagent water. All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Sodium sulfate (granular, anhydrous), Na_2SO_4. Purify by heating at 400°C for 4 hours in a shallow tray, or by precleaning the sodium sulfate with methylene chloride.

5.4 Ammonium acetate, NH_4OOCCH_3, solution (0.1 M). Filter through a 0.45-µm membrane filter (Millipore HA or equivalent).

5.5 Acetic acid, CH_3CO_2H

5.6 Sulfuric acid solution

5.6.1 (1:1, v/v) - Slowly add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of water.

5.6.2 (1:3, v/v) - Slowly add 25 mL H_2SO_4 (sp. gr. 1.84) to 75 mL of water.

5.7 Argon gas, 99+% pure.

5.8 Solvents - Unless otherwise noted, all solvents must be pesticide quality or equivalent.

5.8.1 Methylene chloride, CH_2Cl_2

5.8.2 Toluene, $C_6H_5CH_3$

5.8.3 Acetone, CH_3COCH_3

5.8.4 Diethyl Ether, $C_2H_5OC_2H_5$ - Must be free of peroxides as indicated by test strips (EM Quant, or equivalent). Procedures for removal of peroxides are provided with the test strips. After cleanup, 20 mL of ethyl alcohol preservative must be added to each liter of ether.

5.8.5 Methanol, CH_3OH - HPLC quality or equivalent.

5.8.6 Acetonitrile, CH_3CN - HPLC quality or equivalent.

5.8.7 Ethyl acetate, $CH_3CO_2C_2H_5$

5.9 Standard materials - pure standard materials or certified solutions of each analyte targeted for analysis. Disperse azo dyes must be purified before use according to Sec. 5.10.

WARNING: Tris-BP has been classified as a carcinogen. Purified standard material and stock standard solutions should be handled in a hood.

5.10 Disperse azo dye purification

Two procedures are involved. The first step is the Soxhlet extraction of the dye for 24 hours with toluene and evaporation of the liquid extract to dryness, using a rotary evaporator. The solid is then recrystallized from toluene, and dried in an oven at approximately 100°C. If this step does not give the required purity, column chromatography should be employed. Load the solid onto a 3 x 8 inch silica gel column (Sec. 4.4.4), and elute with diethyl ether. Separate impurities chromatographically, and collect the major dye fraction.

5.11 Stock standard solutions - Standards may be prepared from pure standard materials or may be purchased as certified solutions. Commercially-prepared stock standards may be used if they are certified by the manufacturer and verified against a standard made from pure material.

5.11.1 Prepare stock standard solutions by accurately weighing 0.0100 g of pure material. Dissolve the material in methanol or other suitable solvent (e.g., prepare Tris-BP in ethyl acetate), and dilute to known volume in a volumetric flask.

NOTE: Due to the reactivity of the chlorinated herbicides, the standards must be prepared in acetonitrile. Methylation will occur if standards are prepared in methanol.

If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially-prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

5.11.2 Transfer the stock standard solutions into glass vials with PTFE-lined screw-caps or crimp-tops. Store at 4°C and protect from light. Stock standard solutions

should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards.

5.12 Calibration standards - A minimum of five different concentrations for each parameter of interest should be prepared through dilution of the stock standards with methanol (or other suitable solvent). At least one of the calibration standards should correspond to a sample concentration at or below that necessary to meet the data quality objectives of the project. The remaining concentrations should correspond to the expected range of concentrations found in real samples, or should define the working range of the HPLC-UV or HPLC-TS/MS system (see Method 8000). Calibration standards must be replaced after one or two months, or sooner if comparison with check standards indicates a problem.

5.13 Surrogate standards - The analyst should monitor the performance of the extraction, cleanup (when used), and analytical system, along with the effectiveness of the method in dealing with each sample matrix, by spiking each sample, standard, and blank with one or two surrogates (e.g., organophosphorus or chlorinated phenoxyacid compounds not expected to be present in the sample).

5.14 HPLC/MS tuning standard - Polyethylene glycol 400 (PEG-400), PEG-600, or PEG-800 are recommended as tuning standards. However, analysts may use other tuning standards as recommended by the instrument manufacturer or other documented source. If one of the PEG solutions is used, dilute to 10 percent (v/v) in methanol. Which PEG is used will depend upon analyte molecular weight range: m.w. <500, use PEG-400; m.w. >500, use PEG-600 or PEG-800.

5.15 Internal standards - When the internal standard calibration option is used for HPLC/MS analyses, it is recommended that analysts use stable isotopically-labeled compounds of the same chemical class when they are available (e.g., $^{13}C_6$-carbofuran may be used as an internal standard in the analysis of carbamates).

5.16 Matrix spiking standards - Consult Method 3500 for information on matrix spiking solutions. Prepare a solution containing the analytes of interest in a suitable solvent.

NOTE: The form of the compounds used for spiking should be identical to the form of the target analytes. For the phenoxyacid herbicides in particular, use the acid form of the acid analytes, not the ester form or an ether, as use of these other forms will not represent the performance of the overall extraction, cleanup, and determinative methods relative to the target analytes. Conversely, when the ester forms are of the analytes of interest, e.g., 2,4-D, butoxyethanol ester, use the ester form of the analyte for preparing matrix spiking solutions.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

See the introductory material to Chapter Four, Organic Analytes, Sec. 4.1.

7.0 PROCEDURE

7.1 Sample preparation

Prior to analysis, samples must be extracted using either an appropriate 3500 series method or using specific procedures described in this method.

7.1.1 Samples for analysis of disperse azo dyes and organophosphorus compounds must be prepared by an appropriate 3500 series method prior to HPLC/MS analysis.

7.1.2 Samples for the analysis of Tris(2,3-dibromopropyl)phosphate (Tris-BP) must be prepared according to Sec. 7.2, prior to HPLC/MS analysis.

7.1.3 Samples for the analysis of chlorinated phenoxyacid compounds and their esters should be prepared according to Sec. 7.3, or other appropriate technique, prior to HPLC/MS analysis. TCLP leachates to be analyzed for the phenoxyacid herbicides may also be prepared using solid-phase extraction (SPE), as described in Method 3535.

7.2 Microextraction of Tris-BP

7.2.1 Solid samples

7.2.1.1 Weigh a 1-g portion of the sample into a tared beaker. If the sample appears moist, add an equivalent amount of anhydrous sodium sulfate and mix well. Add 100 µL of Tris-BP (approximate concentration 1000 mg/L) to the sample selected for spiking; the amount added should result in a final concentration of 100 ng/µL in the 1-mL extract.

7.2.1.2 Remove the glass wool plug from a disposable serological pipet. Insert a 1 cm plug of clean silane treated glass wool to the bottom (narrow end) of the pipet. Pack 2 cm of anhydrous sodium sulfate onto the top of the glass wool. Wash pipet and contents with 3 - 5 mL of methanol.

7.2.1.3 Pack the sample into the pipet prepared according to Sec. 7.2.1.2. If packing material has dried, wet with a few mL of methanol first, then pack sample into the pipet.

7.2.1.4 Extract the sample with 3 mL of methanol followed by 4 mL of 50% (v/v) methanol/methylene chloride (rinse the sample beaker with each volume of extraction solvent prior to adding it to the pipet containing the sample). Collect the extract in a 15-mL graduated glass tube.

7.2.1.5 Evaporate the extract to 1 mL using the nitrogen evaporation technique (Sec. 7.5). Record the volume. It may not be possible to evaporate some sludge samples to a reasonable concentration.

7.2.1.6 Determination of percent dry weight - When sample results are to be calculated on a dry weight basis, a second portion of sample should be weighed at the same time as the portion used for analytical determination.

WARNING: The drying oven should be contained in a hood or be vented. Significant laboratory contamination may result from drying a heavily contaminated sample.

Immediately after weighing the sample for extraction, weigh 5 - 10 g of the sample into a tared crucible. Dry this aliquot overnight at 105°C. Allow to cool in a desiccator before weighing. Calculate the % dry weight as follows:

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

This oven-dried aliquot is not used for the extraction and should be disposed of appropriately once the dry weight has been determined.

7.2.2 Aqueous samples

7.2.2.1 Using a 100-mL graduated cylinder, measure 100 mL of sample and transfer it to a 250-mL separatory funnel. Add 200 µL of Tris-BP (approximate concentration 1000 mg/L) to the sample selected for spiking; the amount added should result in a final concentration of 200 ng/µL in the 1-mL extract.

7.2.2.2 Add 10 mL of methylene chloride to the separatory funnel. Seal and shake the separatory funnel three times, approximately 30 seconds each time, with periodic venting to release excess pressure.

NOTE: Methylene chloride creates excessive pressure rapidly; therefore, initial venting should be done immediately after the separatory funnel has been sealed and shaken once. Methylene chloride is a suspected carcinogen, use necessary safety precautions.

7.2.2.3 Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete phase separation. See Section 7 of Method 3510.

7.2.2.4 Collect the extract in a 15-mL graduated glass tube. Concentrate the extract to 1 mL, using nitrogen evaporation (Sec. 7.5).

7.3 Extraction for chlorinated phenoxyacid compounds

Preparation of soil, sediment, and other solid samples should follow the procedures outlined in Method 8151, or other appropriate technique, with the exception of no hydrolysis or esterification is generally performed. However, if the analyst desires to determine all of the phenoxyacid moieties as the acid, hydrolysis may be performed. Sec. 7.3.1 presents an outline of the procedure with the appropriate changes necessary for determination by Method 8321. Sec. 7.3.2 describes the extraction procedure for aqueous samples. TCLP leachates may be extracted using solid-phase extraction, as described in Method 3535.

7.3.1 Extraction of solid samples

7.3.1.1 Add 50 g of soil/sediment sample to a 500-mL, wide-mouth Erlenmeyer flask. Add spiking solutions, if required, mix well and allow to stand for 15 minutes. Add 50 mL of organic-free reagent water and stir for 30 minutes. Determine the pH of the sample with a glass electrode and pH meter, while stirring. Adjust the pH to 2 with cold H_2SO_4 (1:1) and monitor the pH for 15 minutes, with stirring. If necessary, add additional H_2SO_4 until the pH remains at 2.

7.3.1.2 Add 20 mL of acetone to the flask, and mix the contents with the wrist shaker for 20 minutes. Add 80 mL of diethyl ether to the same flask, and shake

again for 20 minutes. Decant the extract and measure the volume of solvent recovered.

7.3.1.3 Extract the sample twice more using 20 mL of acetone followed by 80 mL of diethyl ether. After addition of each solvent, the mixture should be shaken with the wrist shaker for 10 minutes and the acetone-ether extract decanted.

7.3.1.4 After the third extraction, the volume of extract recovered should be at least 75% of the volume of added solvent. If this is not the case, additional extractions may be necessary. Combine the extracts in a 2000-mL separatory funnel containing 250 mL of 5% acidified sodium sulfate. If an emulsion forms, slowly add 5 g of acidified sodium sulfate (anhydrous) until the solvent-water mixture separates. A quantity of acidified sodium sulfate equal to the weight of the sample may be added, if necessary.

7.3.1.5 Check the pH of the extract. If it is not at or below pH 2, add more concentrated H_2SO_4 until the extract is stabilized at the desired pH. Gently mix the contents of the separatory funnel for 1 minute and allow the layers to separate. Collect the aqueous phase in a clean beaker, and the extract phase (top layer) in a 500-mL Erlenmeyer flask with a ground-glass stopper. Place the aqueous phase back into the separatory funnel and re-extract using 25 mL of diethyl ether. Allow the layers to separate and discard the aqueous layer. Combine the ether extracts in the 500-mL Erlenmeyer flask.

7.3.1.6 Add 45 - 50 g acidified anhydrous sodium sulfate to the combined ether extracts. Allow the extract to remain in contact with the sodium sulfate for approximately 2 hours.

NOTE: The drying step is very critical. Any moisture remaining in the ether will result in low recoveries. The amount of sodium sulfate used is adequate if some free flowing crystals are visible when swirling the flask. If all of the sodium sulfate solidifies in a cake, add a few additional grams of acidified sodium sulfate and again test by swirling. The 2-hour drying time is a minimum; however, the extracts may be held overnight in contact with the sodium sulfate.

7.3.1.7 Transfer the ether extract, through a funnel plugged with acid-washed glass wool, into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Use a glass rod to crush caked sodium sulfate during the transfer. Rinse the Erlenmeyer flask and column with 20-30 mL of diethyl ether to complete the quantitative transfer. Reduce the volume of the extract using the macro K-D technique (Sec. 7.5).

7.3.2 Extraction of aqueous samples

7.3.2.1 Using a 1000-mL graduated cylinder, measure 1 liter (nominal) of sample, record the sample volume to the nearest 5 mL, and transfer it to a separatory funnel. If high concentrations are anticipated, a smaller volume may be used and then diluted with organic-free reagent water to 1 liter. Adjust the pH to less than 2 with sulfuric acid (1:1).

7.3.2.2 Add 150 mL of diethyl ether to the sample bottle, seal, and shake for 30 seconds to rinse the walls. Transfer the solvent wash to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water layer for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, and may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Drain the aqueous phase into a 1000-mL Erlenmeyer flask.

7.3.2.3 Repeat the extraction two more times using 100 mL of diethyl ether each time. Combine the extracts in a 500-mL Erlenmeyer flask. (Rinse the 1000-mL flask with each additional aliquot of extracting solvent to make a quantitative transfer.)

7.3.2.4 Proceed to Sec. 7.5 for drying, K-D concentration, solvent exchange, and final volume adjustment.

7.4 Extraction of carbamates

Preparation of aqueous , soil, sediment, and other solid samples must follow an appropriate 3500 series method. The following sections provide general considerations.

7.4.1 One-liter aqueous samples are extracted with methylene chloride using an appropriate 3500 series method.

7.4.2 Forty-gram quantities of solid samples are extracted with methylene chloride using an appropriate 3500 series method.

7.4.3 Concentration steps can be performed using a rotary evaporator or K-D, reducing the final extract to 5-10 mL.

7.4.4 Final concentration of the extract and exchanging the solvent to a 1-mL final volume of methanol may be accomplished using an adaptor on the rotary evaporator. If an adaptor is unavailable, the final concentration may be performed using nitrogen evaporation, in a fume hood.

7.5 Extract concentration techniques

Two procedures are provided for the concentration of extracts: macro-concentration by Kudema-Danish (K-D) and micro-concentration by nitrogen evaporation.

7.5.1 Macro-concentration by K-D

Add one or two clean boiling chips to the flask and attach a three-ball macro-Snyder column. Attach the solvent vapor recovery glassware (condenser and collection device, Sec. 4.6.5) to the Snyder column of the K-D apparatus following manufacturer's instructions. Prewet the Snyder column by adding about 1 mL of diethyl ether to the top. Place the apparatus on a hot water bath (60°-65°C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed in vapor. Adjust the vertical position of the apparatus and the water temperature, as required, to complete the concentration in 15-20 minutes. At the proper rate of distillation the balls of the column will

actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 5 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

7.5.2 Solvent exchange

Prior to analysis, the final extract solvent must be exchanged to methanol or acetonitrile.

7.5.2.1 Transfer the concentrator tube to a nitrogen evaporation device. Add a total of 5 mL of the final solvent of choice (methanol or acetonitrile).

7.5.2.2 Reduce the extract volume according to Sec. 7.5.3 and adjust the final volume to 1 mL (or other volume necessary to achieve the required sensitivity).

7.5.3 Micro-concentration by nitrogen evaporation

7.5.3.1 Place the concentrator tube in a warm water bath (approximately 35°C) and evaporate the solvent volume to the required level using a gentle stream of clean, dry nitrogen (filtered through a column of activated carbon).

CAUTION: Do not use plasticized tubing between the carbon trap and the sample.

7.5.3.2 The internal wall of the tube must be rinsed down several times with the final solvent during the operation. During evaporation, the solvent level in the tube must be positioned to prevent water from condensing into the sample (i.e., the solvent level should be below the level of the water bath). Under normal operating conditions, the extract should not be allowed to become dry.

7.5.4 Transfer the extract to a glass vial with a PTFE-lined screw-cap or crimp-top and store refrigerated at 4°C. Proceed with HPLC analysis.

7.6 HPLC chromatographic conditions

7.6.1 Recommended mobile phases and elution gradients for some groups of analytes are shown in Tables 1 and 2. Analysts should also consult the instrument manufacturer's instructions. In the absence of specific recommendations, the following conditions may be a useful starting point.

Flow rate	0.8 mL/min
Post-column mobile phase	0.1 M ammonium acetate (1% methanol)/(0.1 M ammonium acetate for phenoxyacid compounds)
Post-column flow rate	0.4 mL/min

Optimize the instrumental conditions for resolution of the target analytes and sensitivity. Post-column addition of the MS ionization reagents may not be necessary in all instances, and these reagents may be added to the elution solvents, provided that adequate performance can be demonstrated.

NOTE: Once established, the same operating conditions must be used for both calibrations and sample analyses.

7.6.2 If there is a chromatographic problem from compound retention when analyzing disperse azo dyes, organophosphorus compounds, or tris(2,3-dibromopropyl)phosphate, a 2% constant flow of methylene chloride may be applied as needed. Methylene chloride/aqueous methanol solutions must be used with caution as HPLC eluants. Acetic acid (1%), another mobile phase modifier, can be used with compounds with acid functional groups.

7.6.3 A total flow rate of 1.0 to 1.5 mL/min may be necessary to maintain thermospray ionization, however, consult the instrument manufacturer's instructions and adjust the flow rate as needed.

7.7 Recommended thermospray/MS operating conditions

Prior to analysis of samples, the analyst should evaluate the relative sensitivity of the target compounds to each ionization mode to determine which may provide better sensitivity during analyses. This evaluation may be based on the structures of the analytes or by conducting analyses in each of the two ionization modes. Some groups of target compounds will have much better sensitivity using either positive or negative ionization (e.g., carbamates are generally more sensitive to the positive ionization mode and phenoxyacids are generally more sensitive to the negative ionization mode). When all the analytes of interest for a given application respond adequately in a given ionization mode, a single analysis using that mode may be employed.

7.7.1 Positive ionization mode conditions

Discharge electrode	Off
Filament	On or off (optional, analyte dependent)
Mass range	150 to 450 amu (analyte dependent, expect 1 to 18 amu higher than molecular weight of the compound).
Scan time	1.50 sec/scan
Optional repeller wire or plate	170 to 250 v (sensitivity optimized). See Figure 2 for schematic of source with wire repeller.

7.7.2 Negative ionization mode conditions

Discharge electrode	On
Filament	Off
Mass Range	135 to 450 amu
Scan time	1.50 sec/scan

7.7.3 Thermospray temperatures

Vaporizer control	110 to 130°C
Vaporizer tip	200 to 215°C
Jet	210 to 220°C
Source block	230 to 265°C. (Some compounds may degrade in the source block at higher temperatures, operator should use knowledge of chemical properties to estimate proper source temperature).

7.7.4 Sample injection volume

An injection volume of 20 to 100 µL is normally used. The injection loop must be overfilled by, minimally, a factor of two (e.g., 20-µL sample used to overfill a 10-µL injection loop) when manual injections are performed. If solids are present in the extract, allow them to settle or centrifuge the extract and withdraw the injection volume from the clear layer.

7.8 Calibration

7.8.1 Thermospray/MS system

When an MS detector is employed, the system must be tuned on quadrupole 1 (and quadrupole 3 for triple quadrupoles) for accurate mass assignment, sensitivity, and resolution. It is recommended that this be accomplished using polyethylene glycol (PEG) 400, 600, or 800 (see Sec. 5.14) which have average molecular weights of 400, 600, and 800, respectively. Analysts may use other tuning standards as recommended by the instrument manufacturer or other documented source. If PEGs are used, a mixture of these PEGs can be made such that it will approximate the expected working mass range for the analyses. Use PEG 400 for analysis of chlorinated phenoxyacid compounds. The PEG is introduced via the thermospray interface, circumventing the HPLC.

7.8.1.1 The mass calibration parameters are as follows:

PEG 400 and 600		PEG 800	
Mass range	15 to 765 amu	Mass range	15 to 900 amu
Scan time	0.5 to 5.0 sec/scan	Scan time	0.5 to 5.0 sec/scan

Approximately 100 scans should be acquired, with 2 to 3 injections made. The scan with the best fit to the accurate mass table (see Tables 7 and 8) should be used as the calibration table. If calibrants other than PEG are used, the mass range should be from 15 to approximately 20 amu higher than the highest mass used for calibration. A scan time should be chosen which will give at least 6 scans across the calibrant peak.

7.8.1.2 The low mass range from 15 to 100 amu is covered by the ions from the ammonium acetate buffer used in the thermospray process.

NH_4^+	18 amu
$NH_4^+ \cdot H_2O$	36 amu
$CH_3OH \cdot NH_4^+$	50 amu (methanol)
$CH_3CN \cdot NH_4^+$	59 amu (acetonitrile)
$CH_3OOH \cdot NH_4^+$	78 amu (acetic acid)

The appearance of m/z 50 or 59 depends upon the use of methanol or acetonitrile as the organic modifier. The higher mass range is covered by the ammonium ion adducts

of the various ethylene glycols (e.g., $H(OCH_2CH_2)_nOH$ where n=4, gives the $H(OCH_2CH_2)_4OH{\bullet}NH_4^+$ ion at m/z 212).

7.8.2 Liquid chromatographic system

7.8.2.1 Choose the proper ionization conditions for the MS detector, as outlined in Sec. 7.7. When UV detection is employed in conjunction with the MS detector, establish appropriate operating conditions for the UV detector.

7.8.2.2 Prepare five calibration standards (see Sec. 5.12 and Method 8000). Inject each calibration standard onto the HPLC, using the chromatographic conditions outlined in Table 1. Refer to Sec. 7.0 of Method 8000 for guidance on external and internal calibration options and calibration acceptance criteria. In most cases the $(M^+H)^+$ and $(M^+NH_4)^+$ adduct ions are the only ions of significant abundance. For example, Table 9 lists the retention times and the major ions (>5%) present in the positive ionization thermospray single quadrupole spectra of the organophosphorus compounds.

7.8.2.3 The use of selective ion monitoring (SIM) is acceptable in situations requiring detection limits below the normal range of full spectra analysis. However, SIM may provide a lesser degree of confidence in the compound identification unless multiple ions are monitored for each compound.

7.8.2.4 The use of selective reaction monitoring (SRM) is also acceptable when using triple-quad MS/MS and enhanced sensitivity is needed.

7.8.2.5 If UV detection is being used, integrate the area under the full chromatographic peak for each concentration. Quantitation by HPLC-UV may be preferred if it is known that sample interference and/or analyte coelution are not a problem, or when response of the MS detector is not sufficiently stable for quantitative analyses. In these instances, the MS response may be used for positive qualitative identification of the analytes while the UV response is used for quantitation.

7.8.2.6 The retention time of the chromatographic peak is an important variable in analyte identification. Therefore, the relative retention time of the analyte (versus the internal standard) should be in the range of 0.9 to 1.1.

7.8.3 Calibration verification

At the beginning of each analytical shift, the response of the instrument system must be verified by the analysis of a single standard at the approximate mid-point of the initial calibration range. Consult Method 8000 for information on performing this demonstration and the acceptance criteria that should be employed.

7.9 Sample Analysis

Once the LC system has been calibrated as outlined in Sec. 7.8, it is ready for sample analysis, employing both MS and UV detectors. Depending on the sensitivity necessary for a given project, analyses may be conducted using the MS detector in either the positive or negative ionization modes. The positive ionization mode generally provides greater sensitivity, and may be more appropriate for samples containing very low concentrations of the analytes of interest. However, analyst are advised that some compounds may be detectable in only the negative ionization mode.

7.9.1 An instrument blank (methanol) should be analyzed after the standards, in order to demonstrate that the system is free from contamination.

7.9.2 If performing manual injections, take an appropriate aliquot of the sample as per Sec. 7.7.4. Start the HPLC gradient elution, load and inject the sample aliquot, and start the mass spectrometer data system analysis.

7.9.3 If using an autoinjector, ensure that it is set up properly according to the manufacturer's instructions and that all samples and standards are loaded in the proper order. Start the autoinjector, the HPLC gradient elution, and the mass spectrometer data system.

7.9.4 The concentration of the analyte is determined by using the initial calibration data (see Method 8000) from either the MS or UV detector response. Samples whose concentrations exceed the calibration range must be diluted to fall within the range.

7.9.5 When using MS or MS/MS, and when it is appropriate for the compounds of interest and the project objectives, determinations in both positive and negative ionization analyses may be done on each sample extract.

7.10 Calculations

7.10.1 Using the external or internal standard calibration procedure (Method 8000), determine the identity and quantity of each component peak in the sample reconstructed ion chromatogram which corresponds to the compounds used for calibration processes. See Method 8000 for calculations.

7.10.2 The retention time of the chromatographic peak is an important parameter for the identity of the analyte. However, because matrix interferences can change chromatographic column conditions, the absolute retention times are not as significant as relative retention times (when using internal standards), and the mass spectral patterns are important criteria for analyte identification.

7.10.3 In instances when the TS/MS response exhibits higher variability, the MS response may be used to identify the analytes of interest while the quantitative results are derived from the response of the UV detector.

7.11 Optional MS/MS confirmation

With respect to this method, MS/MS shall be defined as the daughter ion collision activated dissociation acquisition with quadrupole one set on one mass (parent ion), quadrupole two pressurized with argon and with a higher offset voltage than normal, and quadrupole three set to scan desired mass range.

7.11.1 Since the thermospray process often generates only one or two ions per compound, the use of MS/MS is a more specific mode of operation yielding molecular structural information. In this mode, samples can be rapidly screened through direct injection of the sample into the thermospray (e.g., without using the HPLC to separate the sample components).

7.11.2 When using MS/MS, the first quadrupole should be set to the protonated molecule or ammoniated adduct of the analyte of interest. The third quadrupole should be set to scan from 30 amu to just above the mass region of the protonated molecule.

7.11.3 The collision gas pressure (Ar) should be set at about 1.0 mTorr and the collision energy at 20 eV. If these parameters fail to give considerable fragmentation, the settings may be increased to create more and stronger collisions.

7.11.4 For analytical determinations, the base peak of the collision spectrum shall be taken as the quantitation ion. For extra specificity, a second ion should be chosen as a backup quantitation ion.

7.11.5 Perform an initial calibration, as outlined in Sec. 7.8.

7.11.6 MS/MS contamination and interferences

7.11.6.1 If the MS/MS mode is to be used without chromatographic separation (rapid screening), then the method blank analysis must show that the sample preparation and analysis procedures are free of contamination by the analyte of interest or by interfering compounds. Refer to Sec. 8.0 of Method 8000 for guidance on acceptable method blank performance. If contamination is detected in the method blank above acceptable limits, re-extraction and reanalysis of the affected samples is necessary.

7.11.6.2 The MS/MS spectra of a calibration standard and the sample should be compared and the ratios of the three major (most intense) ions examined. These ratios should be approximately the same unless there is an interference. If an interference appears, chromatographic separation must be utilized.

7.11.6.3 The signal of the target analyte in a sample may be suppressed by co-extracted interferences which do not give a signal in the monitored ions. In order to monitor such signal suppression, an internal standard may be spiked into all standards, blanks, and sample extracts at a consistent concentration prior to analysis. The internal standard may be any compound which responds well in the appropriate ionization mode and which is not likely to be found in nature. (Note: Atrazine-d_5 has been used successfully for positive ion analysis, while 2,6-dinitrotoluene-d_3 has been used successfully for negative ion analysis.) The amount spiked should be chosen such that the signal produced is at least 100 times the noise level for the appropriate ion. The signal of the internal standard should be monitored. Reanalysis is required for any sample in which the internal standard peak height varies by more than 30% from the average internal standard height obtained during the five-point calibration. If reanalysis confirms this variance in signal, the sample should be reanalyzed using a chromatographic separation. Quantitation of analyte concentration may be performed using this internal standard. External standard quantitation is also allowed.

7.11.7 The total area of the quantitation ion(s) is calculated and the initial calibration is used to calculate sample results.

7.11.8 MS/MS techniques can also be used to perform structural analysis on ions represented by unassigned m/z ratios. The procedure for compounds of unknown structures is to set up a CAD experiment on the ion of interest. The spectrum generated from this experiment will reflect the structure of the compound by its fragmentation pattern. A trained mass spectroscopist and some history of the sample are usually needed to interpret the spectrum. (CAD experiments on actual standards of the expected compound are necessary for confirmation or denial of that substance.)

7.12 Optional wire-repeller CAD confirmation

7.12.1 See Figure 3 for the correct position of the wire-repeller in the thermospray source block.

7.12.2 Once the wire-repeller is inserted into the thermospray flow, the voltage can be increased to approximately 500 - 700 v. Enough voltage is necessary to create fragment ions, but not so much that shorting occurs.

7.12.3 Continue as outlined in Sec. 7.9.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures necessary to evaluate the HPLC system operation are found in Method 8000 and include evaluation of retention time windows, calibration verification and chromatographic analysis of samples. Check the performance of the entire analytical system daily using data gathered from analyses of blanks, standards, and replicate samples. If any of the chromatographic QC limits are not met, the analyst should examine the LC system for:

- Leaks,
- Proper pressure delivery,
- A dirty guard column; may need replacing or repacking, and
- Possible partial thermospray plugging.

Checking any of the above items will necessitate shutting down the HPLC/TS system, making repairs and/or replacements, and then restarting the analyses. A calibration verification standard should be reanalyzed before any sample analyses, as described in Sec. 7.8.3.

8.3 Initial demonstration of proficiency - Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Method 8000, Sec. 8.0 for information on how to accomplish this demonstration.

8.4 Sample quality control for preparation and analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch and the addition of surrogates to each field sample and QC sample.

8.4.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected

to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair.

8.4.2 A laboratory control sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

8.4.3 See Method 8000, Sec. 8.0 for the details on carrying out sample quality control procedures for preparation and analysis.

8.5 Surrogate recoveries - The laboratory must evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000, Sec. 8.0 for information on evaluating surrogate data and developing and updating surrogate limits.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 Single operator accuracy and precision studies have been conducted using spiked sediment, wastewater, sludge, and water samples. Tables 4, 5, and 6 provide single-laboratory data for Disperse Red 1. Table 1 provides the data for organophosphorus pesticides, Table 11 for Tris-BP, Table 12 for chlorophenoxyacid herbicides, and Tables 14 and 15 for carbamates.

9.2 Table 13 presents multi-laboratory accuracy and precision data for the chlorinated phenoxyacid herbicides. The data summary is based on data from three laboratories that analyzed duplicate solvent solutions at each concentration specified in the table.

9.3 Tables 16 and 17 present the multi-laboratory accuracy and precision data for the carbamates. The data summary is based on data from nine laboratories that analyzed triplicate solvent solutions at each concentration level specified in the tables.

9.4 Table 18 provides data for solid-phase extraction of 2,4-D and 2,4,5-TP spiked into TCLP buffers at two different spiking levels.

10.0 REFERENCES

1. Voyksner, R.D., Haney, C.A., "Optimization and Application of Thermospray High-Performance Liquid Chromatography/Mass Spectrometry", Anal. Chem., 1985, 57, 991-996.

2. Blakley, C.R., Vestal, M.L., "Thermospray Interface for Liquid Chromatography/Mass Spectrometry", Anal. Chem., 1983, 55, 750-754.

3. Taylor, V., Hickey, D.M., Marsden, P.J., "Single Laboratory Validation of EPA Method 8140", EPA-600/4-87/009, U.S. Environmental Protection Agency, Las Vegas, NV, 1987, 144 pp.

4. "Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry", Anal. Chem., 1980, 52, 2242-2249.

5. Betowski, L.D., Jones, T.L., "The Analysis of Organophosphorus Pesticide Samples by HPLC/MS and HPLC/MS/MS", Environmental Science and Technology, 1988.

8. U.S. EPA: 2nd Annual Report on Carcinogens, NTP 81-43, Dec. 1981, pp. 236-237.

9. Blum, A., Ames, B.N., Science 195, 1977, 17.

10. Zweidinger, R.A., Cooper, S.D., Pellazari, E.D., Measurements of Organic Pollutants in Water and Wastewater, ASTM 686.

11. Cremlyn, R., Pesticides: Preparation and mode of Action, John Wiley and Sons, Chichester, 1978, p. 142.

12. Cotterill, E.G., Byast, T.H., "HPLC of Pesticide Residues in Environmental Samples", In Liquid Chromatography in Environmental Analysis, Laurence, J.F., Ed., Humana Press, Clifton, NJ, 1984.

13. Voyksner, R.D., "Thermospray HPLC/MS for Monitoring the Environment", In Applications of New Mass Spectrometry Techniques in Pesticide Chemistry; Rosen, J.D., Ed., John Wiley and Sons: New York, 1987.

14. Yinon, J., Jones, T.L., Betowski, L.D., Rap. Comm. Mass Spectrom., 1989, 3, 38.

15. Shore, F.L., Amick, E.N., Pan, S.T., Gurka, D.F., "Single Laboratory Validation of EPA Method 8150 for the Analysis of Chlorinated Herbicides in Hazardous Waste", EPA/600/4-85/060, U.S. Environmental Protection Agency, Las Vegas, NV, 1985.

16. "Development and Evaluations of an LC/MS/MS Protocol", EPA/600/X-86/328, Dec. 1986.

17. "An LC/MS Performance Evaluation Study of Organophosphorus Pesticides", EPA/600/X-89/006, Jan. 1989.

18. "A Performance Evaluation Study of a Liquid Chromatography/Mass Spectrometry Method for Tris-(2,3-Dibromopropyl) Phosphate", EPA/600/X-89/135, June 1989.

19. "Liquid Chromatography/Mass Spectrometry Performance Evaluation of Chlorinated Phenoxyacid Herbicides and Their Esters", EPA/600/X-89/176, July 1989.

20. "An Interlaboratory Comparison of an SW-846 Method for the Analysis of the Chlorinated Phenoxyacid Herbicides by LC/MS", EPA/600/X-90/133, June 1990.

21. Somasundaram, L., and J.R. Coates, Ed., "Pesticide Transformation Products Fate and Significance in the Environment", ACS Symposium Series 459, Ch. 13, 1991.

22. Single-Laboratory Evaluation of Carbamates, APPL, Inc., Fresno, CA.

23. "Interlaboratory Calibration Study of a Thermospray-Liquid Chromatography/ Mass Spectrometry (TS-LC/MS) Method for Selected Carbamate Pesticides", EPA/600/X-92/102, August 1992.

24. Markell, C., "3M Data Submission to EPA," letter to B. Lesnik, June 27, 1995.

TABLE 1

RECOMMENDED HPLC CHROMATOGRAPHIC CONDITIONS

Analytes	Initial Mobile Phase (%)	Initial Time (min)	Final Gradient (linear)	Final Mobile Phase (%)	Time (min)
Organophosphorus Compounds	50/50 (water/methanol)	0	10	100 (methanol)	5
Azo Dyes	50/50 (Water/CH_3CN)	0	5	100 (CH_3CN)	5
Tris(2,3-dibromopropyl) phosphate	50/50 (water/methanol)	0	10	100 (methanol)	5
Chlorinated phenoxyacid compounds	75/25 (0.1 M NH_4 acetate in 1% acetic acid/ methanol)	2	15	40/60 (0.1 M NH_4 acetate in 1% acetic acid/ methanol)	
	40/60 (0.1 M Ammonium acetate in 1% acetic acid/ methanol)	3	5	75/25 (0.1 M Ammonium acetate in 1% acetic acid/ methanol)	10

TABLE 2

RECOMMENDED HPLC CHROMATOGRAPHIC CONDITIONS FOR CARBAMATES

	Time (min)	Mobile phase A (percent)	Mobile phase B (percent)
Option A	0	95	5
	30	20	80
	35	0	100
	40	95	5
	45	95	5

A = 5 mM ammonium acetate with 0.1 M acetic acid, and

B = methanol, with optional post-column addition of 0.5 M ammonium acetate

	Time (min)	Mobile phase A (percent)	Mobile phase B (percent)
Option B	0	95	5
	30	0	100
	35	0	100
	40	95	5
	45	95	5

A = water with 0.1 M ammonium acetate with 1% acetic acid

B = methanol with 0.1 M ammonium acetate with 1% acetic acid, with optional post-column addition of 0.1 M ammonium acetate.

TABLE 3

COMPOUNDS AMENABLE TO THERMOSPRAY MASS SPECTROMETRY

Disperse Azo Dyes	Alkaloids
Methine Dyes	Aromatic ureas
Arylmethane Dyes	Amides
Coumarin Dyes	Amines
Anthraquinone Dyes	Amino acids
Xanthene Dyes	Organophosphorus Compounds
Flame retardants	Chlorinated Phenoxyacid Compounds
Carbamates	

TABLE 4

PRECISION AND ACCURACY COMPARISONS OF MS AND MS/MS WITH HPLC/UV FOR ORGANIC-FREE REAGENT WATER SPIKED WITH DISPERSE RED 1

	Percent Recovery			
	HPLC/UV	MS	CAD	SRM
Spike 1	82.2 ± 0.2	92.5 ± 3.7	87.6 ± 4.6	95.5 ± 17.1
Spike 2	87.4 ± 0.6	90.2 ± 4.7	90.4 ± 9.9	90.0 ± 5.9
RPD	6.1%	2.5%	3.2%	5.9%

Data from Reference 16.

TABLE 5

PRECISION AND ACCURACY COMPARISONS OF MS AND MS/MS WITH HPLC/UV FOR MUNICIPAL WASTEWATER SPIKED WITH DISPERSE RED 1

	Percent Recovery		
	HPLC/UV	MS	CAD
Spike 1	93.4 ± 0.3	102.0 ± 31	82.7 ± 13
Spike 2	96.2 ± 0.1	79.7 ± 15	83.7 ± 5.2
RPD	3.0%	25%	1.2%

Data from Reference 16.

TABLE 6

RESULTS FROM ANALYSES OF ACTIVATED SLUDGE PROCESS WASTEWATER

	Recovery of Disperse Red 1 (mg/L)		
	HPLC/UV	MS	CAD
5 mg/L Spiking Concentration			
1	0.721 ± 0.003	0.664 ± 0.030	0.796 ± 0.008
1-D	0.731 ± 0.021	0.600 ± 0.068	0.768 ± 0.093
2	0.279 ± 0.000	0.253 ± 0.052	0.301 ± 0.042
3	0.482 ± 0.001	0.449 ± 0.016	0.510 ± 0.091
RPD	1.3%	10.1%	3.6%
0 mg/L Spiking Concentration			
1	0.000	0.005 ± 0.0007	<0.001
1-D	0.000	0.006 ± 0.001	<0.001
2	0.000	0.002 ± 0.0003	<0.001
3	0.000	0.003 ± 0.0004	<0.001
RPD	–	18.2%	–

Data from Reference 16.

TABLE 7

CALIBRATION MASSES AND % RELATIVE ABUNDANCES OF PEG 400

Mass	% Relative Abundance[a]
18.0	32.3
35.06	13.5
36.04	40.5
50.06	94.6
77.04	27.0
168.12	5.4
212.14	10.3
256.17	17.6
300.20	27.0
344.22	45.9
388.25	64.9
432.28	100
476.30	94.6
520.33	81.1
564.35	67.6
608.38	32.4
652.41	16.2
653.41	4.1
696.43	8.1
697.44	2.7

[a] Intensities are normalized to mass 432.

TABLE 8

CALIBRATION MASSES AND % RELATIVE ABUNDANCES OF PEG 600

Mass	% Relative Abundance[a]
18.0	4.7
36.04	11.4
50.06	64.9
77.04	17.5
168.12	9.3
212.14	43.9
256.17	56.1
300.20	22.8
344.22	28.1
388.25	38.6
432.28	54.4
476.30	64.9
520.33	86.0
564.35	100
608.38	63.2
652.41	17.5
653.41	5.6
696.43	1.8

[a] Intensities are normalized to mass 564.

TABLE 9

RETENTION TIMES AND THERMOSPRAY MASS SPECTRA
OF ORGANOPHOSPHORUS COMPOUNDS

Compound	Retention Time (min)	Mass (% Relative Abundance)[a]
Monocrotophos	1:09	241 (100), 224 (14)
Trichlorfon	1:22	274 (100), 257 (19), 238 (19)
Dimethoate	1:28	230 (100), 247 (20)
Dichlorvos	4:40	238 (100), 221 (40)
Naled	9:16	398 (100), 381 (23), 238 (5), 221, (2)
Fensulfothion	9:52	326 (10), 309 (100)
Parathion methyl	10:52	281 (100), 264 (8), 251 (21), 234 (48)
Phorate	13:30	278 (4), 261 (100)
Disulfoton	13:55	292 (10), 275 (100)
Merphos	18:51	315 (100), 299 (15)

[a] For molecules containing Cl, Br and S, only the base peak of the isotopic cluster is listed.

Data from Reference 17.

TABLE 10

SINGLE OPERATOR ACCURACY AND PRECISION FOR LOW CONCENTRATION DRINKING WATER, LOW CONCENTRATION SOIL, MEDIUM CONCENTRATION DRINKING WATER, MEDIUM CONCENTRATION SEDIMENT

Matrix	Compound	Mean Rec. (%)	Std. Dev.	Spike Conc.	Recovery Range (%)	# Analyses
Low conc. drinking water	Dimethoate	70	7.7	5	85 - 54	15
(µg/L)	Dichlorvos	40	12	5	64 - 14	15
	Naled	0.5	1.0	5	2 - 0	15
	Fensulfothion	112	3.3	5	119 - 106	15
	Parathion methyl	50	28	10	105 - 0	15
	Phorate	16	35	5	86 - 0	15
	Disulfoton	3.5	8	5	19 - 0	15
	Merphos	237	25	5	287 - 187	15
Low conc. soil	Dimethoate	16	4	50	24 - 7	15
(µg/kg)	Dichlorvos	ND		50		15
	Naled	ND		50		15
	Fensulfothion	45	5	50	56 - 34	15
	Parathion methyl	ND		100		15
	Phorate	78	15	50	109 - 48	15
	Disulfoton	36	7	50	49 - 22	15
	Merphos	118	19	50	155 - 81	15

TABLE 10
(continued)

Matrix	Compound	Mean Rec. (%)	Std. Dev.	Spike Conc.	Recovery Range (%)	# Analyses
Medium conc. drinking water	Dimethoate	52	4	50	61 - 43	12
(µg/L)	Dichlorvos	146	29	50	204 - 89	12
	Naled	4	3	50	9 - 0	12
	Fensulfothion	65	7	50	79 - 51	12
	Parathion methyl	85	24	100	133 - 37	12
	Phorate	10	15	50	41 - 0	12
	Disulfoton	2	1	50	4 - 0	12
	Merphos	101	13	50	126 - 75	12
Medium conc. sediment	Dimethoate	74	8.5	2	91 - 57	15
(mg/kg)	Dichlorvos	166	25	2	216 - 115	15
	Naled	ND		2		15
	Fensulfothion	72	8.6	2	90 - 55	15
	Parathion methyl	84	9	3	102 - 66	15
	Phorate	58	6	2	70 - 46	15
	Disulfoton	56	5	2	66 - 47	15
	Merphos	78	4	2	86 - 70	12

Data from Reference 17.

TABLE 11

SINGLE OPERATOR ACCURACY AND PRECISION FOR TRIS-BP IN MUNICIPAL WASTE WATER, DRINKING WATER, CHEMICAL SLUDGE

Compound	Matrix	Mean Rec. (%)	Std. Dev.	Spike Conc.	Recovery Range (%)	# Analyses
Tris-BP	Municipal wastewater	25	8.0	2	41 - 9.0	15
	Drinking water	40	5.0	2	50 - 30	12
	Chemical sludge	63	11	100	84 - 42	8

Data from Reference 18.

TABLE 12

SINGLE LABORATORY OPERATOR ACCURACY AND PRECISION
FOR THE CHLORINATED PHENOXYACID HERBICIDES

Compound	Mean Recovery %	Std. Dev.	Spike Conc.	Recovery Range (%)	# Analyses
LOW LEVEL DRINKING WATER			µg/L		
Dicamba	63	22	5	86 - 33	9
2,4-D	26	13	5	37 - 0	9
MCPA	60	23	5	92 - 37	9
MCPP	78	21	5	116 - 54	9
Dichlorprop	43	18	5	61 - 0	9
2,4,5-T	72	31	5	138 - 43	9
Silvex	62	14	5	88 - 46	9
2,4-DB	29	24	5	62 - 0	9
Dinoseb	73	11	5	85 - 49	9
Dalapon	ND	ND	5	ND	9
2,4-D,ester	73	17	5	104 - 48	9
HIGH LEVEL DRINKING WATER					
Dicamba	54	30	50	103 - 26	9
2,4-D	60	35	50	119 - 35	9
MCPA	67	41	50	128 - 32	9
MCPP	66	33	50	122 - 35	9
Dichlorprop	66	33	50	116 - 27	9
2,4,5-T	61	23	50	99 - 44	9
Silvex	74	35	50	132 - 45	9
2,4-DB	83	25	50	120 - 52	9
Dinoseb	91	10	50	102 - 76	9
Dalapon	43	9.6	50	56 - 31	9
2,4-D,ester	97	19	50	130 - 76	9

TABLE 12
(continued)

Compound	Mean Recovery %	Std. Dev.	Spike Conc.	Recovery Range (%)	# Analyses
LOW LEVEL SAND			µg/g		
Dicamba	117	26	0.1	147 - 82	10
2,4-D	147	23	0.1	180 - 118	10
MCPA	167	79	0.1	280 - 78	10
MCPP	142	39	0.1	192 - 81	10
Dichlorprop	ND	ND	0.1	ND	10
2,4,5-T	134	27	0.1	171 - 99	10
Silvex	121	23	0.1	154 - 85	10
2,4-DB	199	86	0.1	245 - 0	10
Dinoseb	76	74	0.1	210 - 6	10
Dalapon	ND	ND	0.1	ND	10
2,4-D,ester	180	58	0.1	239 - 59	7
HIGH LEVEL SAND			µg/g		
Dicamba	153	33	1	209 - 119	9
2,4-D	218	27	1	276 - 187	9
MCPA	143	30	1	205 - 111	9
MCPP	158	34	1	226 - 115	9
Dichlorprop	92	37	1	161 - 51	9
2,4,5-T	160	29	1	204 - 131	9
Silvex	176	34	1	225 - 141	9
2,4-DB	145	22	1	192 - 110	9
Dinoseb	114	28	1	140 - 65	9
Dalapon	287	86	1	418 - 166	9
2,4-D,ester	20	3.6	1	25 - 17	7

TABLE 12
(continued)

Compound	Mean Recovery %	Std. Dev.	Spike Conc.	Recovery Range (%)	# Analyses
LOW LEVEL MUNICIPAL ASH			µg/g		
Dicamba	83	22	0.1	104 - 48	9
2,4-D	ND	ND	0.1	ND	9
MCPA	ND	ND	0.1	ND	9
MCPP	ND	ND	0.1	ND	9
Dichlorprop	ND	ND	0.1	ND	9
2,4,5-T	27	25	0.1	60 - 0	9
Silvex	68	38	0.1	128 - 22	9
2,4-DB	ND	ND	0.1	ND	9
Dinoseb	44	13	0.1	65 - 26	9
Dalapon	ND	ND	0.1	ND	9
2,4-D,ester	29	23	0.1	53 - 0	6
HIGH LEVEL MUNICIPAL ASH			µg/g		
Dicamba	66	21	1	96 - 41	9
2,4-D	8.7	4.8	1	21 - 5	9
MCPA	3.2	4.8	1	10 - 0	9
MCPP	10	4.3	1	16 - 4.7	9
Dichlorprop	ND	ND	1	ND	9
2,4,5-T	2.9	1.2	1	3.6 - 0	9
Silvex	6.0	3.1	1	12 - 2.8	9
2,4-DB	ND	ND	1	ND	9
Dinoseb	16	6.8	1	23 - 0	9
Dalapon	ND	ND	1	ND	9
2,4-D,ester	1.9	1.7	1	6.7 - 0	6

Source: Reference 19.
All recoveries are in negative ionization mode, except for 2,4-D, ester.
ND = Not Detected.

TABLE 13

MULTI-LABORATORY ACCURACY AND PRECISION DATA
FOR THE CHLORINATED PHENOXYACID HERBICIDES

Compound	Spiking Concentration	Mean (% Recovery)[a]	RSD[b]
2,4,5-T	500 mg/L	90	23
2,4,5-T,butoxy ester		90	29
2,4-D		86	17
2,4-DB		95	22
Dalapon		83	13
Dicamba		77	25
Dichlorprop		84	20
Dinoseb		78	15
MCPA		89	11
MCPP		86	12
Silvex		96	27
2,4,5-T	50 mg/L	62	68
2,4,5-T,butoxy ester		85	9
2,4-D		64	80
2,4-DB		104	28
Dalapon		121	99
Dicamba		90	23
Dichlorprop		96	15
Dinoseb		86	57
MCPA		96	20
MCPP		76	74
Silvex		65	71

TABLE 13
(continued)

Compound	Spiking Concentration	Mean (% Recovery)[a]	RSD[b]
2,4,5-T	5 mg/L	90	28
2,4,5-T,butoxy ester		99	17
2,4-D		103	31
2,4-DB		96	21
Dalapon		150	4
Dicamba		105	12
Dichlorprop		102	22
Dinoseb		108	30
MCPA		94	18
MCPP		98	15
Silvex		87	15

[a] Mean of duplicate data from 3 laboratories.
[b] Relative standard deviation of duplicate data from 3 laboratories.
Data from Reference 20.

TABLE 14

SINGLE-LABORATORY EVALUATION OF AVERAGE RECOVERY AND PRECISION DATA FOR WATER[c]

Analyte	Average % Recovery[b]	Standard Deviation	%RSD
Aldicarb sulfoxide	7.6	2.8	37.0
Aldicarb sulfone	56.0	27.1	48.5
Oxamyl[a]	38.9	17.9	45.9
Methomyl	52.0	19.6	37.7
3-Hydroxycarbofuran[a]	22.2	9.3	41.7
Fenuron	72.5	22.0	30.3
Benomyl/Carbendazim	47.3	14.7	31.0
Aldicarb	81.0	13.7	16.9
Aminocarb	109	38.3	35.1
Carbofuran	85.5	10.0	11.7
Propoxur	79.1	13.7	17.3
Monuron	91.8	11.3	12.3
Bromacil	87.6	12.1	13.8
Tebuthiuron	87.1	9.0	10.3
Carbaryl	82.1	13.5	16.5
Fluometuron	84.4	8.3	9.8
Propham	80.7	13.8	17.1
Propachlor	84.3	10.0	11.9
Diuron	90.8	14.1	15.6
Siduron	88.0	9.5	10.8
Methiocarb	93.3	12.8	13.8
Barban	88.1	11.2	12.7
Linuron	87.1	16.8	19.3
Chloropropham	94.9	15.3	16.1
Mexacarbate	79.8	12.9	16.2
Chloroxuron	106	24.9	23.5
Neburon	85.3	12.6	14.8

[a] Values generated from internal response factor calculations.

[b] Nine spikes were performed at three concentrations. The concentrations for Aldicarb sulfoxide, Barban, Chloropropham, and Mexacarbate spike levels were at 25 µg/L, 50 µg/L, and 100 µg/L. All other analyte concentrations were 5 µg/L, 10 µg/L, and 20 µg/L. One injection was disregarded as an outlier. The total number of spikes analyzed was 26.

[c] Data from Reference 22.

TABLE 15

SINGLE-LABORATORY EVALUATION OF AVERAGE RECOVERY AND PRECISION DATA FOR SOIL[b]

Analyte	Average % Recovery[a]	Standard Deviation	%RSD
Aldicarb sulfoxide	66.9	31.3	46.7
Aldicarb sulfone	162	51.4	31.7
Oxamyl	78.9	46.1	58.5
Methomyl	84.9	25.8	30.4
3-Hydroxycarbofuran	105	36.3	34.5
Fenuron	91.9	16.7	18.1
Benomyl/Carbendazim	95.6	18.2	19.0
Aldicarb	97.9	17.0	17.4
Aminocarb	133	44.7	33.6
Carbofuran	109	14.4	13.2
Propoxur	104	16.5	15.9
Monuron	101	12.4	12.3
Bromacil	100	9.0	9.0
Tebuthiuron	104	11.9	11.5
Carbaryl	102	15.5	15.2
Fluometuron	94.5	15.7	16.7
Propham	92.8	12.0	12.9
Propachlor	94.6	10.3	10.9
Diuron	107	17.4	16.2
Siduron	100	12.0	12.0
Methiocarb	107	14.2	13.2
Barban	92.3	15.6	16.9
Linuron	104	13.6	13.1
Chloropropham	105	9.3	8.9
Mexacarbate	77.2	9.8	12.7
Chloroxuron	121	27.3	22.5
Neburon	92.1	16.5	17.9

[a] Nine spikes were performed at three concentrations. The concentrations for Aldicarb sulfoxide, Barban, Chloropropham, and Mexacarbate spike levels were at 0.625 µg/g, 1.25 µg/g, and 2.5 µg/g. All other analyte concentrations were 0.125 µg/g, 0.25 µg/g, and 0.50 µg/g. One injection was disregarded as an outlier. The total number of spikes analyzed was 26.

[b] Data from Reference 22.

TABLE 16

MULTI-LABORATORY EVALUATION OF METHOD ACCURACY
(AFTER OUTLIER REMOVAL)[d]

Analyte	Percent Recovery		
	High-Concentration Samples[a]	Medium-Concentration Samples[b]	Low-Concentration Samples[c]
Aldicarb	98.7	110	52.0
Bendiocarb	81.4	95.0	52.0
Carbaryl	92.0	108	62.0
Carbendazim	125	138	128
Carbofuran	87.8	92.3	72.0
Diuron	79.9	98.8	66.0
Linuron	84.8	93.0	82.0
Methomyl	93.3	90.8	90.0
Oxamyl	83.8	88.0	98.0

[a] Three replicates per laboratory; eight to nine laboratories (per Table 26 of Reference 23). The true concentration is 90 mg/L per compound, except Carbendazim at 22.5 mg/L.

[b] Two replicates per laboratory; eight to nine laboratories (per Table 26 of Reference 23). The true concentration is 40 mg/L per compound except Carbendazim at 10 mg/L.

[c] Three replicates per laboratory; eight to nine laboratories (per Table 26 of Reference 23). The true concentration is 5 mg/L per compound, except Carbendazim at 1.25 mg/L.

[d] Data from Reference 23.

TABLE 17

MULTI-LABORATORY EVALUATION OF METHOD PRECISION (AFTER OUTLIER REMOVAL)[a]

	High Concentration					Medium Concentration					Low Concentration				
Analyte	Avg.	s_r	s_R	$\%RSD_R$	$\%RSD_R$	Avg.	s_r	s_R	$\%RSD_r$	$\%RSD_R$	Avg	s_r	s_R	$\%RSD_r$	$\%RSD_R$
Aldicarb	88.8	11.4	34.4	12.9	38.8	44.1	7.7	17.0	17.5	38.5	2.6	0.9	2.6	33.1	98.2
Bendiocarb	73.3	16.1	39.3	21.9	53.6	38.0	6.6	16.6	17.3	43.7	2.6	0.6	1.6	21.3	61.9
Carbaryl	82.8	11.7	34.0	14.2	41.1	43.1	3.0	15.7	7.0	36.4	3.1	0.7	2.3	23.3	75.8
Carbendazim	28.1	5.6	15.3	19.9	54.4	13.8	1.4	8.9	10.4	64.2	1.6	0.4	1.1	26.1	68.2
Carbofuran	79.0	16.7	35.2	21.2	44.5	36.9	5.0	16.3	13.6	44.3	3.6	0.9	3.3	25.2	91.6
Diuron	71.9	13.1	26.1	18.2	36.3	39.5	2.6	11.8	6.5	29.8	3.3	0.5	2.6	16.2	77.9
Linuron	76.3	8.3	32.5	10.9	42.6	37.2	3.9	13.4	10.5	35.9	4.1	0.6	2.1	15.7	51.4
Methomyl	84.0	10.8	29.4	12.9	35.0	36.3	2.8	15.0	7.8	41.2	4.5	0.7	4.1	15.3	92.9
Oxamyl	75.5	12.4	37.0	16.4	49.1	35.2	3.7	20.8	10.4	59.1	4.9	0.5	4.6	9.7	93.6
Average				16.5	43.9				11.2	43.7				20.7	79.1
Std. Dev.				4.0	7.1				4.1	11.2				7.1	16.3

s_r and s_R are the standard deviations for repeatability and reproducibility, respectively. RSD_r and RSD_R are the corresponding relative standard deviations for repeatability and reproducibility, respectively. The units for average, s_r and s_R are mg/L.

[a] Data from Reference 23.

TABLE 18

SINGLE LABORATORY RECOVERY DATA FOR SOLID-PHASE EXTRACTION OF CHLORINATED HERBICIDES FROM SPIKED TCLP BUFFERS

		Buffer 1		Buffer 2	
Compound	Spike Level (µg/L)	Recovery (%)	RSD	Recovery (%)	RSD
2,4-D	5,000	91	2	79	6
2,4,5-TP	500	93	9	92*	2*
2,4-D	20,000	100	3	99*	1*
2,4,5-TP	2000	103*	2*	78	7

Except where noted with an asterisk, all results are from seven replicates. Those marked with an asterisk are from three replicates.

Data are from Reference 24.

FIGURE 1
SCHEMATIC OF THE THERMOSPRAY PROBE AND ION SOURCE

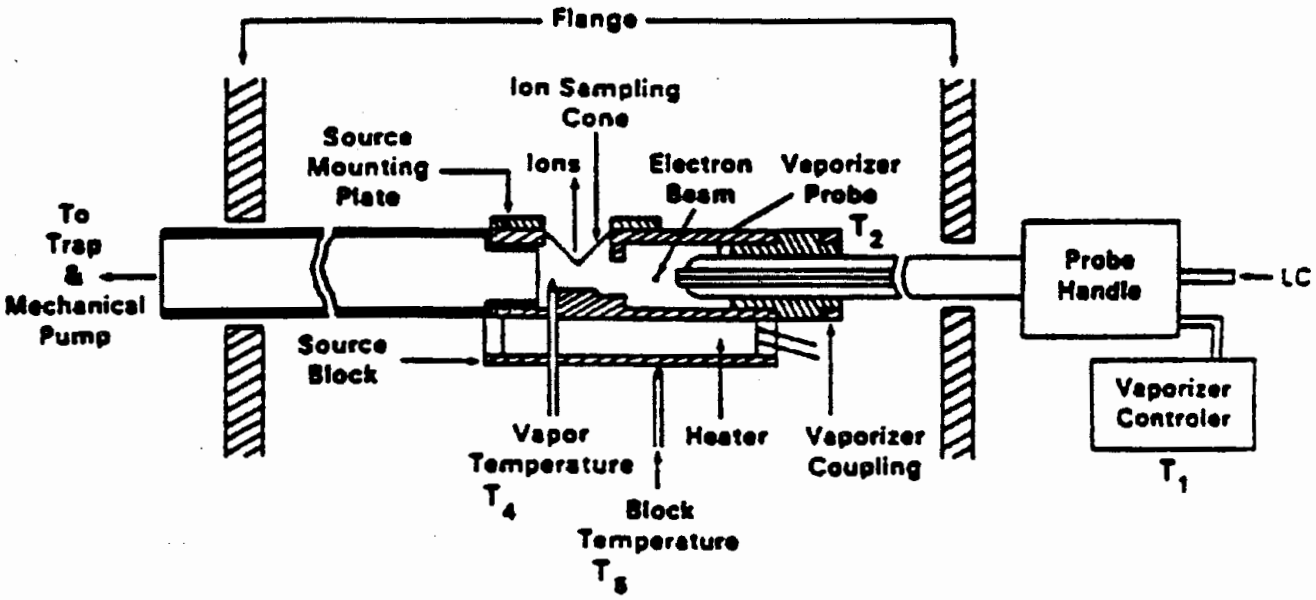


FIGURE 2
THERMOSPRAY SOURCE WITH WIRE-REPELLER
(High sensitivity configuration)

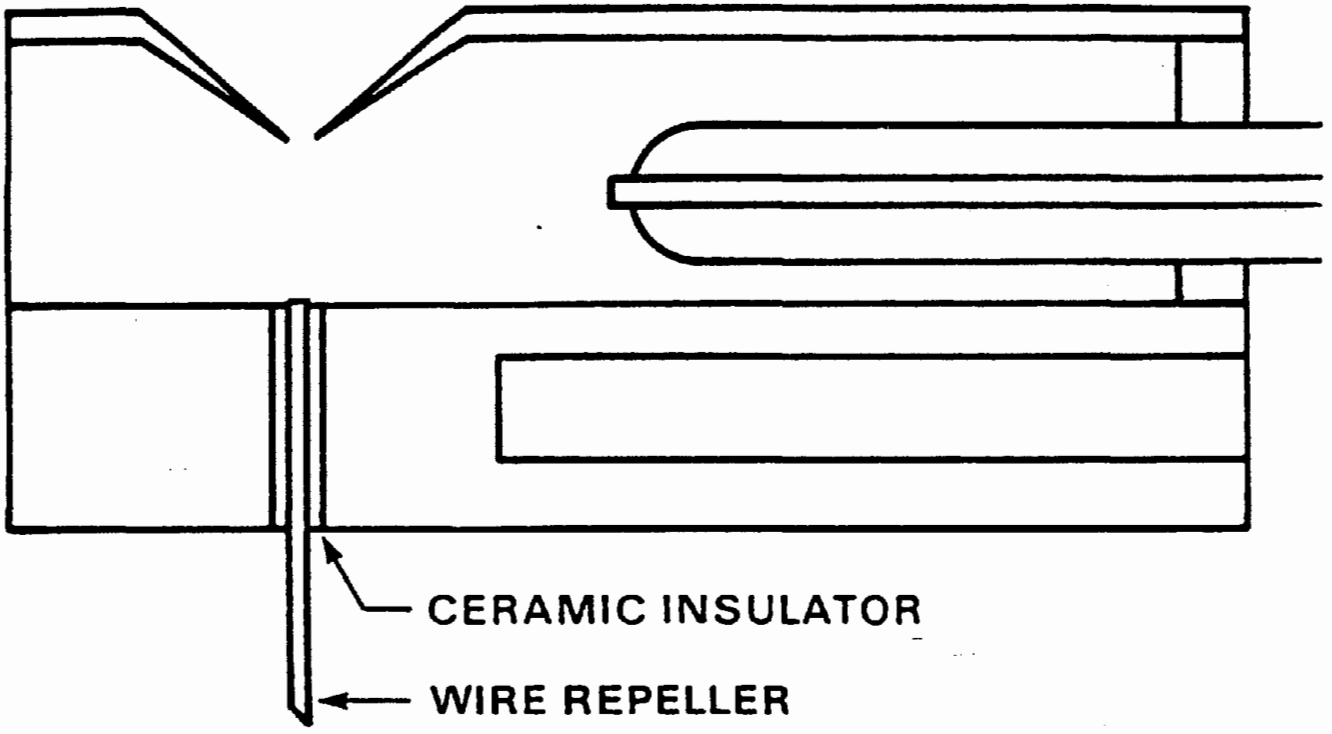
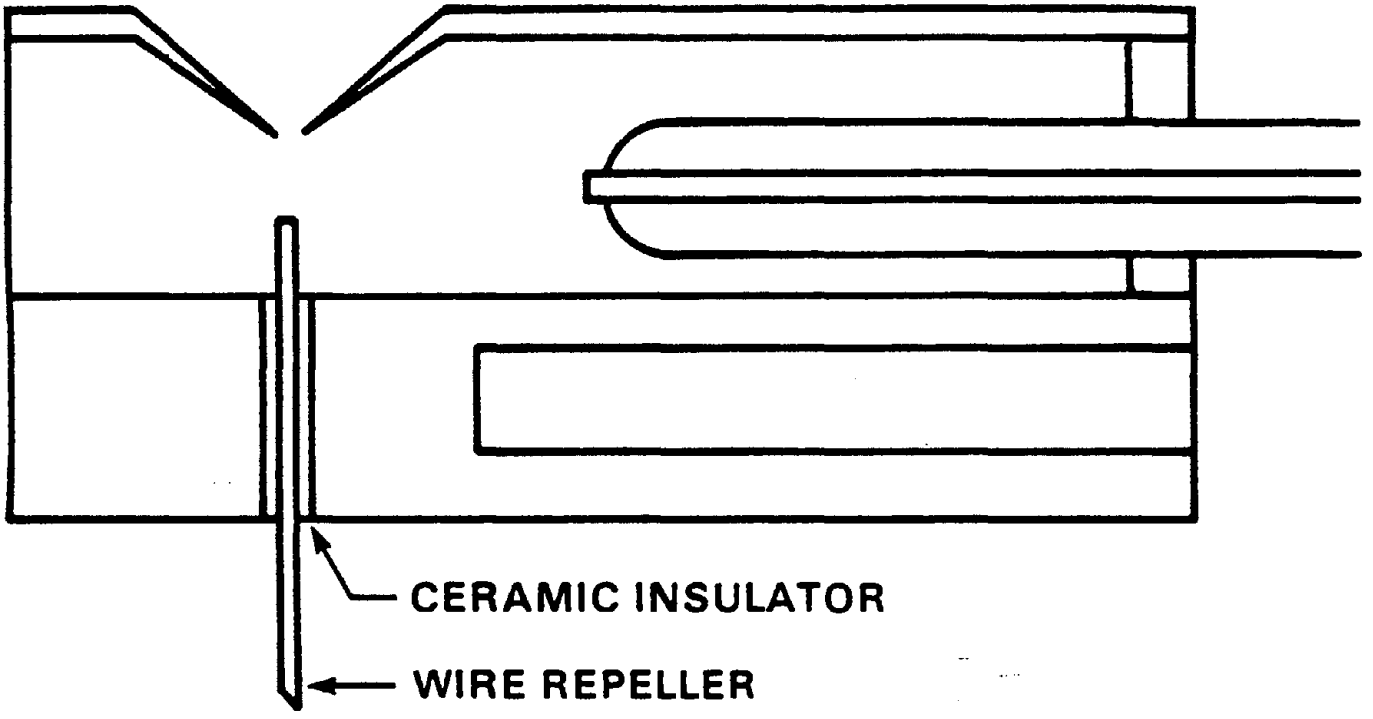
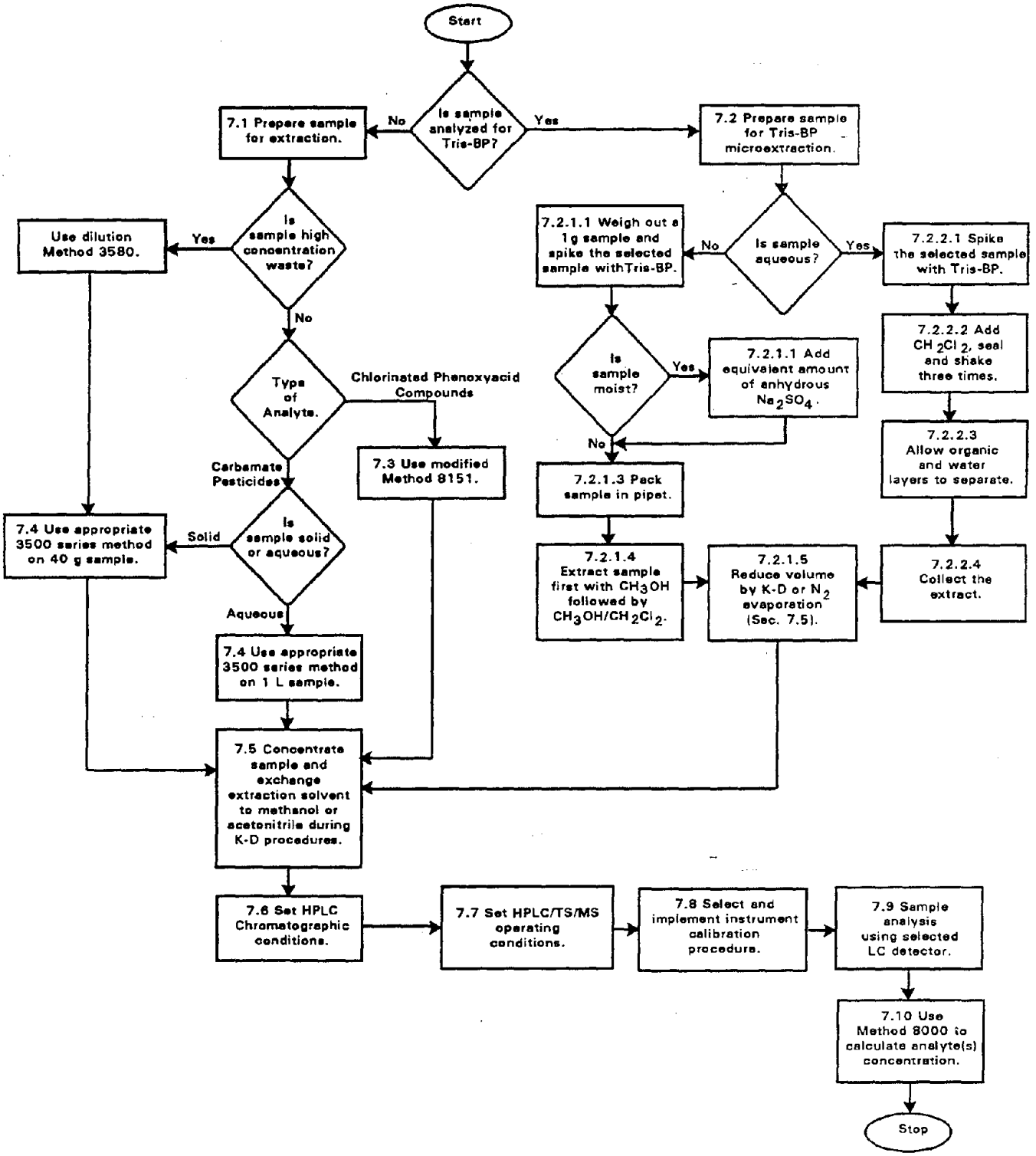


FIGURE 3
THERMOSPRAY SOURCE WITH WIRE-REPELLER
(CAD configuration)



METHOD 8321B
SOLVENT-EXTRACTABLE NONVOLATILE COMPOUNDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/THERMOSPRAY/MASS SPECTROMETRY (HPLC/TS/MS) OR ULTRAVIOLET (UV) DETECTION



METHOD 8330A

NITROAROMATICS AND NITRAMINES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

1.0 SCOPE AND APPLICATION

1.1 Method 8330 is intended for the trace analysis of explosives residues by high performance liquid chromatography using a UV detector. This method is used to determine the concentration of the following compounds in a water, soil, or sediment matrix:

Analyte	Abbreviation	CAS Number
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4
1,3,5-Trinitrobenzene	1,3,5-TNB	99-35-4
1,3-Dinitrobenzene	1,3-DNB	99-65-0
Methyl-2,4,6-trinitrophenylnitramine	Tetryl	479-45-8
Nitrobenzene	NB	98-95-3
2,4,6-Trinitrotoluene	2,4,6-TNT	118-96-7
4-Amino-2,6-dinitrotoluene	4-Am-DNT	1946-51-0
2-Amino-4, 6-dinitrotoluene	2-Am-DNT	35572-78-2
2,4-Dinitrotoluene	2,4-DNT	121-14-2
2,6-Dinitrotoluene	2,6-DNT	606-20-2
2-Nitrotoluene	2-NT	88-72-2
3-Nitrotoluene	3-NT	99-08-1
4-Nitrotoluene	4-NT	99-99-0

1.2 Method 8330 provides a salting-out extraction procedure for low concentrations (parts per trillion, or ng/L) of explosives residues in surface or ground water. Direct injection of diluted and filtered water samples can be used for water samples of higher concentration (See Table 1). Solid-phase extraction, using Method 3535, may also be applied to aqueous samples.

1.3 All of these compounds are either used in the manufacture of explosives or are the degradation products of compounds used for that purpose. When making stock solutions for calibration, treat each explosive compound with caution. See NOTE in Sec. 5.3.1 and Sec. 11.

1.4 The estimated quantitation limits (EQLs) of target analytes determined by Method 8330 in water and soil are presented in Table 1.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC, skilled in the interpretation of chromatograms, and experienced in handling explosive materials. (See Sec. 11.0 on SAFETY.) Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Method 8330 provides high performance liquid chromatographic (HPLC) conditions for the detection of ppb levels of certain explosive residues in water, soil, and sediment. Prior to use of this method, appropriate sample preparation techniques must be used.

2.2 Low-level salting-out method with no evaporation - Aqueous samples of low concentration are extracted by a salting-out extraction procedure with acetonitrile and sodium chloride. The small volume of acetonitrile that remains undissolved above the salt water is drawn off and transferred to a smaller volumetric flask. It is back-extracted by vigorous stirring with a specific volume of salt water. After equilibration, the phases are allowed to separate and the small volume of acetonitrile residing in the narrow neck of the volumetric flask is removed using a Pasteur pipet. The concentrated extract is diluted 1:1 with reagent grade water. An aliquot is separated on a C-18 reversed-phase column, determined at 254 nm, and confirmed on a CN reversed-phase column.

2.3 Solid-phase extraction method - Aqueous samples may also be prepared using solid-phase extraction, as described in Method 3535.

2.4 High-level direct injection method - Aqueous samples of higher concentration can be diluted 1/1 (v/v) with methanol or acetonitrile, filtered, separated on a C-18 reversed-phase column, determined at 254 nm, and confirmed on a CN reversed-phase column. If HMX is an important target analyte, methanol is preferred.

2.5 Soil and sediment samples are extracted using acetonitrile in an ultrasonic bath, filtered and analyzed as described in Sec. 2.3.

3.0 INTERFERENCES

3.1 Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts and/or elevated baselines, causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences.

3.2 2,4-DNT and 2,6-DNT elute at similar retention times (retention time difference of 0.2 minutes). A large concentration of one isomer may mask the response of the other isomer. If it is not apparent that both isomers are present (or are not detected), an isomeric mixture should be reported.

3.3 Tetryl decomposes rapidly in methanol/water solutions, as well as with heat. All aqueous samples expected to contain tetryl should be diluted with acetonitrile prior to filtration and acidified to pH <3. All samples expected to contain tetryl should not be exposed to temperatures above room temperature.

3.4 Degradation products of tetryl appear as a shoulder on the 2,4,6-TNT peak. Peak heights rather than peak areas should be used when tetryl is present in concentrations that are significant relative to the concentration of 2,4,6-TNT.

4.0 APPARATUS AND MATERIALS

4.1 HPLC system

4.1.1 HPLC - equipped with a pump capable of achieving 4000 psi, a 100-µL loop injector and a 254-nm UV detector (Perkin-Elmer Series 3, or equivalent). For the low concentration option, the detector must be capable of maintaining a stable baseline at 0.001 absorbance units full scale.

4.1.2 Recommended columns

4.1.2.1 Primary column - C-18 Reversed-phase HPLC column, 25-cm x 4.6-mm (5 µm) (Supelco LC-18, or equivalent).

4.1.2.2 Secondary column - CN Reversed-phase HPLC column, 25-cm x 4.6-mm (5 µm) (Supelco LC-CN, or equivalent).

4.1.3 Strip chart recorder

4.1.4 Digital integrator (optional)

4.1.5 Autosampler (optional)

4.2 Other equipment

4.2.1 Temperature-controlled ultrasonic bath

4.2.2 Vortex mixer

4.2.3 Balance - capable of weighing ± 0.0001 g

4.2.4 Magnetic stirrer with PTFE stirring bars

4.2.5 Water bath - Heated, with concentric ring cover, capable of temperature control (± 5°C). The bath should be used in a hood.

4.2.6 Oven - Forced air, without heating.

4.3 Materials

4.3.1 High-pressure injection syringe - 500-µL (Hamilton liquid syringe, or equivalent).

4.3.2 Disposable cartridge filters - 0.45-µm PTFE filter.

4.3.3 Pipets - Class A, glass, appropriate sizes.

4.3.4 Pasteur pipets

4.3.5 Scintillation vials - 20-mL, glass.

4.3.6 Vials - 15-mL, glass, PTFE-lined cap.

4.3.7 Vials - 40-mL, glass, PTFE-lined cap.

4.3.8 Disposable syringes - Plastipak, 3-mL and 10-mL or equivalent.

4.3.9 Volumetric flasks - 10-mL, 25-mL, 100-mL, and 1-L, with ground-glass stoppers, Class A.

NOTE: The 100-mL and 1-L volumetric flasks used for magnetic stirrer extraction must be round.

4.3.10 Vacuum desiccator - Glass.

4.3.11 Mortar and pestle - Steel.

4.3.12 Sieve - 30-mesh.

4.3.13 Graduated cylinders - 10-mL, 25-mL, and 1-L.

5.0 REAGENTS

5.1 Reagent grade inorganic chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination.

5.1.1 Acetonitrile, CH_3CN - HPLC grade.

5.1.2 Methanol, CH_3OH - HPLC grade.

5.1.3 Calcium chloride, $CaCl_2$ - Reagent grade. Prepare an aqueous solution containing 5 g/L of calcium chloride.

5.1.4 Sodium chloride, NaCl, shipped in glass bottles - reagent grade.

5.2 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Stock standard solutions

Dry each solid analyte standard to constant weight in a vacuum desiccator in the dark. Place about 0.100 g (weighed to 0.0001 g) of a single analyte into a 100-mL volumetric flask and dilute to volume with acetonitrile. Invert flask several times until dissolved. Store in refrigerator at 4°C in the dark. Calculate the concentration of the stock solution from the actual weight used (nominal concentration = 1,000 mg/L). Stock solutions may be used for up to one year.

NOTE: The HMX, RDX, Tetryl, and 2,4,6-TNT are explosives and the neat material should be handled carefully. See SAFETY in Sec. 11 for guidance. HMX, RDX, and Tetryl reference materials are shipped under water. Drying at ambient temperature requires several days. DO NOT DRY AT ELEVATED TEMPERATURES!

5.4 Intermediate standards solutions

5.4.1 If both 2,4-DNT and 2,6-DNT are to be determined, prepare two separate intermediate stock solutions containing (1) HMX, RDX, 1,3,5-TNB, 1,3-DNB, NB, 2,4,6-TNT, and 2,4-DNT and (2) Tetryl, 2,6-DNT, 2-NT, 3-NT, and 4-NT. Intermediate stock standard solutions should be prepared at 1,000 µg/L, in acetonitrile when analyzing soil samples, and in methanol when analyzing aqueous samples.

5.4.2 Dilute the two concentrated intermediate stock solutions, with the appropriate solvent, to prepare intermediate standard solutions that cover the range of 2.5 - 1,000 µg/L. These solutions should be refrigerated on preparation, and may be used for 30 days.

5.4.3 For the low-level method, the analyst must conduct a detection limit study and devise dilution series appropriate to the desired range. Standards for the low level method must be prepared immediately prior to use.

5.5 Working standards - Calibration standards at a minimum of five concentration levels should be prepared by the dilution of the intermediate standards solutions by 50% (v/v) with 5 g/L calcium chloride solution (Sec. 5.1.3). These solutions must be refrigerated and stored in the dark, and prepared fresh on the day of calibration.

5.6 Surrogate spiking solution - The analyst should monitor the performance of the extraction and analytical system as well as the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard and reagent water blank with one or two surrogates (e.g., analytes not expected to be present in the sample).

5.7 Matrix spiking solutions - Prepare matrix spiking solutions in methanol such that the concentration in the sample is five times the Estimated Quantitation Limit (Table 1). All target analytes should be included.

5.8 HPLC mobile phase - To prepare 1 L of mobile phase, add 500 mL of methanol to 500 mL of organic-free reagent water.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Follow conventional sampling and sample handling procedures as specified for semivolatile organics in Chapter Four.

6.2 Samples and sample extracts must be stored in the dark at 4°C. Holding times are the same as for semivolatile organics.

7.0 PROCEDURE

7.1 Sample preparation

This method addresses both aqueous and solid samples. There are three extraction procedures that may be applied to aqueous samples, depending on the expected level of explosive residue in the sample and the available equipment: a low-level salting-out extraction, a high-level extraction, and solid-phase extraction. It is highly recommended that aqueous process waste samples be screened with the high-level method to determine if the low-level method (1-50 µg/L) is required. Most groundwater samples will fall into the low-level method.

7.1.1 Aqueous low-level method (salting-out extraction)

7.1.1.1 Add 251.3 g of sodium chloride to a 1-L volumetric flask (round). Measure 770 mL of a water sample (using a 1-L graduated cylinder) and transfer it to the volumetric flask containing the salt. Add a stir bar and mix the contents at maximum speed on a magnetic stirrer until the salt is completely dissolved.

7.1.1.2 Add 164 mL of acetonitrile (measured with a 250-mL graduated cylinder) while the solution is being stirred and stir for an additional 15 minutes. Turn off the stirrer and allow the phases to separate for 10 minutes.

7.1.1.3 Remove the acetonitrile (upper) layer (about 8 mL) with a Pasteur pipet and transfer it to a 100-mL volumetric flask (with a round bottom). Add 10 mL of fresh acetonitrile to the water sample in the 1-L flask. Again stir the contents of the flask for 15 minutes followed by 10 minutes for phase separation. Combine the second acetonitrile portion with the initial extract. The inclusion of a few drops of salt water at this point is unimportant.

7.1.1.4 Add 84 mL of salt water (325 g NaCl per 1000 mL of reagent water) to the acetonitrile extract in the 100-mL volumetric flask. Add a stir bar and stir the contents on a magnetic stirrer for 15 minutes, followed by 10 minutes for phase separation. Carefully transfer the acetonitrile phase to a 10-mL graduated cylinder using a Pasteur pipet. At this stage, the amount of water transferred with the acetonitrile must be minimized. The water contains a high concentration of NaCl that produces a large peak at the beginning of the chromatogram, where it could interfere with the HMX determination.

7.1.1.5 Add an additional 1.0 mL of acetonitrile to the 100-mL volumetric flask. Again stir the contents of the flask for 15 minutes, followed by 10 minutes for phase separation. Combine the second acetonitrile portion with the initial extract in the 10-mL graduated cylinder (transfer to a 25-mL graduated cylinder if the volume exceeds 5 mL). Record the total volume of acetonitrile extract to the nearest 0.1 mL. (Use this as the volume of total extract [V_t] in the calculation of concentration after converting to µL). The resulting extract, about 5 - 6 mL, is then diluted 1:1 with organic-free reagent water (with pH <3 if tetryl is a suspected analyte) prior to analysis.

7.1.1.6 If the diluted extract is turbid, filter it through a 0.45-µm PTFE filter using a disposable syringe. Discard the first 0.5 mL of filtrate, and retain the remainder in a PTFE-capped vial for RP-HPLC analysis in Sec. 7.4.

7.1.2 Aqueous high-level method

7.1.2.1 Sample filtration

Place a 5-mL aliquot of each water sample in a scintillation vial, add 5 mL of acetonitrile, shake thoroughly, and filter through a 0.45-µm PTFE filter using a disposable syringe.

7.1.2.2 Discard the first 3 mL of filtrate, and retain the remainder in a PTFE-capped vial for RP-HPLC analysis in Sec. 7.4. HMX quantitation can be improved with the use of methanol rather than acetonitrile for dilution before filtration.

7.1.3 Solid-phase extraction

Aqueous samples containing nitroaromatics and nitramines may also be extracted using solid-phase extraction (SPE) in both disk and cartridge formats. Consult Method 3535 for the procedures to be employed and the apparatus and materials that are required.

7.1.4 Soil and sediment samples

7.1.4.1 Sample homogenization

Dry soil samples in air at room temperature (or less) to a constant weight, being careful not to expose the samples to direct sunlight. Grind and homogenize the dried sample thoroughly in an acetonitrile-rinsed mortar to pass a 30-mesh sieve.

NOTE: Soil samples should be screened by Method 8515 prior to grinding in a mortar and pestle (See Safety Sec. 11.2).

7.1.4.2 Sample extraction

7.1.4.2.1 Place a 2.0-g subsample of each soil sample in a 15-mL glass vial. Add 10.0 mL of acetonitrile, cap with PTFE-lined cap, vortex swirl for one minute, and place in a cooled ultrasonic bath for 18 hours.

7.1.4.2.2 After sonication, allow sample to settle for 30 minutes. Remove 5.0 mL of supernatant, and combine with 5.0 mL of calcium chloride solution (Sec. 5.1.3) in a 20-mL vial. Shake, and let stand for 15 minutes.

7.1.4.2.3 Place supernatant in a disposable syringe and filter through a 0.45-µm PTFE filter. Discard first 3 mL and retain remainder in a PTFE-capped vial for RP-HPLC analysis in Sec. 7.4.

7.2 Chromatographic conditions (recommended)

Primary Column:	C-18 reversed-phase HPLC column, 25-cm x 4.6-mm, 5 µm (Supelco LC-18 or equivalent).
Secondary Column:	CN reversed-phase HPLC column, 25-cm x 4.6-mm, 5 µm (Supelco LC-CN or equivalent).

Mobile Phase:	50/50 (v/v) methanol/organic-free reagent water.
Flow Rate:	1.5 mL/min
Injection volume:	100-µL
UV Detector:	254 nm

7.3 Calibration of HPLC

7.3.1 All electronic equipment is allowed to warm up for 30 minutes. During this period, at least 15 void volumes of mobile phase are passed through the column (approximately 20 min at 1.5 mL/min) and continued until the baseline is level at the UV detector's greatest sensitivity.

7.3.2 Initial calibration - Injections of each calibration standard over the concentration range of interest are made sequentially into the HPLC in random order. Peak heights or peak areas are obtained for each analyte. Employ one of the calibration options described in Method 8000.

7.3.3 Calibration verification - Analyze one mid-point calibration standard, at a minimum, at the beginning of the day, and after every 20 sample extracts (*recommended* after every 10, in order to minimize the number of samples that may be affected by a failing standard), and after the last sample of the day. Calculate the calibration factor for each analyte from the peak height or peak area and compare it with the mean calibration factor obtained for the initial calibration, as described in Method 8000. The calibration factor for the calibration verification must agree within ±15% of the mean calibration factor of the initial calibration. If this criterion is not met, a new initial calibration must be performed, or another of the calibration options described in Method 8000 must be employed.

7.4 HPLC analysis

7.4.1 Analyze the samples using the chromatographic conditions given in Sec. 7.2. All positive measurements observed on the C-18 column must be confirmed by injection onto the CN column.

7.4.2 Method 8000 provides instructions on the analysis sequence, appropriate dilutions, establishing daily retention time windows, and identification criteria. Include a mid-level standard after each group of 20 samples in the analysis sequence. If column temperature control is not employed, special care must be taken to ensure that temperature shifts do not cause peak misidentification.

7.4.3 Table 2 summarizes the estimated retention times on both C-18 and CN columns for a number of analytes analyzable using this method. An example of the separation achieved by Column 1 is shown in Figure 1.

7.4.4 Record the resulting peak sizes in peak heights or area units. The use of peak heights is recommended to improve reproducibility of low level samples.

7.4.5 The calculation of sample concentrations is described in Method 8000.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Quality control procedures to ensure the proper operation of the various sample preparation and/or sample introduction techniques can be found in Method 3500. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures that are necessary to validate the HPLC system operation are found in Method 8000, Sec. 8.0.

8.3 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Method 8000, Sec. 8.0 for information on how to accomplish this demonstration.

8.4 Sample quality control for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and quantitation limit). At a minimum, this includes the analysis of QC samples including a method blank, matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch and the addition of surrogates to each field sample and QC sample.

8.4.1 Before processing any samples, the analyst should demonstrate, through the analysis of a method blank, that interferences from the analytical system, glassware, and reagents are under control. Each time a set of samples is analyzed or there is a change in reagents, a method blank should be analyzed as a safeguard against chronic laboratory contamination. The blanks should be carried through all stages of sample preparation and measurement.

8.4.2 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair.

8.4.3 A Laboratory Control Sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

8.4.4 See Method 8000, Sec. 8.0, for the details on carrying out sample quality control procedures for preparation and analysis.

8.5 Surrogate recoveries

The laboratory must evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000, Sec. 8.0, for information on evaluating surrogate data and developing and updating surrogate limits.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 Table 3 provides the single-laboratory precision based on data from the analysis of blind duplicates of four spiked soil samples and four field-contaminated samples analyzed by seven laboratories.

9.2 Table 4 provides the multi-laboratory error based on data from the analysis of blind duplicates of four spiked soil samples and four field-contaminated samples analyzed by seven laboratories.

9.3 Table 5 provides the multi-laboratory variance of the high-level method for water based on data from nine laboratories.

9.4 Table 6 provides multi-laboratory recovery data from the analysis of spiked soil samples by seven laboratories.

9.5 Table 7 provides a comparison of method accuracy for soil and aqueous samples (high-level method).

9.6 Table 8 provides precision and accuracy data for the salting-out extraction method.

9.7 Table 9 provides data from a comparison of direct injection of groundwater samples with both the salting-out extraction and the solid-phase extraction techniques.

9.8 Table 10 provides data comparing the precision of duplicate samples analyzed by direct injection of groundwater samples with both the salting-out extraction and the solid-phase extraction techniques.

9.9 Table 11 provides a comparison of recovery data for spiked samples analyzed by direct injection of groundwater samples with both the salting-out extraction and the solid-phase extraction techniques.

10.0 REFERENCES

1. Bauer, C.F., T.F. Jenkins, S.M. Koza, P.W. Schumacher, P.H. Miyares and M.E. Walsh (1989). Development of an analytical method for the determination of explosive residues in soil. Part 3. Collaborative test results and final performance evaluation. USACE Cold Regions Research and Engineering Laboratory, CRREL Report 89-9.

2. Grant, C.L., A.D. Hewitt and T.F. Jenkins (1989). Comparison of low concentration measurement capability estimates in trace analysis: Method Detection Limits and Certified Reporting Limits. USACE Cold Regions Research and Engineering Laboratory, Special Report 89-20.

3. Jenkins, T.F., C.F. Bauer, D.C. Leggett and C.L. Grant (1984). Reverse-phased HPLC method for analysis of TNT, RDX, HMX and 2,4-DNT in munitions wastewater. USACE Cold Regions Research and Engineering Laboratory, CRREL Report 84-29.

4. Jenkins, T.F. and M.E. Walsh (1987). Development of an analytical method for explosive residues in soil. USACE Cold Regions Research and Engineering Laboratory, CRREL Report 87-7.

5. Jenkins, T.F., P.H. Miyares and ME. Walsh (1988a). An improved RP-HPLC method for determining nitroaromatics and nitramines in water. USACE Cold Regions Research and Engineering Laboratory, Special Report 88-23.

6. Jenkins, T.F. and P.H. Miyares (1992). Comparison of Cartridge and Membrane Solid-Phase Extraction with Salting-out Solvent Extraction for Preconcentration of Nitroaromatic and Nitramine Explosives from Water. USACE Cold Regions Research and Engineering Laboratory, Draft CRREL Special Report.

7. Jenkins, T.F., P.W. Schumacher, M.E. Walsh and C.F. Bauer (1988b). Development of an analytical method for the determination of explosive residues in soil. Part II: Further development and ruggedness testing. USACE Cold Regions Research and Engineering Laboratory, CRREL Report 88-8.

8. Leggett, D.C., T.F. Jenkins and P.H. Miyares (1990). Salting-out solvent extraction for preconcentration of neutral polar organic solutes from water. Analytical Chemistry, 62: 1355-1356.

9. Miyares, P.H. and T.F. Jenkins (1990). Salting-out solvent extraction for determining low levels of nitroaromatics and nitramines in water. USACE Cold Regions Research and Engineering Laboratory, Special Report 90-30.

10. Jenkins, T. F., Thorne, P. G., Myers, K. F., McCormick, E. F., Parker, D. E., and B. L. Escalon (1995). Evaluation of Clean Solid Phases for Extraction of Nitroaromatics and Nitramines from Water. USACE Cold Regions Research and Engineering Laboratory, Special Report 95-22.

11.0 SAFETY

11.1 Standard precautionary measures used for handling other organic compounds should be sufficient for the safe handling of the analytes targeted by Method 8330. The only extra caution that should be taken is when handling the analytical standard neat material for the explosives themselves and in rare cases where soil or waste samples are highly contaminated with the explosives. Follow the note for drying the neat materials at ambient temperatures.

11.2 It is advisable to screen soil or waste samples using Method 8515 to determine whether high concentrations of explosives are present. Soil samples containing as much as 2% of 2,4,6-TNT have been safely ground. Samples containing higher concentrations should not be ground in the mortar and pestle. Method 8515 is for 2,4,6-TNT, however, the other nitroaromatics will also cause

a color to be developed and provide a rough estimation of their concentrations. 2,4,6-TNT is the analyte most often detected in high concentrations in soil samples. Visual observation of a soil sample is also important when the sample is taken from a site expected to contain explosives. Lumps of material that have a chemical appearance should be suspect and not ground. Explosives are generally a very finely ground grayish-white material.

TABLE 1

ESTIMATED QUANTITATION LIMITS

	Water (µg/L)		
Analytes	Low-Level	High-Level	Soil (mg/kg)
HMX	-	13.0	2.2
RDX	0.84	14.0	1.0
1,3,5-TNB	0.26	7.3	0.25
1,3-DNB	0.11	4.0	0.25
Tetryl	-	4.0	0.65
NB	-	6.4	0.26
2,4,6-TNT	0.11	6.9	0.25
4-Am-DNT	0.060	-	-
2-Am-DNT	0.035	-	-
2,6-DNT	0.31	9.4	0.26
2,4-DNT	0.020	5.7	0.25
2-NT	-	12.0	0.25
4-NT	-	8.5	0.25
3-NT	-	7.9	0.25

TABLE 2

RETENTION TIMES AND CAPACITY FACTORS ON LC-18 AND LC-CN COLUMNS

	Retention time (min)		Capacity factor (k)*	
Analyte	LC-18	LC-CN	LC-18	LC-CN
HMX	2.44	8.35	0.49	2.52
RDX	3.73	6.15	1.27	1.59
1,3,5-TNB	5.11	4.05	2.12	0.71
1,3-DNB	6.16	4.18	2.76	0.76
Tetryl	6.93	7.36	3.23	2.11
NB	7.23	3.81	3.41	0.61
2,4,6-TNT	8.42	5.00	4.13	1.11
4-Am-DNT	8.88	5.10	4.41	1.15
2-Am-DNT	9.12	5.65	4.56	1.38
2,6-DNT	9.82	4.61	4.99	0.95
2,4-DNT	10.05	4.87	5.13	1.05
2-NT	12.26	4.37	6.48	0.84
4-NT	13.26	4.41	7.09	0.86
3-NT	14.23	4.45	7.68	0.88

*Capacity factors are based on an unretained peak for nitrate at 1.71 min on LC-18 and at 2.00 min on LC-CN.

TABLE 3

SINGLE LABORATORY PRECISION OF METHOD FOR SOIL SAMPLES

	Spiked Soils			Field-Contaminated Soils		
Analyte	Mean Conc. (mg/kg)	SD	%RSD	Mean Conc. (mg/kg)	SD	%RSD
HMX	46	1.7	3.7	14	1.8	12.8
				153	21.6	14.1
RDX	60	1.4	2.3	104	12	11.5
				877	29.6	3.4
1,3,5-TNB	8.6	0.4	4.6	2.8	0.2	7.1
	46	1.9	4.1	72	6.0	8.3
2,4,6-TNT	40	1.4	3.5	7.0	0.61	9.0
				669	55	8.2
1,3-DNB	3.5	0.14	4.0	1.1	0.11	9.8
2,4-DNT	5.0	0.17	3.4	1.0	0.44	42.3
Tetryl	17	3.1	17.9	2.3	0.41	18.0

Source: Reference 1.

TABLE 4

MULTILABORATORY ERROR OF METHOD FOR SOIL SAMPLES

	Spiked Soils			Field-Contaminated Soils		
Analyte	Mean Conc. (mg/kg)	SD	%RSD	Mean Conc. (mg/kg)	SD	%RSD
HMX	46	2.6	5.7	14	3.7	26.0
				153	37.3	24.0
RDX	60	2.6	4.4	104	17.4	17.0
				877	67.3	7.7
1,3,5-TNB	8.6	0.61	7.1	2.8	0.23	8.2
	46	2.97	6.5	72	8.8	12.2
2,4,6-TNT	40	1.88	4.7	7.0	1.27	18.0
				669	63.4	9.5
1,3-DNB	3.5	0.24	6.9	1.1	0.16	14.5
2,4-DNT	5.0	0.22	4.4	1.0	0.74	74.0
Tetryl	17	5.22	30.7	2.3	0.49	21.3

Source: Reference 1.

TABLE 5

MULTILABORATORY VARIANCE OF METHOD FOR WATER SAMPLES[a]

Analyte	Mean Conc. (µg/L)	SD	%RSD
HMX	203	14.8	7.3
RDX	274	20.8	7.6
2,4-DNT	107	7.7	7.2
2,4,6-TNT	107	11.1	10.4

[a] Nine Laboratories

TABLE 6

MULTILABORATORY RECOVERY DATA FOR SPIKED SOIL SAMPLES

	Concentration (µg/g)						
Laboratory	HMX	RDX	1,3,5-TNB	1,3-DNB	Tetryl	2,4,6-TNT	2,4-DNT
1	44.97	48.78	48.99	49.94	32.48	49.73	51.05
3	50.25	48.50	45.85	45.96	47.91	46.25	48.37
4	42.40	44.00	43.40	49.50	31.60	53.50	50.90
5	46.50	48.40	46.90	48.80	32.10	55.80	49.60
6	56.20	55.00	41.60	46.30	13.20	56.80	45.70
7	41.50	41.50	38.00	44.50	2.60	36.00	43.50
8	52.70	52.20	48.00	48.30	44.80	51.30	49.10
True Conc	50.35	50.20	50.15	50.05	50.35	50.65	50.05
Mean Conc	47.79	48.34	44.68	47.67	29.24	49.91	48.32
Std. Dev.	5.46	4.57	3.91	2.09	16.24	7.11	2.78
% RSD	11.42	9.45	8.75	4.39	55.53	14.26	5.76
% Diff.*	5.08	3.71	10.91	4.76	41.93	1.46	3.46
Mean % Recovery	95	96	89	95	58	98	96

* Between true value and mean determined value.
Source: Reference 1.

TABLE 7

COMPARISON OF METHOD ACCURACY FOR SOIL AND AQUEOUS SAMPLES
(HIGH CONCENTRATION METHOD)

	Recovery (%)	
Analyte	Soil Method*	Aqueous Method**
2,4-DNT	96.0	98.6
2,4,6-TNT	96.8	94.4
RDX	96.8	99.6
HMX	95.4	95.5

* Data from Reference 1.
** Data from Reference 3.

TABLE 8

PRECISION AND ACCURACY DATA FOR THE SALTING-OUT EXTRACTION METHOD

Analyte	# Samples	%RSD	Mean Recovery (%)	Highest Concentration Tested
HMX	20	10.5	106	1.14
RDX	20	8.7	106	1.04
1,3,5-TNB	20	7.6	119	0.82
1,3-DNB	20	6.6	102	1.04
Tetryl	20	16.4	93	0.93
2,4,6-TNT	20	7.6	105	0.98
2-Am-DNT	20	9.1	102	1.04
2,4-DNT	20	5.8	101	1.01
1,2-NT	20	9.1	102	1.07
1,4-NT	20	18.1	96	1.06
1,3-NT	20	12.4	97	1.23

All tests were performed in reagent water.
Source: Reference 6.

TABLE 9

COMPARISON OF DIRECT ANALYSIS OF GROUNDWATER SAMPLES CONTAINING NITROAROMATICS WITH SALTING-OUT AND SOLID-PHASE EXTRACTION TECHNIQUES

		Analyte Concentration (µg/L)								
Sample	Technique	HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
1	Direct									
	Salting-out	1.04	2.45				0.47		0.36	0.32
	SPE-Cart.	1.00	1.33				0.44		0.29	0.30
	SPE-Disk	0.93	1.35				0.57		0.28	0.56
2	Direct	94	79							
	Salting-out	54.2	63.8			0.3	0.33		3.08	1.36
	SPE-Cart.	64.0	83.1			0.3	0.34		3.34	2.27
	SPE-Disk	57.1	71.8			0.3	0.29		2.89	2.05
3	Direct	93	91							
	Salting-out	85.7	75.3			0.2	0.19		2.43	1.31
	SPE-Cart.	93.1	88.8			0.2	0.17		2.49	1.65
	SPE-Disk	78.9	74.7			0.2	0.13		1.99	1.89
4	Direct	45	14							
	Salting-out	45.7	16.4		0.17	0.3	0.13		2.18	1.21
	SPE-Cart.	48.0	21.6			0.2	0.19		2.31	1.42
	SPE-Disk	40.8	18.9			0.2	0.13		2.07	1.64
5	Direct									
	Salting-out	0.76	5.77						0.13	0.05
	SPE-Cart.	1.16	6.48						0.16	0.05
	SPE-Disk	1.19	6.11						0.16	0.14
6	Direct									
	Salting-out	10.5	6.17				0.10		0.71	0.33
	SPE-Cart.	11.5	7.03				0.10		0.79	0.40
	SPE-Disk	10.3	6.34				0.07		0.82	0.70

TABLE 9
(continued)

Sample	Technique	Analyte Concentration (µg/L)								
		HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
7	Direct	134	365							
	Salting-out	75.4	202				0.98		8.12	1.80
	SPE-Cart.	115	308				1.51		11.3	3.44
	SPE-Disk	109	291				1.41		9.81	3.30
8	Direct									
	Salting-out	0.61	10.9							
	SPE-Cart.	0.64	11.9							
	SPE-Disk	0.64	11.0							
9	Direct	25	13							
	Salting-out	30.2	12.1						1.14	0.56
	SPE-Cart.	31.2	12.7						1.50	0.79
	SPE-Disk	27.5	11.0						1.34	0.79
10	Direct									
	Salting-out	0.33	7.12							
	SPE-Cart.	0.62	8.23							
	SPE-Disk	0.26	7.60							
14	Direct		13							
	Salting-out		5.98							
	SPE-Cart.		12.0							
	SPE-Disk		11.6							
16	Direct		40							
	Salting-out	0.58	28.7			0.04			0.39	0.13
	SPE-Cart.	0.77	33.8			0.03			0.43	0.17
	SPE-Disk	0.66	32.7			0.03			0.44	0.22
18	Direct	165	58						9	7
	Salting-out	141	39.1			0.80	0.96		8.5	5.62

TABLE 9
(continued)

Sample	Technique	Analyte Concentration (µg/L)								
		HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
	SPE-Cart.	152	44.4			0.93	0.88		9.5	7.01
	SPE-Disk	138	40.9			0.90	0.99		9.3	6.03
19	Direct	173	76				17		59	54
	Salting-out	172	69.5			2.6	23.1	1.20	65.2	56.4
	SPE-Cart.	142	75.6		0.11	2.5	20.9	1.08	57.7	50.5
	SPE-Disk	136	72.7		0.11	2.4	20.3	1.23	55.0	48.0
21	Direct	252	157	5			110		47	65
	Salting-out	227	132	6.62	0.30		102		42.6	56.5
	SPE-Cart.	238	146	6.90	0.33		104		48.0	63.5
	SPE-Disk	226	141	6.45	0.31		102		47.0	61.8
22	Direct	218	40							
	Salting-out	201	35.9						2.20	1.90
	SPE-Cart.	203	36.5						2.74	2.24
	SPE-Disk	199	35.8						2.78	2.08
24	Direct									
	Salting-out	2.15	7.54							
	SPE-Cart.	2.47	8.91							
	SPE-Disk	2.34	8.84							
25	Direct									
	Salting-out									
	SPE-Cart.		0.59							
	SPE-Disk		0.63							
27	Direct	112	608	8			180		10	8
	Salting-out	82.8	429	4.45	0.79		137		7.71	6.20
	SPE-Cart.	91.0	510	9.53	0.90		149		8.25	7.67
	SPE-Disk	77.3	445	7.37	0.79		128		8.16	6.33

TABLE 9
(continued)

		Analyte Concentration (µg/L)								
Sample	Technique	HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
28	Direct	325	102				14		51	40
	Salting-out	290	87.5	0.37	0.10		13.9		42.3	33.5
	SPE-Cart.	319	109	0.87	0.17		22.0		56.2	45.0
	SPE-Disk	249	85.7	0.65	0.13		17.2		43.0	34.5
29	Direct									
	Salting-out									
	SPE-Cart.		0.43							
	SPE-Disk		0.28							
31	Direct									
	Salting-out									
	SPE-Cart.		0.21							
	SPE-Disk		0.23							
32	Direct									
	Salting-out									
	SPE-Cart.									
	SPE-Disk	0.38								

An additional 11 samples (11, 12, 13, 15, 17, 20, 23, 26, 30, 31, and 33) were analyzed in which none of the analytes were detected by any of the techniques. Therefore, the non-detect results are not shown here. Similarly, for those samples that are shown here, the fields are left blank for the analytes that were not detected.

All data are taken from Reference 10.

TABLE 10

RELATIVE PERCENT DIFFERENCE BETWEEN DUPLICATE SAMPLE ANALYSES

		Relative Percent Difference (%)								
Sample	Technique	HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
4	Direct	0	24							
	Salting-out	0	15		6	100	8		18	11
	SPE-Cart.	1	12			0	45		8	5
	SPE-Disk	3	8			0	17		2	1
29	Direct									
	Salting-out									
	SPE-Cart.		26							
	SPE-Disk		7							
LCS	Direct	1	0	0			1	1		
	Salting-out	4	4	4			3	3		
	SPE-Cart.	6	1	7			6	6		
	SPE-Disk	5	7	7			13	6		

All data are taken from Reference 10.

TABLE 11

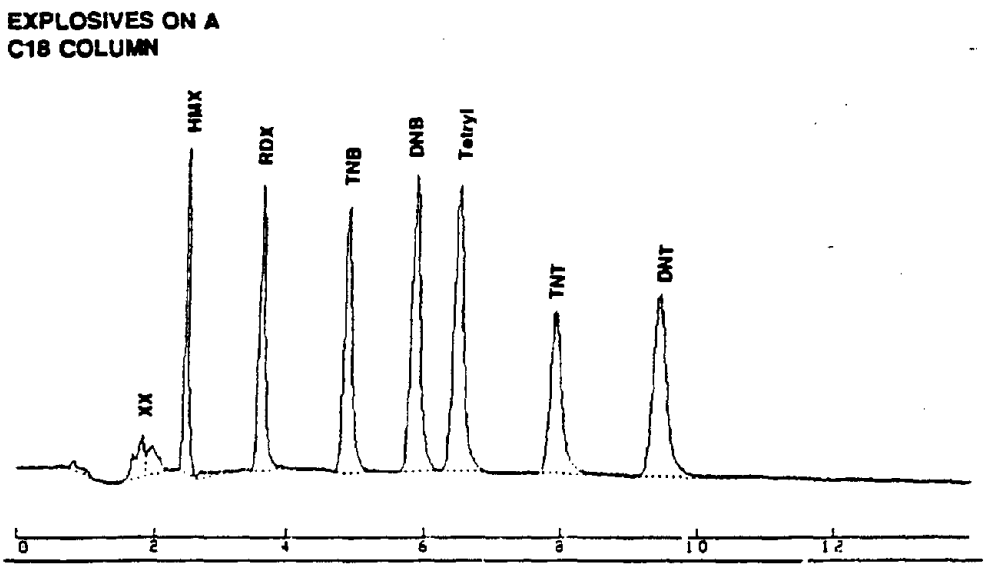
RECOVERY OF ANALYTES FROM SPIKED SAMPLES

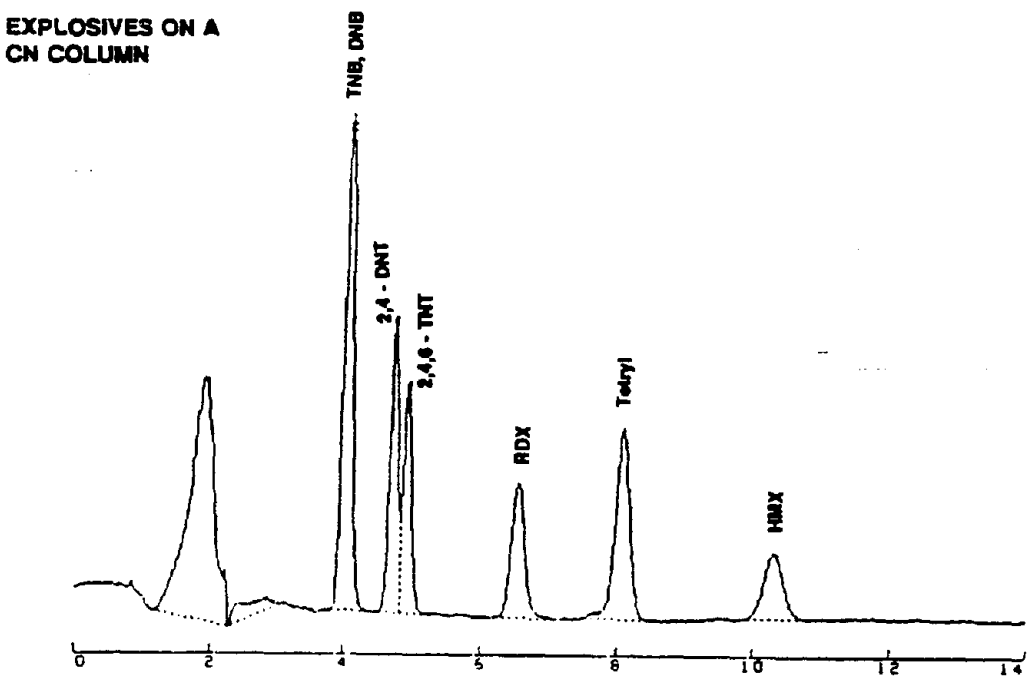
		Percent Recovery (%)				
Sample	Technique	HMX	RDX	TNB	TNT	24D
LCS1	Direct	99.5	98.5	95.6	96.5	98.1
	Salting-out	94.2	91.2	92.9	83.2	92.1
	SPE-Cart.	99.0	101.0	96.6	94.1	95.1
	SPE-Disk	92.5	95.6	89.3	88.6	86.9
LCS2	Direct	98.8	98.2	95.9	97.2	99.2
	Salting-out	91.0	95.0	89.0	81.0	89.0
	SPE-Cart.	93.5	100.0	83.0	89.1	89.3
	SPE-Disk	88.0	102.0	83.0	78.0	82.0
29	Direct	95.0	95.5	95.2	92.8	93.0
	Salting-out	107.0	89.0	85.0	89.0	65.0
	SPE-Cart.	103.0	107.0	104.0	05.0	102.0
	SPE-Disk	80.0	78.0	76.0	78.0	77.0
4	Direct	105.5	105.0	103.0	104.0	105.0
	Salting-out	23*	191*	76.0	83.0	76.0
	SPE-Cart.	351*	95*	92.2	91.1	93.7
	SPE-Disk	308*	49.5*	87.4	85.6	90.8

All data are taken from Reference 10.

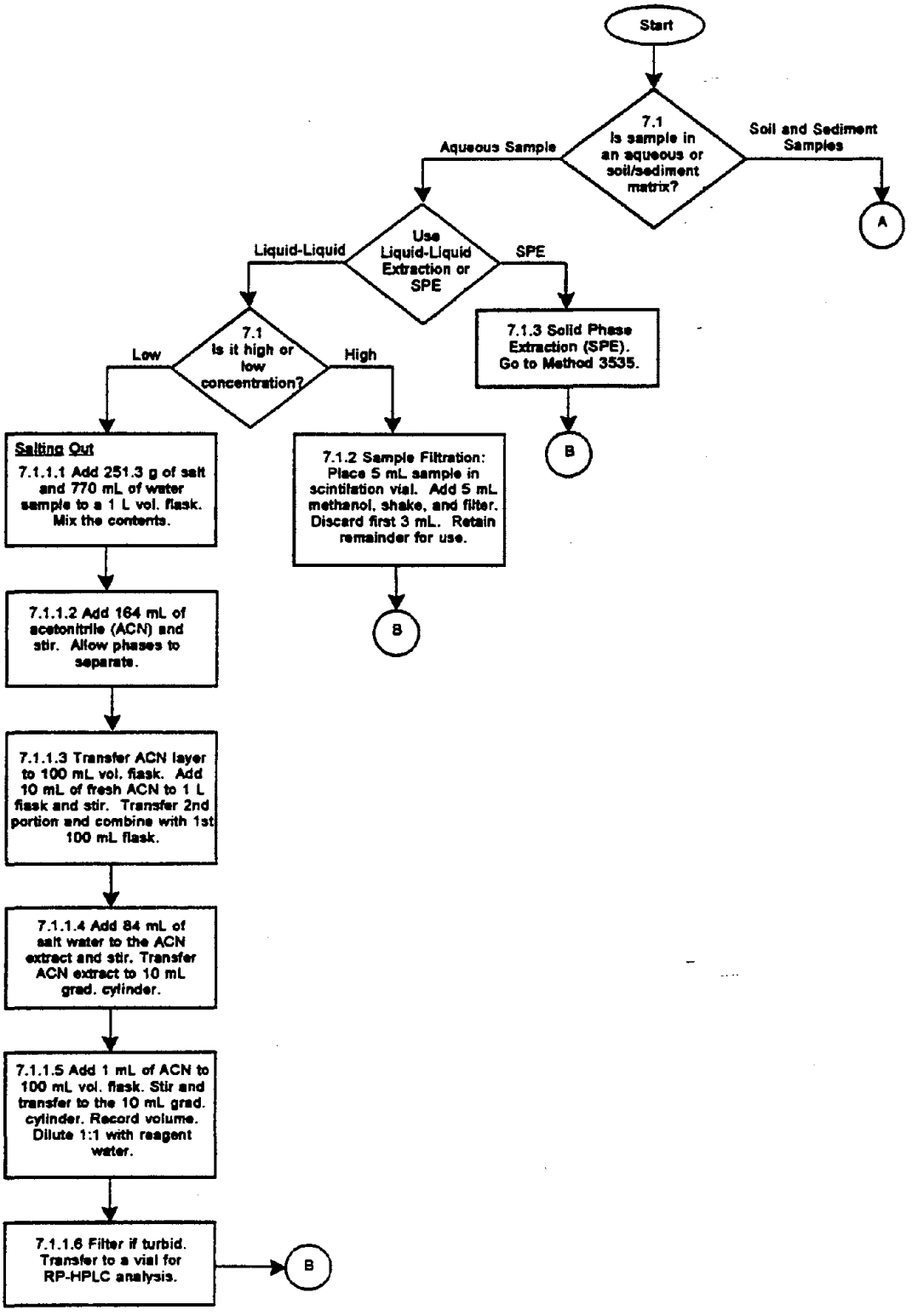
* Results for these analytes in Sample 4 are believed to result from spiking levels that are very similar to the background concentrations of these analytes in this sample (see Reference 10).

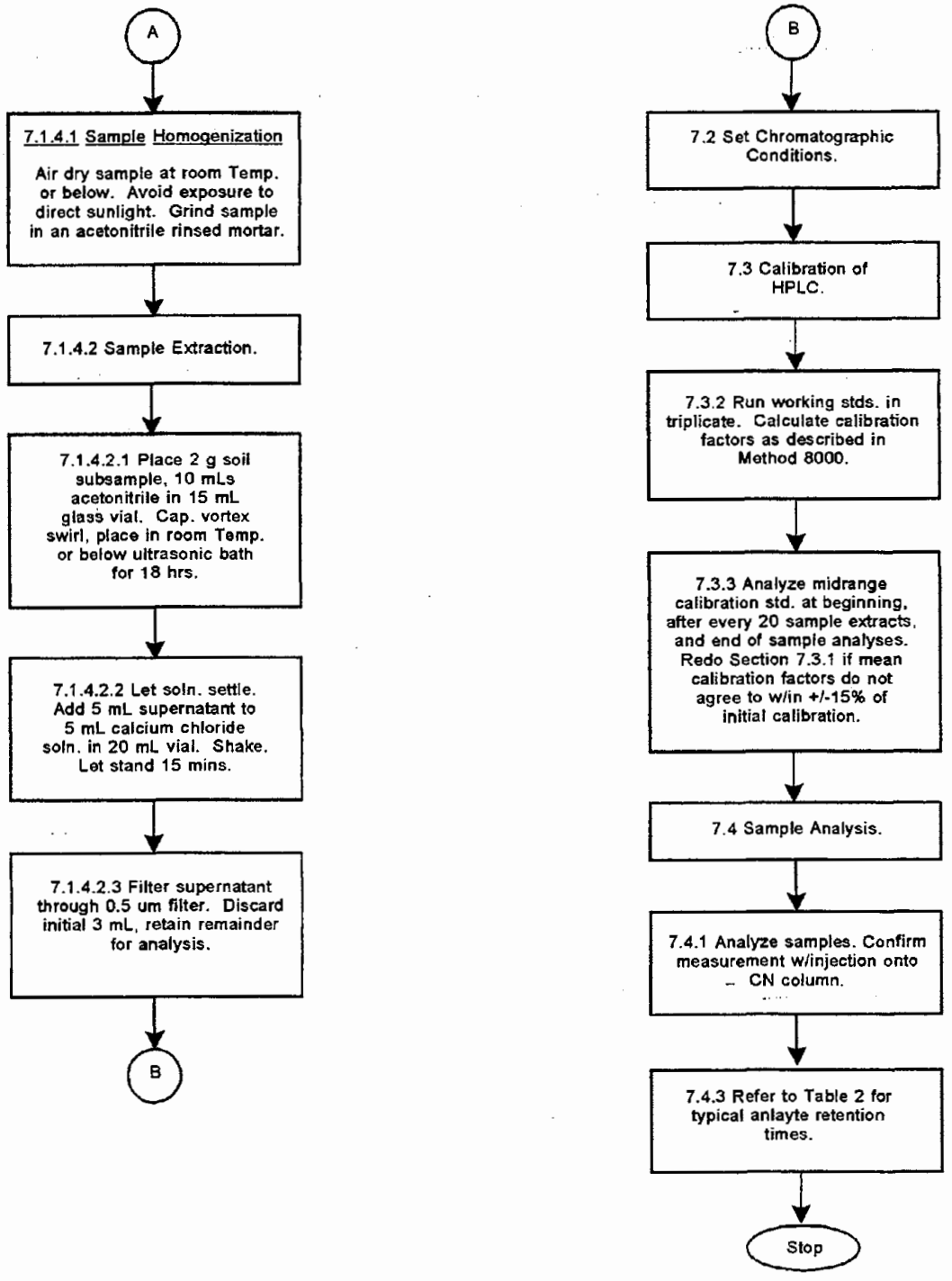
FIGURE 1
EXAMPLE CHROMATOGRAMS





METHOD 8330A
NITROAROMATICS AND NITRAMINES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)




A
7.1.4.1 Sample Homogenization
Air dry sample at room Temp. or below. Avoid exposure to direct sunlight. Grind sample in an acetonitrile rinsed mortar.
7.1.4.2 Sample Extraction.
7.1.4.2.1 Place 2 g soil subsample, 10 mLs acetonitrile in 15 mL glass vial. Cap. vortex swirl, place in room Temp. or below ultrasonic bath for 18 hrs.
7.1.4.2.2 Let soln. settle. Add 5 mL supernatant to 5 mL calcium chloride soln. in 20 mL vial. Shake. Let stand 15 mins.
7.1.4.2.3 Filter supernatant through 0.5 um filter. Discard initial 3 mL, retain remainder for analysis.
B
B
7.2 Set Chromatographic Conditions.
7.3 Calibration of HPLC.
7.3.2 Run working stds. in triplicate. Calculate calibration factors as described in Method 8000.
7.3.3 Analyze midrange calibration std. at beginning, after every 20 sample extracts, and end of sample analyses. Redo Section 7.3.1 if mean calibration factors do not agree to w/in +/-15% of initial calibration.
7.4 Sample Analysis.
7.4.1 Analyze samples. Confirm measurement w/injection onto CN column.
7.4.3 Refer to Table 2 for typical anlayte retention times.
Stop

4.3 DETERMINATION OF ORGANIC ANALYTES

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgements necessary to meet the data quality objectives or needs for the intended use of the data.

4.3.4 INFRARED METHODS

The following methods are included in this section:

Method 8410:	Gas Chromatography/Fourier Transform Infrared (GC/FT-IR) Spectrometry for Semivolatile Organics: Capillary Column
Method 8430:	Analysis of Bis(2-chloroethyl) Ether and Hydrolysis Products by Direct Aqueous Injection GC/FT-IR
Method 8440:	Total Recoverable Petroleum Hydrocarbons by Infrared Spectrophotometry

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4.3.5 MISCELLANEOUS SPECTROMETRIC METHODS

The following method is included in this section:

Method 8520: Continuous Measurement of Formaldehyde in Ambient Air

4.4 IMMUNOASSAY METHODS

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgements necessary to meet the data quality objectives or needs for the intended use of the data.

The following methods are included in this section:

Method 4000:	Immunoassay
Method 4010A:	Screening for Pentachlorophenol by Immunoassay
Method 4015:	Screening for 2,4-Dichlorophenoxyacetic Acid by Immunoassay
Method 4020:	Screening for Polychlorinated Biphenyls by Immunoassay
Method 4030:	Soil Screening for Petroleum Hydrocarbons by Immunoassay
Method 4035:	Soil Screening for Polynuclear Aromatic Hydrocarbons by Immunoassay
Method 4040:	Soil Screening for Toxaphene by Immunoassay
Method 4041:	Soil Screening for Chlordane by Immunoassay
Method 4042:	Soil Screening for DDT by Immunoassay
Method 4050:	TNT Explosives in Soil by Immunoassay
Method 4051:	Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Soil by Immunoassay
Method 4670:	Triazine Herbacides as Atrazine in Water by Quantitative Immunoassay

METHOD 4670

TRIAZINE HERBICIDES AS ATRAZINE IN WATER BY QUANTITATIVE IMMUNOASSAY

1.0 SCOPE AND APPLICATION

1.1 This method describes a procedure for the quantitative determination of atrazine (CAS# 1912-24-9) and other triazine herbicides in water using a competitive immunoassay. The method provides a single quantitative result, reported as atrazine, for all compounds detected. However, the extent to which other triazine herbicides and other compounds are detected may vary between commercial testing products (see Secs. 1.4 and 4.0).

1.2 Testing products are commercially available from several manufacturers. The testing product evaluated by EPA for this method employs a competitive immunoassay. Other products differ in a number of respects, including the format of the test (tubes versus microtiter plates), the reagents used, and the specific steps in the test procedure.

1.3 The method detection limit (MDL) submitted by the manufacturer of the testing product described in Sec. 6.2 was 0.03 µg/L for drinking water samples. The actual detection limit may be highly dependent on the sample matrix and analyst's performance.

1.4 Since immunoassay methods use antibody molecules that can bind to more than the target analyte, an immunoassay has a tendency to overestimate the concentration of the target analyte when other analytes are present that may bind with the antibody. The commercially-available testing product evaluated for this method is based on an immunochemical reaction that will also respond to other triazine compounds. These other triazine compounds are often included in pesticide formulations containing atrazine. Thus, the specificity of this procedure for atrazine is partly a function of the cross-reactivity of those other compounds (see Table 1). Therefore, as with other analytical techniques such as single-column gas chromatography, it is advisable to confirm positive test results near or above a regulatory action limit when the presence of other triazines is suspected.

1.5 This method is restricted to use by or under the supervision of analysts trained in the performance and interpretation of immunoassay methods. Each analyst must demonstrate the ability to generate acceptable results with this method (see Sec. 9.5).

2.0 SUMMARY OF METHOD

2.1 An accurately measured volume of sample (as little as 200 µL for some testing products) is mixed with a volume of enzyme-atrazine conjugate reagent in a test tube or a microtiter plate that has an anti-atrazine antibody immobilized on the surface, or in a vessel to which particles (magnetic particles for one testing product) with an immobilized antibody on the surface are added. The conjugate "competes" with the atrazine present in the sample for binding to the immobilized anti-atrazine antibody. The mixture is incubated at the temperature, and for the time, described in the manufacturer's instructions. (Testing products may employ other solid-phase support configurations, or even eliminate the solid-phase support. The summary here is intended to be generic and not to limit the development of other testing products).

2.2 Unbound conjugate and sample analyte that may be present in the tubes or wells are removed by washing with organic-free reagent water or wash solution specified by the manufacturer. A signal-generating substrate/chromogen reagent is added and the tube or plate is incubated as described in the manufacturer's instructions. In the case of the testing product described in Sec. 6.2, a magnetic field is applied to the tubes to retain the magnetic particle coated with antibody and any bound enzyme conjugate present during the wash step. (Other testing products may use different configurations).

2.3 In an enzyme immunoassay, a stop solution is added to the tubes or wells of the plate to terminate the signal generating activity of the enzyme conjugate reagent. The absorbance is measured at a wavelength specified by the manufacturer. The test is interpreted by measuring the signal produced by a sample and determining the concentration from a dose-response curve constructed from standards tested at the same time. For a competitive immunoassay, the color (signal) developed during the test is inversely proportional to the concentration of atrazine in the sample.

3.0 DEFINITIONS

The definitions associated with immunoassay procedures are given in Method 4000 and in the glossary at the end of this method.

4.0 INTERFERENCES

4.1 Compounds that are chemically similar may cause a positive test result (false positive) for atrazine. This phenomenon is known as cross-reactivity. The testing product used in preparation of this method has been evaluated for cross-reactivity by the manufacturer. Table 1 provides the concentration at which known cross-reactants will give a comparable response to that of atrazine when present in the sample.

4.1.1 The presence of cross-reacting compounds will result in an increase in the calculated concentration of the sample being analyzed and therefore influence the incidence of false positive results. Thus, from the standpoint of monitoring compliance with a regulatory action limit, cross-reactivity is not a significant concern for test results *below* the action limit.

4.1.2 As with techniques such as single-column gas chromatography, in instances where the presence of other triazine compounds is known or suspected, it may be advisable to confirm positive results near or above the regulatory action limit using another analytical technique. However, false negative results are generally not a concern with immunoassay techniques.

4.2 Non-specific interferences such as sample pH, temperature, osmolarity, solvents, surfactants, and the presence of metal ions can effect immunoassay performance. Samples should be tested at the pH and temperature range specified by the testing product manufacturer. Review the product literature with regard to other potential interferences.

4.3 Storage temperatures may alter the useful life of the testing product reagents and supplies. Follow the manufacturer's directions for storage and use of all reagents and supplies.

5.0 SAFETY

No extraordinary safety measures are required. However, safety procedures consistent with good laboratory practices should be employed. Some reagents may contain dilute acid solutions. Avoid contact with eyes, skin, and mucous membranes.

6.0 EQUIPMENT AND SUPPLIES

6.1 Each commercially-available testing product will supply or specify the apparatus and materials necessary for successful completion of the test. Most testing products supply the equipment and supplies specific to the immunoassay, including the tubes or plates containing the immobilized antibody, and the immunochemical reagents. Do *not* mix the equipment, supplies, and reagents from the testing products for different analytes, or from the testing products from different manufacturers. Testing products contain immunochemical reagents that are evaluated by the manufacturer on a lot-specific basis. Do not mix the reagents from one lot with those from another lot unless expressly allowed by the manufacturer. Other equipment that may be required, but is not supplied with the testing product, includes common laboratory items such as precision pipetting devices, vortex mixers, etc.

6.2 The immunoassay testing product listed below has been submitted to EPA, evaluated by the Agency, and found to meet the performance specifications necessary for inclusion in SW-846. Additional testing products may be available from other manufacturers or in different formats. As additional testing products are evaluated by EPA and found to provide equivalent performance, information will be made available by the Office of Solid Waste regarding all those testing products that are capable of meeting the performance specifications in this method. However, this procedure will not be revised solely to include information on additional testing products.

Atrazine RaPID Assay® (Ohmicron Environmental Diagnostics, Inc.).

7.0 REAGENTS AND STANDARDS

As with the equipment and supplies, each commercially-available testing product will supply or specify the reagents necessary for successful completion of the test. This includes the calibrators (standards) employed in the immunoassay. As noted in Sec. 6.1, do *not* mix the equipment, supplies, and reagents from the testing products for different analytes, or from the testing products from different manufacturers. Store all reagents and standards according to the manufacturer's instructions, and, where applicable, discarding any which have exceeded the expiration date assigned by the manufacturer.

In addition, in order to demonstrate the method performance described in Sec. 9, the following reagents and standards will be required.

7.1 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One. Organic-free reagent water is used for the preparation of the initial demonstration of capability test, the laboratory control sample, and other quality control tests. These tests are in addition to any control material(s) supplied by the manufacturer.

7.2 Atrazine spiking solution - a solution of atrazine in a water-miscible solvent is required for spiking into organic-free reagent water to prepare the initial demonstration of proficiency test, the laboratory control sample, and other quality control tests. This solution may be provided by the

manufacturer. If not provided, the laboratory should prepare a spiking solution or purchase one from a commercial source. Consult the manufacturer's instructions regarding solvents that may interfere with the testing product and do not use them. The concentration of this solution should be approximately 0.3 µg/mL, such that a 100 µL volume spiked into a 10 mL volume of reagent water will yield a concentration of 3 µg/L. Other volumes and concentrations may be employed, provided that the laboratory can demonstrate that the volume of solvent used does not affect the test performance.

7.3 Solutions for adjusting the pH of samples before extraction, where such pH adjustment is specified by the manufacturer.

7.3.1 Sulfuric acid solution (1:1 v/v), H_2SO_4 - Slowly add 50 mL of H_2SO_4 (sp. gr. 1.84) to 50 mL of organic-free reagent water.

7.3.2 Sodium hydroxide solution (2N), NaOH - Dissolve 8 g NaOH in organic-free reagent water and dilute to 100 mL.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation, and storage requirements may vary by EPA program and may be specified in the regulation that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining the sample collection, preservation, and storage requirements.

8.1 Sample Collection

The immunoassay testing products employ very small (< 1 mL) sample volumes. Therefore, sample collection procedures should focus on the volume necessary to ensure that the sample represents the source.

8.1.1 Samples should be collected in pre-cleaned glass containers.

8.1.2 When sampling from a water tap, open the tap and allow the system to flush until the water temperature has stabilized (usually 2 to 5 minutes). Adjust the flow to about 500 mL/min, and collect samples from the flowing stream. When sampling from an open body of water, fill the sample container with water from a representative area.

8.2 Sample preservation

8.2.1 If residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample to the bottle, prior to collecting the sample.

8.2.2 Retard microbiological degradation by adjusting the pH of the samples to <2 with hydrochloric acid at the time of sample collection. Before analysis, readjust the pH of the samples to the pH specified by the manufacturer with 2N NaOH. The pH of the entire collected sample should be adjusted, not just the small volume utilized for the analysis.

8.3 Sample storage - Samples should be stored at 4 ± 2°C until analysis, but must be warmed to the temperature specified by the manufacturer for analysis.

8.4 EPA has not conducted holding time studies relative to immunoassay.

9.0 QUALITY CONTROL

As noted in Sec. 1.2, the specific formats of the commercially-available testing products vary by manufacturer. As a result, those testing products evaluated and accepted by EPA represent performance-based analytical methods. **Therefore, it is imperative that the manufacturer's instructions and specifications be followed closely.** Follow the manufacturer's instructions for the testing product being used for quality control procedures specific to the testing product used. The following discussion of quality control requirements relies heavily of the analyst's knowledge and understanding of the manufacturer's instructions.

9.1 Initial calibration

An initial calibration must be performed concurrent with the analysis of any samples, as described in Sec. 10.

9.2 Calibration verification

Calibration verification is not performed in the traditional sense because the initial calibration standards are analyzed with each batch of samples each time the analyses are performed.

9.3 Routine Quality Control

Routine quality control procedures associated with this method include the analyses of standards, matrix spike samples, laboratory control samples, method blanks, and duplicate or replicate analyses (as specified by the manufacturer). All of the analyses described below must be conducted simultaneously, e.g., as part of the same batch of samples. A batch of samples consists of up to 20 field samples prepared and analyzed at the same time, *or* the maximum number of samples that can be analyzed along with the standards, controls, and other analyses specified by the manufacturer using a single testing product, *whichever is fewer.* The batch must include any duplicate or replicate analyses specified by the manufacturer as well as all additional quality control tests specified by EPA in this procedure.

9.3.1 Calibration standards must be analyzed concurrently with each batch of samples processed.

9.3.2 Matrix spike (MS) samples must be analyzed with each batch of samples processed. The matrix spike samples should contain atrazine at the regulatory limit of interest (e.g., the MCL for the Drinking Water Program). The sample chosen for spiking should be representative of the field samples being analyzed.

9.3.3 The analyst must evaluate the accuracy of the assay by analyzing a laboratory control sample (LCS) consisting of organic-free reagent water sample spiked at the regulatory limit of concern for atrazine. For the Drinking Water Program, the LCS must be spiked at 3 µg/L (the MCL for atrazine) with the spiking solution in Sec. 7.2. The mean recovery (bias) of the assay must be between 80-120%. If the manufacturer does not supply the spiking solution described in Sec. 7.2, or if another regulatory limit is relevant, then the laboratory is responsible for purchasing or preparing an appropriate spiking solution and performing this test. An LCS must be prepared and analyzed with each batch of samples analyzed.

NOTE: Spiking at 3 µg/L may require that the sample be diluted to be within the calibration range for some testing products, however, it provides data regarding the bias (if any) at the regulatory threshold, as well as indications of the analyst's proficiency at making dilutions.

9.3.4 A method blank, consisting of a volume of organic-free reagent water (see 7.1) equal to that of a field sample, must be analyzed with each batch of samples processed. The method blank should not contain any detectable atrazine.

9.3.5 Samples should be analyzed in duplicate or triplicate, as instructed by the manufacturer. The number of replicate analyses is specified by the manufacturer, and is a function of the overall precision of the particular testing product. If the manufacturer determines that, in order to achieve the precision claimed by the manufacturer, a given number of replicate analyses must be performed, then the laboratory must employ the specified number of replicate analyses.

9.4 Sample Dilutions

If the sample concentration is outside of the calibrated range demonstrated by the initial calibration and as specified by the manufacturer, then the sample must be diluted to within the calibration range and re-tested. As employed in these testing products, the calibration range specified by the manufacturer is based on a B/B_0 in the 0.2-0.8 (20-80%) range. Given the nature of the competitive immunoassay, the sample cannot be diluted *after* color development. Thus, a diluted aliquot of the original sample must be prepared and analyzed.

NOTE: The B/B_0 range of 0.2-0.8 is *narrower* than the simple concentration range of the calibration standards. Therefore, the decision to dilute a sample for reanalysis must be based on an evaluation of the B/B_0 value of the sample, and *not* on a simple comparison of the concentration in the sample and the highest standard in the calibration.

9.5 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with the testing product that it utilizes, by generating data of acceptable accuracy and precision for a reference sample containing atrazine in a clean matrix. The laboratory must also repeat this demonstration whenever new staff are trained or significant changes in instrumentation are made.

9.5.1 The reference sample is prepared from a spiking solution containing the analyte of interest (see Sec. 7.2). Given the very small sample volume required for the immunoassay, a single 10-mL aliquot will provide sufficient volume for multiple tests and minimizes the difficulties involved in spiking small volumes of organic-free reagent water. Prepare a new aliquot each time the initial demonstration is to be performed.

9.5.2 Prepare an aliquot of organic-free reagent water, spiking it with the solution in Sec. 7.2 to yield a concentration of 3 µg/L. Mix the aliquot well and allow the spiked sample to stand for at least one hour.

9.5.3 Analyze at least four replicate subsamples of the spiked organic-free reagent water aliquot using the same procedures used to analyze actual samples (Sec. 11). Analyze the number of replicates of each subsample specified by the manufacturer, e.g., if the manufacturer specifies triplicate analyses of samples, then analyze 12 replicates (4 x 3) of the spiked sample.

9.5.4 Calculate the mean recovery ($\bar{X}$), and the standard deviation of the recoveries using the total number of replicate results, as described in Sec. 12.6.

9.5.5 Given the total number of replicate analyses performed, the mean recovery ($\bar{X}$) should be in the range of 90-110% and the relative standard deviation should be no more than 10% of the mean recovery. If the results fall outside of these acceptance limits, recheck all calculations. If no errors are found, repeat the demonstration until the specifications are met.

9.6 Other Quality Control Considerations

9.6.1 Do not use testing products past their expiration date.

9.6.2 Do *not* mix the equipment, supplies, and reagents from the testing products for different analytes, or from the testing products from different manufacturers.

9.6.3 Use the testing products within the storage temperature and operating temperature limits specified by the manufacturer.

10.0 CALIBRATION AND STANDARDIZATION

10.1 The analyst must perform an initial calibration. This calibration is performed concurrently with the analysis of samples.

10.1.1 The initial calibration must consist of standards (calibrators) at a minimum of three concentrations that describe the quantitation range of the assay and should preferably span the regulatory limit of interest (e.g., for drinking water, the maximum contaminant level [MCL] is 3.0 µg/L). The standards must fall within the B/B_0 range of 0.2 to 0.8. The calibrators are generally provided by the product manufacturer. Calibration curves where all the calibrators are below the regulatory limit are allowed, but will require dilution and reanalysis of samples when the sample concentration is near the regulatory limit. Calibration curves where *all* the calibrators are *above* the applicable regulatory limit may *not* be employed for compliance monitoring.

10.1.2 The testing product must also contain a "zero standard" or diluent solution that contains none of the target analytes. This solution is used to generate the B_0 value, but must not be used as one of the three standards specified in Sec. 10.1.1.

10.1.3 When the entire dose response of a competitive immunoassay testing product (the absorbance of the solution or other signal specified by the manufacturer) is plotted on the y-axis against the concentration of the calibration standard on the x-axis, the resulting calibration curve will be hyperbolic when plotted on rectilinear paper, sigmoidal when plotted on semi-log paper, and linear when a Logit-log transformation of the data is employed and plotted on rectilinear paper. In addition, since the immunoassay is competitive, the blank (zero standard) will yield the highest response, with the color development inversely proportional to the standard concentration.

A plot of either the Logit B/B_0 or the Logit of the signal (absorbance units) versus the natural log of concentration is a widely used representation of the calibration data that generally yields a linear response curve. It is the basis of most computerized data analysis

algorithms for competitive binding assays. The Logit B/B_0 is calculated according to the following formula:

$$\text{Logit } (B/B_0) = \log_e \left(\frac{\frac{B}{B_0}}{1 - \frac{B}{B_0}} \right) = \log_e \left(\frac{B}{B_0 - B} \right)$$

where:

$\log_e$ = Natural log or logarithm base e
B = Response of the standard or sample
B_0 = Response of the zero standard

When Logit B/B_0 is plotted against the natural log ($\log_e$) of concentration, the results approximate a straight line with a negative slope (see Figure 1c). The transformed calibration data can then be characterized by the slope, intercept, correlation coefficient, and standard error of the line. The following sections describe the use of the Logit-log transformation of the data to prepare a calibration curve. Manufacturer's may provide software that performs these calculations and, if provided, such software should be employed according to the manufacturer's instructions.

10.1.3.1 The commercially-available testing products may specify the analysis of standards in duplicate, or even in triplicate in some testing products. Thus, a three-point initial calibration may generate six to nine calibration points. Calculate the mean response (absorbance) at each concentration, and use this in all subsequent calculations.

10.1.3.2 Following the Logit B/B_0 and log transformations described in Sec. 10.1.3, construct a first order regression line (e.g., $y = mx + b$) using Logit B/B_0 as the dependent variable (y-axis) and the $\log_e$ concentration as the independent variable (x-axis). Since the slope of the line is negative, the regression cannot be forced through the origin, as the zero standard will yield the highest response and a value of 1.0 for B/B_0. The standards used to construct the regression line all must have B/B_0 values (prior to the Logit transformation) that fall within the 0.2-0.8 range.

The correlation coefficient of the regression (r) must be at least 0.98 in order to employ the calibration curve (manufacturers may provide more stringent linearity requirements for their testing products). If r is less than 0.98, check the expiration dates of all reagents, review the procedures to ensure that all standards were incubated for the same time specified by the manufacturer, and perform a new calibration.

10.2 By convention, the working range of an immunoassay calibration curve is defined as the range of B/B_0 from 0.2 to 0.8 (or $\%B/B_0$ from 20% to 80%). Samples may be quantitated only within the working range of the curve.

10.3 As noted in Sec. 9, a new initial calibration curve must be constructed with each batch of samples assayed.

11.0 PROCEDURE

Follow the manufacturer's instructions for the test being used. These instructions are summarized in Secs. 11.1 through 11.3, however, given the difference in test formats and reagents, the discussion is generic in nature. Where the manufacturer's instructions contradict these instructions or where these instructions do not apply to a specific testing product, **follow the manufacturer's instructions.**

11.1 Prepare the samples and standards

11.1.1 Bring samples, controls, and reagents to ambient temperature. Verify that the ambient temperature is consistent with the manufacturer's recommendations and limitations for the method. Do not attempt to perform tests outside of the temperature range specified by the manufacturer.

11.1.2 Check the pH of the samples. If necessary, adjust the pH to the range specified by the manufacturer, using 2N NaOH.

11.2 Prepare the spectrophotometer, photometer, or signal measurement equipment specified by the manufacturer.

11.3 Assay samples

11.3.1 Dispense the standards, controls, and samples into the container specified by the manufacturer. Be certain to include the replicate analyses specified by the manufacturer and the routine quality control samples specified in Sec. 9.3 (also in replicate if samples are analyzed in replicate). Determine the maximum number of standards, controls, and samples that can be analyzed simultaneously and limit the number of field samples accordingly.

11.3.2 Dispense the enzyme conjugate reagent into each container as specified by the manufacturer.

11.3.3 Dispense the antibody capture reagent (where appropriate) as specified by the manufacturer.

11.3.4 Immunoassay methods employ kinetic and chromogenic reactions that are temperature sensitive. As a result, take care to perform the assay in the temperature range recommended by the manufacturer. Failure to follow temperature recommendations can lead to anomalous test results.

NOTE: Do not attempt to process more samples simultaneously than specified by the manufacturer, as the additional processing time will lead to different incubation times for the samples and standards being tested and will produce erroneous results.

11.3.5 Wash each tube or well with washing reagents, as directed by the manufacturer.

11.3.6 Dispense the signal generating and signal terminating reagents (e.g., substrate/chromogen reagent and stop solutions) to each container in accordance with the manufacturer's instructions. Pay careful attention to the incubation times specified by the

manufacturer. Failure to follow incubation time recommendations can lead to erroneous results.

11.3.7 Interpret the test results within the time specified by the manufacturer. Follow the manufacturer's instructions for determining the sample concentration. For instance, read absorbance values (or optical density) at wavelength(s) specified by the manufacturer. Follow the manufacturer's quality control and data acceptance instructions.

12.0 DATA ANALYSIS AND CALCULATIONS

As with the specific formats of the testing products and the reagents and supplies, the specifics of the required calculations may vary by manufacturer. Some testing products may provide measuring devices such as optical density readers or spectrophotometers and may include software for performing all the necessary calculations. Other testing products may require the analyst to plot results manually, using graph paper that may or may not be provided with the testing product, and determine sample results by interpolation from a standard curve. Whichever approach is used, the laboratory records (bench notes, etc.) should clearly indicate how the results were obtained and records specific to each determination, whether in hard copy or in electronic form, should be retained by the laboratory to substantiate the results.

12.1 Follow the manufacturer's instructions regarding calculation of all testing product results. Use the calibration curve generated concurrently with the sample analyses.

12.2 Where replicate test results are generated for samples or standards, calculate the mean concentration ($\overline{C}$) as:

$$\text{mean concentration} = \overline{C} = \frac{\sum_{i=1}^{n} C_i}{n}$$

where C_i is the concentration in each replicate and n is the number of replicate analyses.

12.3 For duplicate test results, calculate the relative percent difference (RPD) according to the following equation:

$$RPD = \frac{|C_1 - C_2|}{\frac{(C_1 + C_2)}{2}} \times 100$$

where C_1 and C_2 are the concentrations of the two replicate determinations.

12.4 When the manufacturer's instructions specify the analyses of three or more replicates, calculate the standard deviation (SD) and the relative standard deviation (RSD) of the replicate results for each sample, according to the following equations:

$$SD = \sqrt{\frac{\sum_{i-1}^{n}(C_i - \bar{C})^2}{n-1}} \qquad SD = \frac{SD}{\bar{C}} \times 100$$

where C_i is the concentration in each replicate, $\bar{C}$ is the mean concentration, and n is the number of replicate analyses.

12.5 Accuracy is estimated from the recovery of spiked analytes from the matrix of interest. Laboratory performance in a clean matrix is estimated from the recovery of analytes in the LCS. Calculate the recovery of each spiked analyte in the matrix spike, matrix spike duplicate (if performed) and LCS according to the following formula.

$$\text{Recovery} = \%R = \frac{C_s - C_u}{C_n} \times 100$$

where:

C_s = Measured concentration of the spiked sample aliquot
C_u = Measured concentration of the unspiked sample aliquot (use 0 for the LCS)
C_n = Nominal (theoretical) concentration of the spiked sample aliquot

12.6 For the initial demonstration of proficiency (Sec. 9.6) calculate the mean recovery ($\bar{X}$), and the standard deviation of the recoveries, using the results from all replicate analyses of the four subsamples. Use the equation in Sec. 12.4 for standard deviation, substituting recovery for concentration.

13.0 METHOD PERFORMANCE

13.1 Table 1 summarizes the cross-reactivity of other triazines relative to atrazine for the testing product listed in Sec. 6.2. Other testing products may have different cross-reactivity characteristics.

13.2 Table 2 summarizes the single laboratory MDL data submitted by the manufacturer for the testing product in Sec. 6.2.

13.3 Table 3 summarizes the results of a collaborative study of the immunoassay testing product described in Sec. 6.2 conducted under the auspices of the AOAC and described in Reference 3.

13.4 Figure 1 (a-c) provides three graphical representations of the calibration of atrazine using a competitive binding immunoassay such as those described here.

14.0 POLLUTION PREVENTION

Analysis for atrazine using immunoassay conforms with EPA's pollution prevention goals. Little, if any, solvent is used and minimal waste is generated.

15.0 WASTE MANAGEMENT

Laboratory waste management procedures must be consistent with federal, state, and local regulations.

16.0 REFERENCES

1. "Principles of Competitive Protein-Binding Assays," Dell, W.O., Franchimont, P., John Wiley and Sons, New York, 1983.

2. "Immunoassay Analysis and GC/MS Confirmation for Residues of Atrazine in Water Samples from a Field Study Conducted by the State of Wisconsin," Project No. 101174, Report No. ABR-91069, CIBA-GEIGY Corporation, April 6, 1992.

3. "Determination of Atrazine in Water by a Magnetic Particle Immunoassay: Collaborative Study," Hayes, Mary C., Jourdan, Scott W., and Herzog, David P., *JAOAC*, 79(2): 530-538, 1996.

4. "Performance Characteristics of a Novel Magnetic-particle-based Enzyme-linked Immunosorbent Assay for the Quantitative Analysis of Atrazine and Related Triazines in Water Samples," Rubio, Fernando M., Itak, Jeanne M., Scutellaro, Adele M., Selisker, Michele Y., and Herzog, David P., *Food & Agricultural Immunology*, 3: 113-125, 1991.

5. "Comparison of an Enzyme Immunoassay and Gas Chromatography/Mass Spectrometry for the Detection of Atrazine in Surface Waters," Gruessner, Barry, Shambaugh, Nathaniel C., and Watzin, Mary, C., *Environmental Science and Technology*, 29: 251-254, 1995.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain Tables 1 through 3 and Figure 1.

TABLE 1

CROSS-REACTIVITY OF RaPID ASSAY TO RELATED COMPOUNDS

Compound	CAS #	Percent Reactivity Relative to Atrazine
Atrazine	1912-24-9	100
Ametryn	834-12-8	185
Prometryn	7287-19-6	113
Propazine	139-40-2	97
Prometon	1610-18-0	32
De-ethylated atrazine	6190-65-4	22
Simazine	122-34-9	15
Terbutryn	886-50-0	13
Terbuthylazine	5915-41-3	5
Hydroxy atrazine	2163-68-0	0.5
De-isopropylated atrazine	1007-28-9	0.3
Cyanazine	21725-46-2	<0.1

TABLE 2

METHOD DETECTION LIMIT (µg/L)

Product	n	Spike Level	Std. Dev.	MDL[1]
RaPID Assay	10	0.1	0.0105	0.03

[1] The manufacturer reported MDL results for 10 replicates but used the Student's *t* value of 3.143, for seven replicates, in performing the calculations. The value shown above was corrected to the appropriate *t* value of 2.821.

TABLE 3

SUMMARY STATISTICS OF COLLABORATIVE STUDY OF RaPID ASSAY (Source: Ref. 3)

Sample Type	Spike Conc. (µg/L)[1]	n	Mean Conc. (µg/L)	Single Analyst RSD	Overall RSD	Recovery (%)[2]
Reagent Water	0.00	14	0.02	--	--	--
Municipal Tap Water	0.00	14	0.02	--	--	--
	0.15	14	0.16	19.1	39.6	107
	1.00	13	1.13	9.09	15.6	113
	3.00	14	2.85	9.08	9.08	95
Well Water	0.00	14	0.00	--	--	--
	0.15	13	0.15	16.6	43.4	100
	1.00	14	1.05	9.53	11.5	105
	3.00	14	2.88	9.95	9.95	96
Surface Water	0.00	14	0.02	--	--	--
	0.15	14	0.17	27.7	39.0	113
	1.00	14	1.06	8.22	16.2	106
	3.00	13	3.44	11.7	19.1	114
Field-Contaminated	0.00	14	0.24	29.9	35.8	--
Sample 1	0.60	14	0.93	9.27	17.9	115
	2.00	14	2.19	9.13	13.8	98
	4.00	14	3.48	8.29	9.28	81
Field-Contaminated	0.00	14	0.47	20.1	30.8	--
Sample 2	0.80	13	1.28	10.0	19.5	101
	2.00	14	3.15	7.74	18.4	134
	4.00	14	4.03	9.52	12.5	89

[1] Data for the two field-contaminated water samples represent the amount of atrazine added to the sample and the mean concentration and RSD data represent the amount found in excess of the background field contamination.

[2] Recovery not calculated for unspiked samples.

FIGURE 1

CALIBRATION DATA FROM A COMPETITIVE IMMUNOASSAY

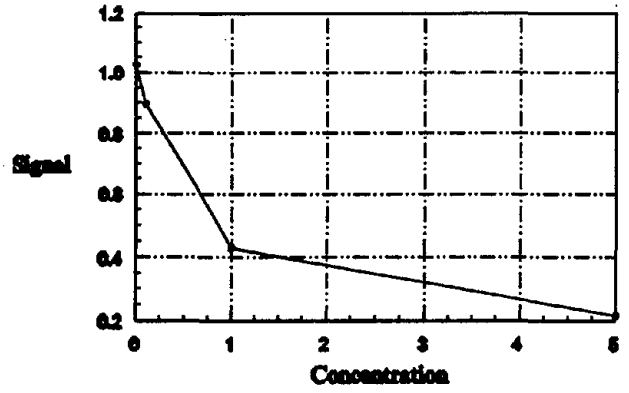


Figure 1a - Generalized plot of immunoassay signal (test response) versus concentration of calibration standard (µg/L).

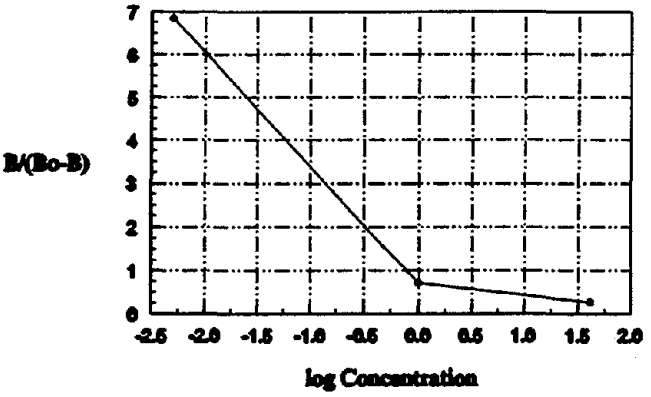


Figure 1b - Generalized plot of $B/(B_0-B)$ versus log concentration of calibration standard.

FIGURE 1
(continued)

CALIBRATION DATA FROM A COMPETITIVE IMMUNOASSAY

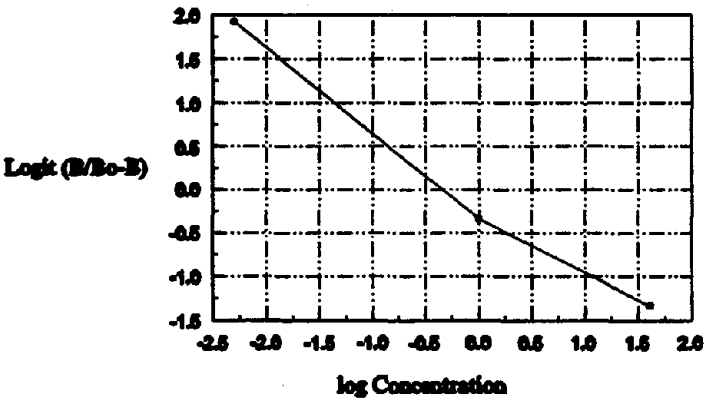


Figure 1c - Generalized plot of Logit [B/(B_0-B)] versus log concentration of calibration standard.

GLOSSARY OF TERMS

Antibody - A binding protein which is produced in response to an antigen, and which has the ability to bond with the antigen that stimulated its production.

$\%B/B_0$ - an indication of the displacement characteristics of the conjugate from the antibody at specified concentrations of the target compound.

$$\%\frac{B}{B_0} = \frac{\text{Response of the standard or sample}}{\text{Response of the zero standard}} \times 100$$

Competitive Immunoassay - An immunoassay method involving an *in-vitro* competitive binding reaction.

Cross-Reactivity - The relative concentration of an untargeted substance that would produce a response equivalent to a specified concentration of the targeted compound. In a quantitative immunoassay, it provides an indication of the concentration of cross-reactant that would produce a positive response. Cross-reactivity for individual compounds is often calculated as the ratio of target substance concentration to the cross-reacting substance concentration at 50% inhibition of the immunoassay's maximum signal times 100%.

Dose-Response Curve - Representation of the signal generated by an immunoassay (y axis) plotted against the concentration of the target compound (x axis) in a series of standards of known concentration. When plotting a competitive immunoassay in a rectilinear format, the dose-response will have a hyperbolic character. When the log of concentration is used, the plot assumes a sigmoidal shape, and when the log of signal is plotted against the Logit transformation of concentration, a straight line plot is produced.

ELISA - *Enzyme Linked Immunosorbent Assay* is an enzyme immunoassay method that uses an immobilized reagent (*e.g.*,antibody adsorbed to a plastic tube), to facilitate the separation of targeted analytes (antibody-bound components) from non-target substances (free reaction components) using a washing step, and an enzyme conjugate to generate the signal used for the interpretation of results.

Enzyme Conjugate - A molecule produced by the coupling of an enzyme molecule to an immunoassay component that is responsible for acting upon a substrate to produce a detectable signal.

Enzyme Immunoassay - An immunoassay method that uses an enzyme conjugate reagent to generate the signal used for interpretation of results. The enzyme mediated response may take the form of a chromogenic, fluorogenic, chemiluminescent or potentiometric reaction. (*see Immunoassay and ELISA*)

False Negatives - A negative interpretation of the method containing the target analytes at or above the detection level. Ideally, an immunoassay test product should produce no false negatives. The false negative rate can be estimated by analyzing split samples using both the test product and a reference method.

False Positives - A positive interpretation for a sample is defined as a positive response for a sample that contains analytes below the action level.

Immunoassay - An analytical technique that uses an antibody molecule as a binding agent in the detection and quantitation of substances in a sample. (*see Enzyme Immunoassay and ELISA*)

Immunogen - A substance having a minimum size and complexity, and that is sufficiently foreign to a genetically competent host to stimulate an immune response.

Logit - A logarithmic transformation of data normalized to the highest observed response. For the competitive immunoassay described in this procedure, the Logit transformation is calculated as:

$$\text{Logit } (B/B_0) = \log_e \left(\frac{\frac{B}{B_0}}{1 - \frac{B}{B_0}} \right) = \log_e \left(\frac{B}{B_0 - B} \right)$$

Natural Log - The logarithm, base e, of a number. The natural logarithm may also be represented as "ln" or "$\log_e$."

4.5 MISCELLANEOUS SCREENING METHODS

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgements necessary to meet the data quality objectives or needs for the intended use of the data.

The following methods are included in this section:

Method 3820:	Hexadecane Extraction and Screening of Purgeable Organics
Method 8515:	Colorimetric Screening Method for Trinitrotoluene (TNT) in Soil
Method 9074:	Turbidmetric Screening Method for Total Recoverable Pertoleum Hydrocarbons in Soil
Method 9078:	Screening Test Method for Polychlorinated Biphenyls in Soil
Method 9079:	Screening Test Method for Polychlorinated Biphenyls in Transformer Oil

METHOD 9074

TURBIDIMETRIC SCREENING METHOD FOR TOTAL RECOVERABLE PETROLEUM HYDROCARBONS IN SOIL

1.0 SCOPE AND APPLICATION

1.1 This method may be used to screen soil samples to determine the total amount of recoverable petroleum hydrocarbon contamination in soil including a wide range of fuels, oils, and greases. The turbidimetric approach in this method is designed to quickly screen soil samples using a system calibrated with a blank and a single calibration standard.

1.2 The definition of total recoverable petroleum hydrocarbons for this method can be found in the section on definitions (Sec. 3.0).

1.3 This screening technique is specifically designed to be used in the field but may also have some screening applications in the laboratory. The system analysis range is 10-2000 ppm for most hydrocarbons.

1.4 This method is considered a screening technique because of the broad spectrum of hydrocarbons it detects. The method may be especially useful in quickly determining that a site does not contain hydrocarbon contamination. However, it cannot be used to determine specific hydrocarbon compounds or groups of compounds that may be part of a larger hydrocarbon mixture. As with other screening techniques, it is advisable to confirm a certain percentage of both positive and negative test results, especially when near or above a regulatory action limit or when the presence of background or interfering hydrocarbons is suspected. The limitations of this procedure are described in more detail in the section on interferences (Sec. 4.0).

1.5 This method does not address the evaporation of volatile petroleum hydrocarbon mixtures (i.e. gasoline) during sample collection, preparation, and analysis. Although the screening kit can be used to qualitatively detect volatile hydrocarbons, it is NOT recommended that the system be used to quantitatively determine volatile petroleum hydrocarbons unless evaporation during sample handling is addressed, appropriate response factor corrections are made, and method performance is demonstrated on real world samples.

1.6 This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 A 10 ± 0.1 g sample of soil is extracted with a solvent mixture composed primarily of methanol. The resulting mixture is allowed to settle and the free liquid is decanted into the barrel of a filter-syringe assembly. The liquid is filtered through a 0.2-µm filter into a vial containing an aqueous emulsifier development solution. The filtered sample is allowed to develop for 10 minutes. During the development, any hydrocarbons present precipitate out and become suspended in solution.

2.2 The developed sample is placed in a turbidimeter that has been calibrated using a blank and a single calibration standard. A beam of yellow light at 585 nm is passed through the

sample and the scattering of light through the suspension at 90° is measured. The concentration of total recoverable petroleum hydrocarbons present is calculated relative to the standard curve.

3.0 DEFINITIONS

3.1 See Sec. 5.0 of Chapter 1 and the manufacturer's instructions for definitions associated with this analytical procedure.

3.2 For the purpose of this method, "total recoverable petroleum hydrocarbons" is defined as those hydrocarbons that are recovered using the solvent-specific extraction procedure provided with this kit. Since there is no cleanup step to separate any co-extracted naturally occurring hydrocarbons from the petroleum hydrocarbons, elevated turbidimetric readings are likely without performing background correction. See the interferences section (Sec. 4.0) for additional details.

4.0 INTERFERENCES

4.1 This method is considered a screening technique because of the broad spectrum of hydrocarbons it detects. It cannot distinguish between co-extracted naturally occurring hydrocarbons and petroleum hydrocarbons. Using background correction and/or a selected response factor discussed in the manufacturer's instructions, an analyst may be able to eliminate some of the interferences caused by co-extracted naturally occurring hydrocarbons. However, it is very difficult to find a truly clean, representative sample for use as a background.

4.2 This method has been shown to be susceptible to interference from vegetable oils (positive interference). It is anticipated that co-extracted naturally occurring oils from vegetative materials would be one of the most probable positive interferants found in the field. To demonstrate this interference, standard soil samples were spiked with corn oil at levels of 50 to 1000 ppm and tested with PetroFLAG™ system. Soil samples spiked with mineral oil were also analyzed for comparison. These data indicate that, over the range tested, the slope of the PetroFLAG™ vegetable oil response is approximately 18% of the response of the mineral oil standard. Supporting data are presented in Table 2.

4.3 This method has been shown to be susceptible to interference from water (negative interference). To demonstrate this interference, soils were spiked with diesel fuel at 100 ppm. The samples were then spiked with varying amounts of water, up to saturation. The samples were analyzed using the PetroFLAG™ system and the results were below that expected for the spike added. The low bias may be due to a decrease in extraction efficiency in samples containing large amounts of water, as a result of dilution of the extraction solvent. Supporting data are presented in Table 3.

4.4 This method has been shown to NOT be significantly affected by up to 5% sodium chloride contamination. Supporting data are presented in Table 6.

4.5 This method has been shown to NOT be significantly affected by up to 1000 ppm of common surfactants such as trisodium phosphate (TSP), soap, and sodium dodecyl sulfate (SDS). Supporting data are presented in Tables 7, 8, and 9.

4.6 Polycyclic aromatic hydrocarbons (PAHs) are a class of compounds present in many hydrocarbon mixtures that are detected by the PetroFLAG system. These compounds are often targeted because of their toxic characteristics and may be present individually as soil contaminants.

However, the response of the individual PAHs varies greatly from compound to compound. Therefore, use of the PetroFLAG system to quantitate individual PAHs is not recommended without good knowledge of the site and after adjusting the analytical approach. Quantitation of PAHs as part of a larger hydrocarbon fraction, such as diesel fuel, is recommended. Supporting data are presented in Table 12.

4.7 The PetroFLAG™ analyzer can be used at temperatures from 4°C to 45°C. The analyzer is equipped with an on-board temperature sensor to measure the ambient temperature at which measurements are being made. The software uses this temperature reading to correct the optical drift caused by temperature fluctuations.

4.8 Temperature at which the calibration is run should be recorded because of the effect temperature has on the suspension. This can be done by taking a reading without inserting a vial. If, during sample analysis, the temperature fluctuates more than ±10°C from the temperature at the calibration, the calibration should be rerun at the new temperature.

5.0 SAFETY

Safety practices appropriate for handling potentially contaminated hazardous or toxic samples and extraction solvents should be employed.

6.0 EQUIPMENT AND SUPPLIES

PetroFLAG™ Hydrocarbon Analysis System, (Dexsil Corporation, One Hamden Park Drive, Hamden, CT), or equivalent. Each commercially-available test kit will supply or specify the apparatus and materials necessary for successful completion of the test.

7.0 REAGENTS AND STANDARDS

Each commercially-available test kit will supply or specify the reagents necessary for successful completion of the test. Reagents should be labeled with appropriate expiration dates, and reagents should not be employed beyond such dates.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

8.2 Soil samples may be contaminated, and should therefore be considered hazardous and handled accordingly. All samples should be collected using a sampling plan that addresses the considerations discussed in Chapter Nine.

8.3 To achieve accurate analyses, soil samples should be well homogenized prior to testing. The hydrocarbons may not be evenly distributed in a soil sample and extensive mixing is necessary to assure homogeneity.

NOTE: It is strongly recommended that any free aqueous liquid be decanted from samples prior to analysis with the PetroFLAG system. Free aqueous liquid will dilute the extraction solvent and produce a negative interference.

NOTE: When users of the PetroFLAG system wish to report their results on a dry weight basis, additional representative samples should be collected for percent moisture determination. See the extraction Methods 3540 or 3550 for the procedure for determining percent moisture.

9.0 QUALITY CONTROL

9.1 Follow the manufacturer's instructions for quality control procedures specific to the test kit used. Additional guidance on quality control is provided in Chapter One.

9.2 Use of replicate analyses, particularly when results indicate concentrations near the action level, is recommended to refine information gathered with the kit.

9.3 Method 9074 is intended for use as a screening procedure in either the field or a fixed laboratory. Wherever it is employed, a quality assurance program appropriate for a screening procedure should be employed as a means of documenting the quality of the resulting data.

10.0 CALIBRATION AND STANDARDIZATION

See the PetroFLAG™ Hydrocarbon Analyzer User's Manual for instruction on generating an initial calibration curve using the PetroFLAG™ analyzer. Contact the manufacturer for specific details on the calibration calculations programmed into the PetroFLAG™ analyzer.

11.0 PROCEDURE

Follow the manufacturer's instructions in the PetroFLAG™ Hydrocarbon Analyzer User's Manual to extract, develop, and analyze soil samples. Those test kits used must meet or exceed the performance specifications indicated in Tables 1 through 3.

12.0 DATA ANALYSIS AND CALCULATIONS

Consult the PetroFLAG™ Hydrocarbon Analyzer User's Manual for the procedure used to generate concentration readings from samples using the PetroFLAG™ analyzer. Contact the manufacturer for specific details on the concentration calculations programmed into the PetroFLAG™ analyzer.

13.0 METHOD PERFORMANCE

13.1 Method Detection Limits were determined using a modification of the procedures in Chapter One and in 40 CFR, Part 136. The procedure was modified slightly because the instrument automatically subtracts an average blank value for each analysis (blank analysis is part of the calibration procedure of the PetroFLAG™ test system). Two sets of seven samples each were prepared, one set spiked with 30 ppm of diesel fuel, and one set spiked with 30 ppm of used motor oil. The standard deviation (SD) of the results for each oil type were calculated. The method detection limit (MDL) was determined by multiplying the SD by the Student's *t* value (3.143). These data are presented in Table 1. The MDL for diesel fuel was 13 ppm and for used motor oil was 18.6 ppm (Ref 1).

13.2 Samples of a standard soil were prepared by spiking with either diesel fuel or used motor oil at 100 ppm intervals from 100 ppm to 1000 ppm. Each sample was analyzed in duplicate by the PetroFLAG™ system and by Methods 3550 and 8015B. The results are shown in Table 4. These data were analyzed using regression analysis. The results of the regression analysis are also provided in Table 4. In addition, an analysis of variance (ANOVA) analysis was performed. The F-statistic from the ANOVA revealed a significant bias between the two methods, with the PetroFLAG™ providing consistently higher values for both types of contamination. The results confirm that the kit design is intentionally conservative, in that it favors a high bias in order to avoid reporting false negative results (Ref. 1).

13.3 Precision and bias were determined by analysis of variance (ANOVA) of the results obtained from spiked soil samples. Four sets of spiked samples were prepared, containing either diesel fuel or used motor oil at two different concentrations (200 and 1000 ppm). Each analyte at each concentration was analyzed in duplicate 10 times (e.g., 20 replicates of each). The results were transformed into recovery data. The ANOVA used these transformed data. The results are presented in Table 5. The F-statistic for the diesel fuel analysis indicate a slight day effect for these samples. The F-statistic seems to be driven more by the very low value of the mean square error within days rather than by any large value for the mean square error between days (Ref. 1).

13.4 The response of the PetroFLAG System to a soil spiked with 500 ppm of diesel fuel and 0 to 5% of dry sodium chloride is provided in Table 6 (Ref. 2).

13.5 The responses of the PetroFLAG System to a soil spiked with 500 ppm of diesel fuel and up to 1000 ppm of common surfactants such as trisodium phosphate (TSP), soap, and sodium dodecyl sulfate (SDS), are presented in Tables 7, 8, and 9 (Ref. 2).

13.6 Performance of the PetroFLAG™ system on anthracene from 100 to 2000 ppm and on creosote from 100 to 1000 ppm are presented in Tables 10 and 11, respectively. An explanation of the erratic performance of anthracene is provided in the Table 10 narrative (Ref. 2).

13.7 The performance of the PetroFLAG system for several PAHs relative to the mineral oil calibrator on soil is presented in Table 12 (Ref. 4).

13.8 Performance of the PetroFLAG™ system on Jet-A from 40 to 2808 ppm (Ref. 4) and on gasoline from 1000 to 4070 ppm (Ref. 2) are provided in Tables 13 and 14, respectively. An explanation of the performance of Jet-A and gasoline are provided in the narrative in Tables 13 and 14.

14.0 POLLUTION PREVENTION

This method does not use any halogenated solvents and may be used to help reduce the number of samples sent to the laboratory under certain project scenarios. Traditional laboratory extraction methods (i.e. Soxhlet or sonication) would generally require much larger volumes of solvent to extract the sample.

15.0 WASTE MANAGEMENT

Waste management procedures must be consistent with federal, state, and local regulations.

16.0 REFERENCES

1. Data Validation Package, *Testing for Petroleum Hydrocarbons in Soil by Turbimetric Analysis*, PetroFLAG™ Test System, DEXSIL Corp., Hamden, CT.

2. Supplementary Validation Data, *Additional Analyte and Contaminant Testing Data for the PetroFLAG™ Hydrocarbon Analysis System*, DEXSIL Corp., Hamden, CT, August 24, 1995.

3. PetroFLAG™ Hydrocarbon Analyzer User's Manual, DEXSIL Corp., Hamden, CT.

4. Supplementary Data Validation Package III, *Additional Analyte Testing Data for Petroleum Hydrocarbons in Soil by Turbimetric Analysis - PetroFLAG™ Test System*, DEXSIL Corp., Hamden, CT, June 20, 1997.

5. Supplementary Data Validation Package IV, *Polycyclic Aromatic Hydrocarbon Response data for Method 9074 Petroleum Hydrocarbons in Soil by Turbimetric Analysis - PetroFLAG™ Test System*, DEXSIL Corp., Hamden, CT, August 22, 1997.

17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain Tables 1 through 14.

TABLE 1

METHOD DETECTION LIMIT FOR PetroFLAG TEST SYSTEM

Trial #	30 ppm diesel fuel	30 ppm motor oil
1	34	35
2	24	41
3	28	40
4	34	53
5	36	46
6	32	48
7	30	42
Average (ppm)	31.03	43.6
SD (ppm)	4.12	5.91
MDL (ppm)	13.0	18.6

Data from Reference 1.

TABLE 2

RELATIVE RESPONSE OF VEGETABLE OILS AS AN INTERFERANT

Analyte Spike Concentration (ppm)	Mineral Oil Response (ppm)	Vegetable Oil Response[a] (ppm)
50	55	30
100	100	45
200	189	94
500	504	111
1000	947	208

[a] The vegetable oil samples were analyzed using the PetroFLAG system set to response factor 10. The slope of the PetroFLAG vegetable oil response is approximately 18% of the response of the mineral oil standard. This means that a sample containing 5,560 ppm vegetable oil would provoke a response equivalent to that given by 1,000 ppm mineral oil.

Data from Reference 1.

TABLE 3

EFFECT OF WATER ON PetroFLAG RESULTS

% Water Saturation (% Water)	% Recovery of Mineral Oil[a]
0 (0)	100
5 (1)	94
25 (5)	98
50 (10)	95
100 (20)	85

[a] Soil sample spiked with 100 ppm of mineral oil. (Ref. 1)

TABLE 4

COMPARISON OF PetroFLAG AND GC TEST RESULTS

Spike Conc. (µg/g)	PetroFLAG (µg/g)		3550/8015B (µg/g)	
Diesel Fuel	Trial 1	Trial 2	Trial 1	Trial 2
100	112	116	73	82
200	230	248	158	156
300	312	370	242	218
400	420	455	299	275
500	538	564	342	344
600	626	654	460	439
700	774	790	509	494
800	910	900	612	607
900	1091	977	678	614
1000	1182	1062	646	649
Corr Coef	0.999		0.992	
Slope	1.126		0.679	
Intercept	-2.8		30.5	
Motor Oil	Trial 1	Trial 2	Trial 1	Trial 2
100	121	128	123	82
200	243	292	200	200
300	381	408	301	275
400	428	497	341	343
500	531	554	441	452
600	654	668	534	528
700	717	771	609	652
800	880	883	711	746
900	931	1052	835	881
1000	1014	1098	887	846
Corr Coef	0.998		0.997	
Slope	1.02		0.887	
Intercept	50.9		20.5	

Data from Reference 1.

TABLE 5

ANOVA RESULTS FOR SPIKED PETROLEUM HYDROCARBON SAMPLES

Analyte/Concentration	n	Mean ($\bar{x}$)	Variance (σ_{n-1}^2)	Standard Deviation (σ_{n-1})	Standard Error ($\sigma_{\bar{x}}$)
Diesel, 200 ppm	20	1.09	0.0059	0.0768	0.0172
Diesel, 1000 ppm	20	1.00	0.00430	0.0656	0.0147
Motor Oil, 200 ppm	20	1.12	0.00266	0.0515	0.0115
Motor Oil, 1000 ppm	20	0.937	0.000919	0.0303	0.00678

Data from Reference 1.

TABLE 6

RESPONSE OF PetroFLAG SYSTEM WITH VARIOUS LEVELS OF SODIUM CHLORIDE[a]

	% Sodium Chloride				
	0	0.5	1.0	2.0	5.0
PetroFLAG Response (ppm)	518	539	529	516	524

[a] A series of soil samples consisting of sand, clay, and topsoil was spiked with 500 ppm of diesel fuel and varying levels of dry sodium chloride (NaCl) from 0 to 5 percent. The samples were analyzed using the PetroFLAG system set to response factor 5 (Ref. 2).

TABLE 7

RESPONSE OF PetroFLAG SYSTEM WITH VARIOUS TSP CONCENTRATIONS[a]

	TSP Concentration (ppm)				
	0	100	200	500	1000
PetroFLAG Response (ppm)	522	511	512	500	492

[a] Response of the PetroFLAG system for soil containing 500 ppm of diesel fuel and various levels of trisodium phosphate(TSP), a common surfactant. The samples were analyzed using the PetroFLAG system set to response factor 5 (Ref. 2).

TABLE 8

RESPONSE OF PetroFLAG SYSTEM WITH VARIOUS SOAP CONCENTRATIONS[a]

	Soap Concentration (ppm)				
	0	100	200	500	1000
PetroFLAG Response (ppm)	500	494	488	502	528

[a] Response of the PetroFLAG system for soil containing 500 ppm of diesel fuel and various levels of soap (non-ionic and anionic surfactants). The samples were analyzed using the PetroFLAG system set to response factor 5 (Ref. 2).

TABLE 9

RESPONSE OF PetroFLAG SYSTEM WITH VARIOUS SDS CONCENTRATIONS[a]

	SDS Concentration (ppm)				
	0	100	200	500	1000
PetroFLAG Response (ppm)	472	474	488	486	496

[a] Response of the PetroFLAG system for soil containing 500 ppm of diesel fuel and various levels of sodium dodecyl sulfate, a surfactant. The samples were analyzed using the PetroFLAG system set to response factor 5 (Ref. 2).

TABLE 10

RESPONSE OF PetroFLAG SYSTEM WITH VARIOUS AMOUNTS OF ANTHRACENE[a]

	Anthracene Conc. (ppm)				
	100	200	500	1000	2000
PetroFLAG Response (ppm)	798	1376	1641	1380	1735

[a] Response of the PetroFLAG system for soil containing various levels of anthracene. The results show that the PetroFLAG system returns a strong response to anthracene. The response to anthracene is higher than response to the calibrator, therefore, the meter displays a reading over-estimating the concentration. For concentrations greater than 200 ppm, the turbidity developed exceeds the recommended level (i.e. a reading greater than 1000 on response factor 10). To obtain accurate results the user should rerun the sample using a smaller sample size. This will bring the results into linear range. The samples were analyzed using the PetroFLAG system set to response factor 10 (Ref. 2).

TABLE 11

RESPONSE OF PetroFLAG SYSTEM WITH VARIOUS AMOUNTS OF CREOSOTE[a]

	Creosote Conc. (ppm)			
	100	200	500	1000
PetroFLAG Response (ppm)	103	210	538	1043

[a] Response of the PetroFLAG system for soil containing various levels of creosote. The samples were analyzed using the PetroFLAG system set to response factor 8 (Ref. 2).

TABLE 12

RELATIVE RESPONSE OF PetroFLAG SYSTEM TO VARIOUS POLYCYCLIC AROMATIC HYDROCARBONS[a]

Compound	Spike Level in ppm (Matrix Used)	PetroFLAG Reading in ppm (Rf 10)	Response Relative to Mineral Oil Calibrator
Anthracene	100 (Soil)	798	8
Benzo[a]pyrene	50 (Soil)	180	3.6
Chrysene	16 (Solvent)	172	11
Fluoranthene	200 (Solvent)	101	0.5
Pyrene	200 (Solvent)	216	1.1

[a] The data for anthracene and benzo(a)pyrene were generated by spiking each compound onto a composite sandy clay loam soil and homogenizing the sample for later analysis. The soil sample size was 10 g. The soil spiking procedure used for anthracene and benzo(a)pyrene produced inconsistent results for the other PAH compounds. These compounds (chrysene, flouranthene, and pyrene), which are very soluble in the extraction solvent, were spiked directly into the extraction solvent and analyzed. All of the PAHs samples were analyzed on response factor 10 (the correct response factor for mineral oil). The data indicate that, for example, using a standard sample size analyzed on response factor 10 (the correct response factor for mineral oil), a 100 ppm anthracene sample read 798 ppm. The PetroFLAG response to the above analytes is equal to or greater than the calibrator in all cases except for fluoranthene which has a response equivalent to diesel fuel.

NOTE: When analyzing soils containing anthracene, benzo(a)pyrene, or chrysene the PetroFLAG meter will read over range for concentrations of 250, 550, and 180 ppm respectively. These soils can be analyzed using a 1 gram sample size to increase the maximum quantifiable concentration.

TABLE 13

RESPONSE OF PetroFLAG SYSTEM WITH VARIOUS AMOUNTS OF JET-A[a]

	Jet-A Conc. (ppm)							
	0	40	79	198	397	793	1586	2776
PetroFLAG Response (ppm)	54	110	162	208	368	700	1592	2808

[a] Response of the PetroFLAG system for soil containing various levels of Jet-A. The composite soils were prepared from two types of clay-loam soil and sand. The component soils were air dried and sieved to remove particles larger than 850 µm and then mixed in the ratio 2:1:1, followed by tumbling for one hour. The soil was weighed out into 10 g aliquots. Each of the soil aliquots was spiked by direct injection of Jet-A fuel onto the soil using a microliter syringe, mixed, and analyzed by the PetroFLAG system with the instrument set to response factor 4. The coefficient of determination (r^2) for the Jet-A data was 0.997, indicating that the PetroFLAG response was linear over the range 40 ppm to 2808 ppm (Ref. 4).

TABLE 14

RESPONSE OF PetroFLAG SYSTEM WITH VARIOUS AMOUNTS OF WEATHERED GASOLINE[a]

	Weathered Gasoline Conc. (ppm)			
	1000	2040	3050	4070
PetroFLAG Response (ppm)	285	1780	4335	6870

[a] Response of the PetroFLAG system for soil containing various levels of weathered gasoline (50% evaporated). The manufacturer recommends that PetroFLAG be used to qualitatively detect gasoline at these levels. It is not recommended that PetroFLAG be used quantitatively for gasoline unless significant response factor corrections are made and evaporation of the target hydrocarbons is addressed. The samples were analyzed using the PetroFLAG system set to response factor 2 (Ref. 2).

CHAPTER FIVE

MISCELLANEOUS TEST METHODS

Prior to employing the methods in this chapter, analysts are advised to consult the disclaimer statement at the front of this manual and the information in Chapter Two for guidance on the allowed flexibility in the choice of apparatus, reagents, and supplies. In addition, unless specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in each procedure is provided by EPA as guidance to be used by the analyst and the regulated community in making judgements necessary to meet the data quality objectives or needs for the intended use of the data.

The following methods are found in Chapter Five:

Method 5050:	Bomb Preparation Method for Solid Waste
Method 9010B:	Total and Amenable Cyanide: Distillation
Method 9012A:	Total and Amenable Cyanide (Automated Colorimetric, with Off-Line Distillation)
Method 9013:	Cyanide Extraction Procedure for Solids and Oils
Method 9014:	Titrimetric and Manual Spectrophotometric Determinative Methods for Cyanide
Method 9020B:	Total Organic Halides (TOX)
Method 9021:	Purgeable Organic Halides (POX)
Method 9022:	Total Organic Halides (TOX) by Neutron Activation Analysis
Method 9023:	Extractable Organic Halides (EOX) in Solids
Method 9030B:	Acid-Soluble and Acid-Insoluble Sulfides: Distillation
Method 9031:	Extractable Sulfides
Method 9034:	Titrimetric Procedure for Acid-Soluble and Acid-Insoluble Sulfides
Method 9035:	Sulfate (Colorimetric, Automated, Chloranilate)
Method 9036:	Sulfate (Colorimetric, Automated, Methylthymol Blue, AA II)
Method 9038:	Sulfate (Turbidimetric)
Method 9056:	Determination of Inorganic Anions by Ion Chromatography
Method 9057:	Determination of Chloride from HCl/Cl_2 Emission Sampling Train (Methods 0050 and 0051) by Anion Chromatography
Method 9060:	Total Organic Carbon
Method 9065:	Phenolics (Spectrophotometric, Manual 4-AAP with Distillation)
Method 9066:	Phenolics (Colorimetric, Automated 4-AAP with Distillation)
Method 9067:	Phenolics (Spectrophotometric, MBTH with Distillation)
Method 9070:	Total Recoverable Oil & Grease (Gravimetric, Separatory Funnel Extraction)
Method 9071A:	Oil and Grease Extraction Method for Sludge and Sediment Samples
Method 9075:	Test Method for Total Chlorine in New and Used Petroleum Products by X-Ray Fluorescence Spectrometry (XRF)
Method 9076:	Test Method for Total Chlorine in New and Used Petroleum Products by Oxidative Combustion and Microcoulometry

Method 9077:	Test Methods for Total Chlorine in New and Used Petroleum Products (Field Test Kit Methods)
Method A:	Fixed End Point Test Kit Method
Method B:	Reverse Titration Quantitative End Point Test Kit Method
Method C:	Direct Titration Quantitative End Point Test Kit Method
Method 9131:	Total Coliform: Multiple Tube Fermentation Technique
Method 9132:	Total Coliform: Membrane-Filter Technique
Method 9210:	Potentiometric Determination of Nitrate in Aqueous Samples with Ion-Selective Electrode
Method 9211:	Potentiometric Determination of Bromide in Aqueous Samples with Ion-Selective Electrode
Method 9212:	Potentiometric Determination of Chloride in Aqueous Samples with Ion-Selective Electrode
Method 9213:	Potentiometric Determination of Cyanide in Aqueous Samples and Distillates with Ion-Selective Electrode
Method 9214:	Potentiometric Determination of Fluoride in Aqueous Samples with Ion-Selective Electrode
Method 9215:	Potentiometric Determination of Sulfide in Aqueous Samples and Distillates with Ion-Selective Electrode
Method 9216:	Potentiometric Determination of Nitrate in Aqueous Samples with Ion-Selective Electrode
Method 9250:	Chloride (Colorimetric, Automated Ferricyanide AAI)
Method 9251:	Chloride (Colorimetric, Automated Ferricyanide AAII)
Method 9253:	Chloride (Titrimetric, Silver Nitrate)
Method 9320:	Radium-228

METHOD 9216

POTENTIOMETRIC DETERMINATION OF NITRITE
IN AQUEOUS SAMPLES WITH ION-SELECTIVE ELECTRODE

1.0 SCOPE AND APPLICATION

1.1 This method can be used for measuring nitrite in drinking water, wastewater, and reagent waters. If this method is used for other types of water samples (i.e., surface water, ground water, etc.), method precision and accuracy must be demonstrated for each matrix type.

1.2 The method detection limit is 0.05 mg/L of nitrite as nitrogen. Nitrite concentrations from 0.05 to 20 mg/L may be measured.

1.3 Ion selective electrodes (ISEs) must be used carefully and results must be interpreted cautiously. An ISE may be affected by numerous analytical interferences which may either increase or decrease the apparent analyte concentration, or which may damage the ISE. Effects of most interferences can be minimized or eliminated by adding appropriate chemical reagents to the sample. Obtaining the most accurate results, therefore, requires some knowledge of the sample composition.

NOTE: Manufacturers usually include a list of interferences in the instruction manual accompanying an ISE, along with recommended methods for minimizing or eliminating effects of these interferences.

2.0 SUMMARY OF METHOD

2.1 This method uses a nitrite-selective electrode. All standards and samples are mixed with an equal volume of nitrite interference suppressor solution (NISS). A calibration curve is constructed by recording the nitrite calibration standard readings using an appropriate meter or by manual plotting. Samples are then read in the same manner, and the concentrations reported by the meter or read from the graph.

3.0 DEFINITIONS

Refer to Chapter Three for the applicable definitions.

4.0 INTERFERENCES

4.1 Some anions, if present at high enough levels, are electrode interferences and will cause measurement errors. Table 1 displays the levels of possible interferences causing a 10% error. NISS is mixed in an equal volume with standards as well as with samples. For example, 25 mL of sample would be mixed with 25 mL of NISS. This procedure ensures that samples and standards are properly buffered, have a similar background and that no correction factor is needed for the dilution. Figure 1 shows how the nitrite electrode response changes with pH. This is compensated for by the addition of the NISS. Selectivity is mathematically demonstrated by the following equation:

$$E = E' + s \log[c_j + \sum K_{ij} c_j^{Z_j}]$$

Where: E' = Reference potential

s = Slope

c_j = Primary ion concentration

K_{ij} = Selectivity Coefficient

c_j^{Zj}=Interfering ion concentration

Zj = charge ratio of interfering ion

Successful analytical conditions depend upon:

$$c_j >> \sum K_{ij}\, c_j^{Zj}$$

4.2 Temperature changes affect electrode potentials. Therefore, standards and samples must be equilibrated at the same constant temperature (± 1°C).

CAUTION: Use hood to avoid exposure to toxic gases released during acidification.

4.3 The user should be aware of the potential of interferences from colloidal substances and that, if necessary, the samples may be filtered.

4.4 Standard electrode filling solutions containing high levels of KCl should not be used as the reference electrode filling solution.

4.5 If electrodes are exposed to samples with nitrite concentrations greater than 20 mg/L, their response may become very sluggish when again measuring at a lower concentration. If this occurs, soak the electrodes for 8-12 hours in a mixture of the 0.5 mg/L standard and NISS.

5.0 SAFETY

5.1 Refer to Chapter Three for additional guidance on safety protocols.

5.2 It is the responsibility of the user to prepare, handle, and dispose of electrolyte solutions in accordance with all applicable federal, state, and local regulations.

6.0 EQUIPMENT AND SUPPLIES

6.1 A pH/mV meter capable of reading to 0.1 mV or an ISE meter.

6.2 Nitrite ISE (Orion 93-46 or equivalent) and double-junction reference electrode (Orion 90-01 or equivalent).

6.3 Thermally isolated magnetic stirrer, fluorocarbon (PFA or TFM)-coated magnetic stir bar, and stopwatch.

6.4 Volumetric flasks, 100 mL and 1 L - Class A.

6.5 Volumetric pipets, 5 mL, 10 mL and 50 mL - Class A

7.0 REAGENTS AND STANDARDS

7.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 Reagent water. All references to water in this method refer to reagent water, as defined in Chapter One.

7.3 ISA adjustor solution (2M, $(NH_4)_2SO_4$): Dissolve 26.4 g of ammonium sulfate in reagent water to make 100 mL of solution.

7.4 Nitrite reference electrode filling solution (Orion 900046 or equivalent).

7.5 Nitrite interference suppressor solution (NISS) (Orion 934610 or equivalent).

7.6 1000 mg/L nitrite as N, stock standard - Weigh out 4.93 g of ACS reagent grade sodium nitrite that has been dried for 24 hours in a desiccator. Place in a clean one L volumetric flask. Add approximately 200 mL of reagent water and mix to dissolve. Add two drops of NaOH and make to volume. Mix by inverting 20 times.

7.7 100 mg/L nitrite as N, stock standard - Pipet 10.0 mL of the 1000 mg/L standard into a clean 100 mL volumetric flask. Make to volume and mix well. Replace this standard monthly.

7.8 10 mg/L nitrite as N, stock standard - Pipet 10.0 mL of the 100 mg/L standard into a clean 100 mL volumetric flask. Make to volume and mix well. Replace this standard weekly.

7.9 5 mg/L nitrite as N, stock standard - Pipet 50.0 mL of the 10 mg/L standard solution into a clean 100 mL volumetric flask. Make to volume with reagent water and mix well. This standard should be replaced daily.

7.10 1 mg/L nitrite as N, stock standard - Pipet 10.0 mL of the 10.0 mg/L standard into a clean 100 mL volumetric flask. Make to volume with reagent water and mix well. This standard should be replaced daily.

7.11 0.5 mg/L nitrite as N, stock standard - Pipet 5.00 mL of the 10.0 mg/L standard into a clean 100 mL volumetric flask. Make to volume with reagent water and mix well. This standard should be replaced daily.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

8.2 Samples should be stored at 4 °C and must be analyzed within 48 hours of collection.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for specific quality control procedures.

9.2 Initial calibration verification standard (ICV): After performing the calibration step (Section 10.0), verify calibration by analyzing an ICV. The ICV contains a known nitrite concentration at the mid-range of the calibration standards and is from an independent source. ICV recovery must be 90-110 percent. If not, the source of error must be found and corrected. An acceptable ICV must be analyzed prior to sample analysis. The ICV also serves as a laboratory control sample.

9.3 Continuing calibration verification standard (CCV): After every 10 samples, and after the final sample, a CCV must be analyzed. The CCV contains a known nitrite concentration at mid-calibration range. CCV recovery must be 90-110 percent. If not, the error source must be found and corrected. If ISE calibration has changed, all samples analyzed since the last acceptable CCV must be re-analyzed.

9.4 Reagent blank: After the ICV and after every CCV, a reagent blank must be analyzed. A reagent blank is 25 mL of reagent water with 25 mL of NISS added. The indicated reagent blank concentration must be less than 1 mg/L nitrite. If not, the contamination source must be found and corrected. All samples analyzed since the last acceptable reagent blank must be re-analyzed.

9.5 Matrix spike: Follow the matrix spike protocols presented in Chapter One. The spike concentration must be 10 times the detection limit and the volume added must be negligible (less than or equal to one-thousandth the sample aliquot volume). Spike recovery must be 75-125 percent. If not, samples must be analyzed by the method of standard additions.

10.0 CALIBRATION AND STANDARDIZATION

10.1 When using a nitrate ISE and a separate double-junction reference electrode, ensure that reference electrode inner and outer chambers are filled with solutions recommended by the manufacturer. Equilibrate the electrodes for at least one hour in a 100 mg/L nitrite standard before use.

10.2 Calibrate the nitrite ISE using standards that narrowly bracket the expected sample concentration. If the sample concentration is unknown, calibrate with 0.5 mg/L, 1.0 mg/L, and 5.0 mg/L nitrite standards. Add 25.0 mL of a standard solution and 25 mL of the NISS into a 100 mL beaker to make each calibration standard.

10.3 Add a fluorocarbon (PFA or TFM)-coated magnetic stir bar, place the beaker on a magnetic stir plate, and stir at slow speed (no visible vortex). Immerse the electrode tips to just above the rotating stir bar. If using an ISE meter, calibrate the meter in terms of nitrite concentration following the manufacturer's instructions. If using a pH/mV meter, record the meter reading (mV) as soon as the reading is stable, but in no case should the time exceed five minutes after immersing the electrode tips.

10.4 Prepare a calibration curve by plotting measured potential (mV) as a function of the logarithm of nitrite concentration. For corrective action, consult the ISE operating manual.

11.0 PROCEDURE

11.1 Allow samples and standards to equilibrate to room temperature.

11.2 Prior to and between analyses, rinse the electrodes thoroughly with reagent water and gently shake off excess water. Low-level measurements are faster if the electrode tips are first immersed five minutes in reagent water.

11.3 Add 25.0 mL of sample and 25.0 mL of NISS to a 100-mL beaker. Add a fluorocarbon (PFA or TFM)-coated magnetic stir bar. Place the beaker on a magnetic stir plate and stir at a slow speed (no visible vortex). Immerse the electrode tips to just above the rotating stir bar. Record the meter reading (mV or concentration) as soon as the reading is stable, but in no case should the time exceed five minutes after immersing the electrode tips. If reading mV, determine nitrite-nitrogen concentration from the calibration curve.

11.4 When analyses have been completed, rinse the electrodes thoroughly and store them in a 100 mg/L nitrate standard solution. If the electrodes will not be used more than one day, drain the reference electrode internal filling solutions, rinse with reagent water, and store dry.

12.0 DATA ANALYSIS AND CALCULATIONS

Results must be reported in units commensurate with their intended use and all dilutions must be taken into account when computing final results.

13.0 METHOD PERFORMANCE

13.1 Figure 2 displays a typical calibration curve for nitrite at 0.5 mg/L, 1 mg/L, and 5 mg/L. Figure 3 displays a low level calibration curve for nitrite at 0.05 mg/L, 0.1 mg/L, 0.2 mg/L, and 0.5 mg/L.

13.2 Table 1 displays the levels at which known interferences may impact the analysis. Refer to Sec. 4.0 for a discussion on interferences.

13.3 The following documents may provide additional guidance and insight on this method and technique:

13.3.1 "Determination of Nitrite in Foods and Wastewater Using a Nitrite-Selective Electrode", S.J.West, X.Wen, M.S.Frant, N.A.Chaniotakis, Pittsburgh Conference, March 1994.

13.3.2 "Determination of Nitrate, Nitrite, and Ammonia in Advanced Secondary Effluent by Means of Ion-Selective Electrodes", S.J.West, X.Wen, Pittsburgh Conference, March 1994.

13.3.3 Model 93-46 Nitrite Electrode Instruction Manual, ATI Orion, Boston MA, 1994.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/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.

14.2 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 Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits 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 Sec. 14.2.

16.0 REFERENCES

1. Applications Laboratory Report, "Tests in Water Samples by Nitrite Electrode and 'Standard Methods' Colorimetric Analysis", ATI Orion, Boston MA, April 1995.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain Table 1, Figures 1 through 3, and a flow diagram of the method procedures.

TABLE 1

NITRITE ELECTRODE SELECTIVITY DATA

Interfering Ion	log K_{ij}	10% Error Ratio (ppm)
Hydroxide	2.8	-
Fluoride	-3.1	170
Chloride	-3.1	320
Chlorate	-3.4	1600
Perchlorate	-3.1	830
Bromide	-3.0	570
Iodide	-1.2	15
Sulfate	-4.1	1100
Nitrate (N)	-3.3	200
Phosphate	-4.0	9500
Polyphosphate	-4.4	3400
Bicarbonate	-3.3	870
Acetate	-3.2	720
Lactate	-4.9	Very high
Phthalate	-2.5	380
Ascorbate	-4.2	Very high
Salicylate	-0.8	7.0

Source: Reference 1.

FIGURE 1

NITRITE ELECTRODE pH RESPONSE

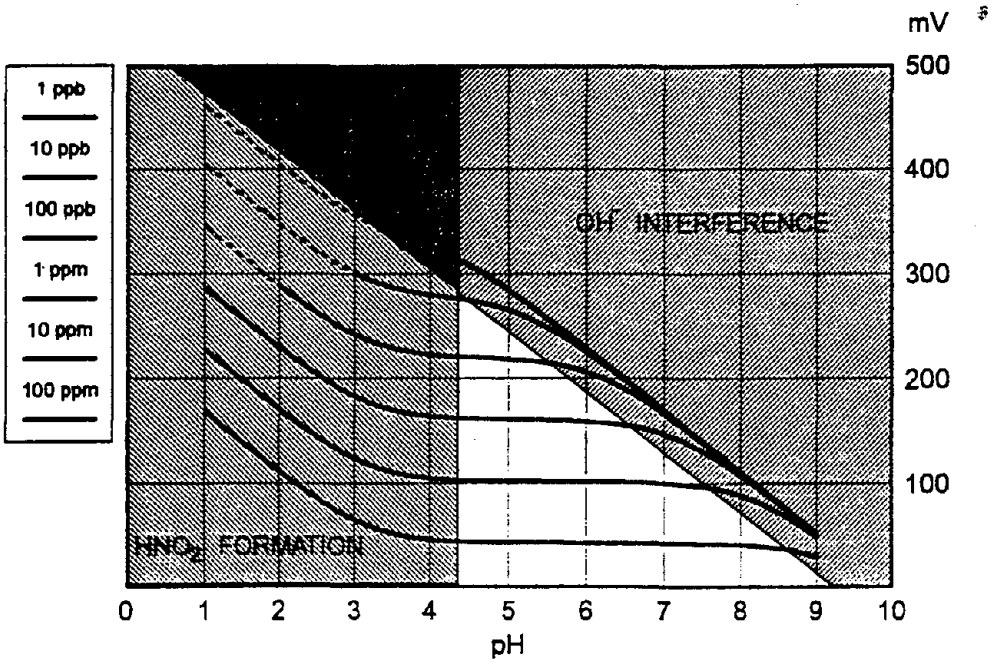


FIGURE 2

CALIBRATION CURVE FOR STANDARD LEVEL OF NITRITE

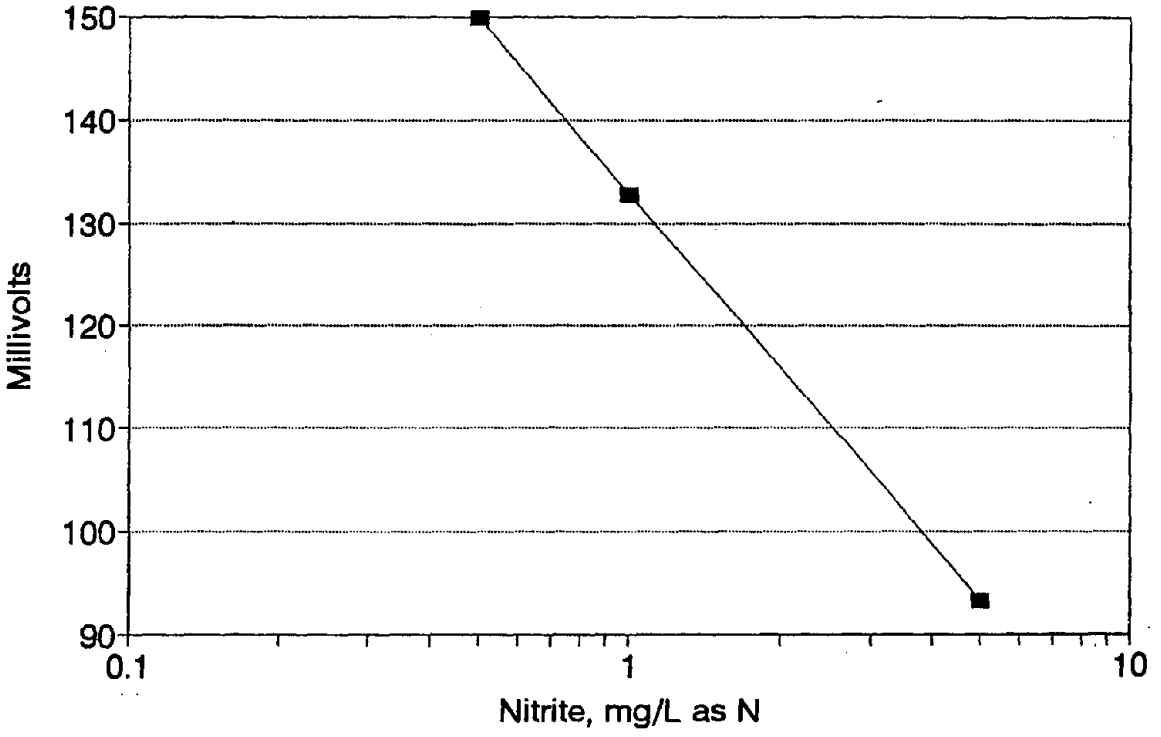
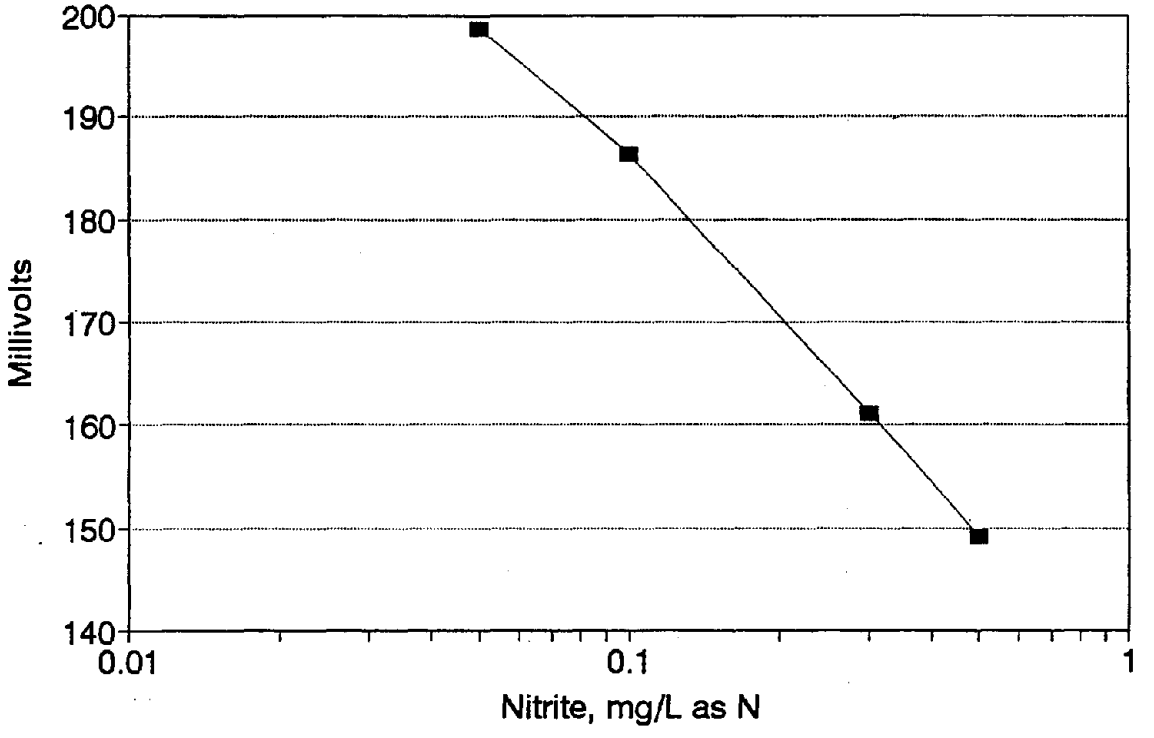


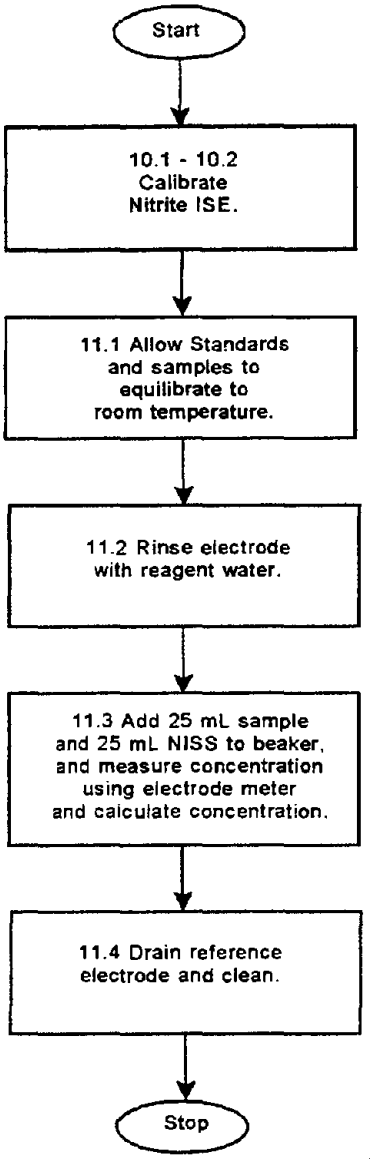
FIGURE 3

CALIBRATION CURVE FOR LOW LEVEL NITRITE METHOD



METHOD 9216

POTENTIOMETRIC DETERMINATION OF NITRITE IN AQUEOUS SAMPLES WITH ION-SELECTIVE ELECTRODE



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