Results of a Study Investigating the Plant Uptake of Explosive Residues From Compost of Explosives-Contaminated Soil Obtained from the Umatilla Army Depot Activity Hermiston, Oregon

Prepared for

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November 1998

TVA Contract No. RG-99719V
Report No. SFIM-AEC-ET-CR-98043

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION **Unclassified**	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT **Unlimited**
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S) **SFIM-AEC-ET-CR-98043**
6a. NAME OF PERFORMING ORGANIZATION **Tennessee Valley Authority** 6b. OFFICE SYMBOL (*if applicable*) **CEB 4C-M**	7a. NAME OF MONITORING ORGANIZATION **U.S. Army Environmental Center** **Pollution Prevention and Environmental Technology Division**
6c. ADDRESS (*City, State, and ZIP Code*) **TVA Reservation** **Post Office Box 1010** **Muscle Shoals, Alabama 35662-1010**	7b. ADDRESS (*City, State, and Zip Code*)
8a. NAME OF FUNDING /SPONSORING ORGANIZATION **U.S. Army Environmental Center** 8b. OFFICE SYMBOL (*if applicable*) **SFIM-AEC-ETD**	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER **TVA Contract No. RG-99719V**
8c. ADDRESS (*City, State, and ZIP Code*)	10. SOURCE OF FUNDING NUMBERS PROGRAM. ELEMENT NO / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.
11. TITLE (*Include Security Classification*) **Results of a Study Investigating the Plant Uptake of Explosive Residues from Compost of Explosives-Contaminated Soil Obtained from the Umatilla Army Depot Activity**	
12. PERSONAL AUTHOR(S) **J. J. Maddox, R. A. Almond, M. F. Broder, J. J. Hoagland, D. A. Kelly, W. J. Rogers, R. C. Strickland, and Wayne Sisk**	
13a. TYPE OF REPORT **Final**	13b. TIME COVERED FROM 6/17/96 TO 9/31/98
14. DATE OF REPORT (*Year, Month, Day*) **1998, November**	15. PAGE COUNT
16. SUPPLEMENTARY NOTATION	

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (*Continue on reverse if necessary and identify by block number*)
Results report examining the uptake of explosives or explosive by-products by plants in composted explosives-contaminated soil.

19. ABSTRACT (*Continue on reverse if necessary and identify by block number*)

This report describes the results of a Plant Uptake Study conducted to determine if plants would take up explosives or explosive by-products from composted explosives-contaminated soil. The study was conducted using compost produced during the remediation of an explosives-contaminated site at the U.S. Army's Umatilla Army Depot Activity at Hermiston, Oregon. This compost was shipped to the Tennessee Valley Authority's Environmental Research Facility in Muscle Shoals, Alabama, where the study was conducted. The report concludes that the plants did not cause an increase in levels of explosives or explosive degradation by-products in the compost nor did they take up explosives or explosive by-products.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT **X** UNCLASSIFIED/UNLIMITED ___ SAME AS RPT ___ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION **Unclassified**
22a. NAME OF RESPONSIBLE INDIVIDUAL **Wayne Sisk**	22b. TELEPHONE (*Include Area Code*) **(410) 436-6851** 22c. OFFICE SYMBOL **SFIM-AEC-ETD**

EXECUTIVE SUMMARY

The Plant Uptake Study examines whether or not the compost produced from remediating explosives-contaminated soils might be of concern. Specifically, the study was conducted to determine if any bound explosive compounds or degradation by-products remaining in the composted soil could be:

- Released by plants;
- Taken up by plants which, in turn, might be consumed by livestock or people; and
- Leached from the compost when stockpiled or land-applied.

The study was conducted by the Tennessee Valley Authority's (TVA) Environmental Research Center (ERC) in Muscle Shoals, Alabama.

This report examines the potential uptake of explosives and explosive by-products by plants growing in compost produced from the composting of explosives-contaminated soil at the Umatilla Army Depot Activity (UMADA), Hermiston, Oregon. The study involved the measurement of the uptake of explosive residues by plants, as well as the development of analytical methods for detecting explosives and explosive by-products in compost and plant tissues. The study described herein began on June 17, 1996.

The leaching issue is addressed in another report entitled "*Results of a Microbial Weathering Study of Composted Explosives-Contaminated Soil Obtained from the Umatilla Army Deport Activity, Hermiston Oregon,*" USAEC Report No. SFIM-AEC-ET-CR-98042. The Microbial Weathering Study was limited to determining if the explosives or explosive by-products would leach when exposed to natural weather conditions over a three-year period.

Uncontaminated soils, composting amendments, and treated compost used during the Plant Uptake Study were obtained from UMADA in 1996 and shipped to the TVA's ERC in Muscle Shoals, Alabama. Treated compost is defined here to mean compost produced from composting explosives-contaminated soil. The uncontaminated soil and composting amendments were used to make a control compost which did not contain explosive contaminants. At the time the treated compost was obtained, the Army was remediating

explosives-contaminated soil at UMADA using windrow composting. Previous treatability studies conducted by the Army at UMADA indicated that composting was both an effective and economical method for reducing TNT and RDX to levels below the state of Oregon's action limit of 30 ppm for each explosive. Analysis of the soil prior to remediation indicated that it contained between 6,000-8,000 ppm total explosives. Approximately 90% of this was TNT, 8% RDX, and 2% other explosives. The treated compost was analyzed for explosives and explosive by-products prior to initiating the study. These analyses revealed that the compost received by TVA initially had very low levels of explosives. TNB, TNT, and RDX had average concentrations of 245, 64, and 20 ug/Kg (ppb), respectively.

To conduct the Plant Uptake Study, a control compost was produced by mixing uncontaminated UMADA soil with the composting amendments obtained from UMADA. The control compost produced was then placed in a large pile and both the control and treated composts were monitored until they were mature enough to support plant growth. Evaluation of compost maturity was based on the measurement of several factors including:

- Nitrate-to-ammonium ratios,
- Carbon-to-nitrogen ratios,
- Respiration rates, and
- Odor.

After the composts had matured, a Seed Germination Study was conducted with three plant species (radish, kale, and lettuce) to determine the extent to which the composts needed to be diluted with uncontaminated soil in order to ensure the plants would germinate. Prior to conducting the Main Plant Uptake Study, a Preliminary Plant Uptake Study was conducted with two plant species (radish and kale) to further quantify the appropriate soil-to-compost ratio to ensure that plants would mature in the mixtures used.

During the Main Plant Uptake Study, a total of eight plant varieties (seven plant species) were used, including four cool season plants (five varieties) and three warm season plants. The plants were chosen to cover a broad range of plant types such as fruit-bearing crops, root crops, leafy vegetable crops, nodulating plants, monocots, and dicots. Cool season crops studied included: chives (*Allium schoenoprasum* variety Cebolletas), redtop (*Agrostis alba*), alfalfa

(*Medicago sativa* variety Vernal), and two barleys (*Hordeum vulgare* variety Parmunky and variety Starling). Warm season crops included: sorghum (*Sorghum bicolor* variety FFR 321 DR), bush snapbeans (*Phaseolus vulgaris* variety Habichulas), and tomato (*Lycopersicion esculentum* variety Patio). To conduct the study, the plants were grown in pots containing both treated and control composts in uncontaminated UMADA soils at a soil-to-compost ratio of 5:1 by weight.

In both the Preliminary and Main Plant Uptake Studies, the plant parts including shoots, roots, grain, and fruit were analyzed for explosives and explosive by-products in nine plant species. These plant species included:

- Radish (*Raphanus sativus* L. variety Cherry Belle),
- Kale (*Brassica oleracea* L. variety Siberian),
- Bush snapbean (*Phaseolus vulgaris* L. variety Habichulas),
- Tomato (*Lycopersicon esculentum* Mill. variety Patio),
- Alfalfa (*Medicago sativa* variety Vernal) pasture crop,
- Chives (*Allium schoenoprasum* variety Cebolletas),
- Redtop (*Agrostis alba*),
- Sorghum (*Sorghum bicolor* variety FFR 321DR), and
- Winter Barley (*Hordeum vulgare* variety Parmunky).

Two other plants were used during the project, but were not subjected to explosive or explosive by-product analyses. These included: lettuce (*Lactuca sativa* variety Bibb), which was used to test seed germination during the Seed Germination Study, and a winter barley (*Hordeum vulgare* variety Starling), which was used as a backup in the event that the Parmunky variety did not produce grain during the Main Plant Uptake Study.

No explosives or explosive by-products were detected above the Method Detection Limits (MDL) in any of the plant samples analyzed.

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ABBREVIATIONS

2A-DNT	2-Amino-4,6-dinitrotoluene
4A-DNT	4-Amino-2,6-dinitrotoluene
2,4-DANT	2,4-Diamino-6-nitrotoluene
2,6-DANT	2,6-Diamino-4-nitrotoluene
2,4-DNT	2,4-Dinitrotoluene
2,6-DNT	2,6-Dinitrotoluene
3,5-DNA	3,5-Dinitroaniline
1,3-DNB	1,3-Dintirobenzene
AA	Atomic Absorbtion
ANOVA	Analysis of Variance
BSI	Bioremediation Services, Inc.
CEB	Chemical Engineering Building
C:N	Carbon-to-Nitrogen Ratio
CRREL	Cold Regions Research and Engineering Laboratory
DN-4,4-AZT	Dinitro-4,4'-azoxytoluene
DNT	Dinitrotoluene
DoD	Department of Defense
EC	Electrical Conductivity
EPA	Environmental Protection Agency
ERC	Environmental Research Center
FIA	Flow Injection Analyzer
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
HPLC	High Performance Liquid Chromatography
IC	Ion Chromatography
ICP	Inductively Coupled Plasma
LSD	Least Significant Difference
MDL	Method Detection Limit
NH_4-N	Ammonium Nitrogen
NO_3	Nitrate
(NO_3+NO_2)-N	Nitrate + Nitrite Nitrogen
PO_4	Orthophosphate
PO_4-P	Orthophosphate – Phosphorus
QA	Quality Assurance
QC	Quality Control
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
TKN	Total Kjeldahl Nitrogen
TNT	2,4,6 Trinitrotoluene
TOC	Total Organic Carbon
TVA	Tennessee Valley Authority
UMADA	Umatilla Army Depot Activity
USACE	U.S. Army Corps of Engineers
USAEC	U.S. Army Environmental Center
USEPA	United States Environmental Protection Agency
WES	Waterways Experiment Station

SECTION 1.0
INTRODUCTION

1.1 Background

Contamination of soils and sediments with explosives is a concern at many military facilities where explosives have been produced and handled. Over time, the soils and sediments at these facilities have become contaminated with a variety of explosives such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3.5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and N-methyl-N-2,4,6-tetranitroaniline (tetryl). Incineration methods can be used to remediate the soils at these sites. However, the U.S. Army Environmental Center (USAEC) is investigating alternative remediation technologies because of public resistance to the use of incineration and because of its high cost. Among the most promising of these alternatives is composting.

Previous USAEC demonstrations have indicated that soil remediation using composting techniques is cost-effective. Furthermore, destruction efficiencies for TNT, RDX, and HMX are high and the compost produced has little or no toxicity.[Ref. 1] However, the USAEC was concerned that undetected explosive residuals may be present within the compost and that these residuals could represent potential risk to the health of individuals or livestock if they came into contact with the compost. Specific concerns were:

- That explosive material residues may be taken up by plants which, in turn, might be consumed by humans or livestock, and
- That land-applied compost would leach explosive residuals.

These concerns were based on the difficulty of detecting explosive materials in compost and the consequent difficulty in establishing that the explosives have indeed been destroyed. The USAEC felt that these concerns should be addressed before it could recommend unrestricted use of the composted soil. Therefore, the USAEC funded this project to study the potential for the uptake of explosives by plants and leaching of explosives from composts. This report describes project findings in the area of explosives uptake by plants. The findings on explosives leaching are documented in another report entitled "*Results of a Microbial*

Weathering Study of Composted Explosives-Contaminated Soil Obtained from the Umatilla Army Deport Activity, Hermiston, Oregon," USAEC Report No. SFIM-AEC-ET-CR-98042.[Ref. 2]

This project was executed under an agreement between the USAEC and the Tennessee Valley Authority (TVA). The USAEC was the lead agency while the TVA provided technical expertise in plant uptake, explosives residue analysis, and composting. During the project, TVA grew selected plants in compost which had previously been derived from explosives-contaminated soil. The plants and compost were then subjected to chemical analysis to determine if any explosives were taken up by the plants. The compost used during the project was obtained from the Umatilla Army Depot Activity (UMADA) in Hermiston, Oregon. This compost was produced as part of a soil remedial action program conducted at UMADA. All of the test work was conducted at TVA's facilities in Muscle Shoals, Alabama.

1.2 Current Practice and Alternatives

Currently, incineration is the most common method of remediating explosives-contaminated soil and sediment. Incineration is an effective treatment; however, treatment costs are high, particularly for small sites. For a small site with 2,000 tons of contaminated soil, incineration can cost as high as $1,200 per ton.[Ref. 1] In addition, the public has been increasingly resistant to incineration as a remediation technology.

Composting is one of the more attractive alternative technologies to treat explosives-contaminated soil and sediment. In composting, soil contaminated with a degradable contaminant is combined with a biodegradable carbon source and a bulking agent. The bulking agent increases the compost's porosity, oxygen content, and water-holding capacity. The mixture is usually placed within a containment structure to prevent contaminant migration and protect the compost from extreme weather. Here, the biodegradable contaminants are degraded naturally by the action of microorganisms which form organic and inorganic transformation products and heat during the process. Once the contaminant concentrations are reduced to appropriate levels and the compost exhibits little or no toxicity, the compost can be returned to the land or used to revegetate a site. Prior work with poultry

litter compost has revealed that mature compost requires dilution with soil if used to grow potted plants.

Composting is an attractive remediation technology due to its:

- Simplicity of operation,
- Lower cost, and
- High public acceptability.

Composting is most attractive when the finished compost can be returned to revegetate a site, applied to range lands, or applied to crop lands. However, the USAEC has been concerned that plants may take up explosive residues bound to the compost and/or that these residues might leach from the compost as it weathers in the environment. To address these concerns, the following questions needed to be answered:

- When is the compost mature enough for land application and plant growth?
- Are available analytical methods adequate for detecting explosives and explosive by-products in plants and compost?
- Will plants take up significant amounts of bound explosives and explosive by-products from compost?
- What is the potential for contaminants to leach from compost as it weathers in the environment?

Two recent studies have evaluated composted explosives-contaminated soil for its suitability for land application and have suggested that the composting process has limitations.

- Scientists at Oak Ridge National Laboratory (Oak Ridge, Tennessee) examined the effect of compost on leaf photosynthesis, foliar pigment concentrations, nodulation, acetylene reduction rates in soybean, and germination of lettuce, *Arabidopsis*, cabbage, and clover. Results showed that composted TNT-contaminated soil led to chlorosis and lower photosynthesis rates in soybean leaves, as well as less nodulation of the roots. TNT-contaminated compost also appeared to inhibit germination.[Ref 3]

- Scientists at the U.S. Army Corps of Engineers' (USACE) Waterways Experiment Station (WES), Vicksburg, Mississippi, recovered more than 80% of the explosives and explosive by-products in the cellulose, humin, fulvic acid, and humic acid fractions of compost-contaminated soil. The rest was extractable with acetonitrile or ether.[Ref 4]

The studies above were the basis for the design of the plant uptake and weathering studies in this project. Compost derived from treated explosives-contaminated soil will be referred to as treated compost throughout the remainder of this document.

1.3 Project Objectives

The objectives of the Plant Uptake Study were:

- To assess the amount of time needed to mature the compost before plant growth would occur, and
- To determine whether explosive residues from treated compost are taken up by plants.

The evaluation of compost maturity was based on the measurement of:

- Proportion of nitrate to ammonium,
- Carbon-to-nitrogen ratio,
- Respiration rate, and
- Odor.

The evaluation of plant uptake of explosives and explosive by-products from compost and effects on plant growth were based on analysis of:

- Specific explosives in plant tissues,
- Known explosives transformation products in plant tissues,
- Plant growth characteristics, and
- The plant's ability to germinate.

1.4 Approach

This project was executed in the following six phases:

- Planning, Permitting, and Materials Acquisition (Phase I). During this phase, a test plan was written and approved; an environmental review was conducted; the state of Alabama was notified about the project; and compost produced from contaminated soil, uncontaminated soil, and compost amendments were obtained from UMADA.

- Compost Production and Maturity Test (Phase II). During this phase, a control compost was produced at TVA's facility and both the control and treated composts were monitored to determine when they had matured. The control compost was made by TVA from the uncontaminated soil and amendments obtained from UMADA in Phase I.

- Microbial Weathering Study (Phase III). The purpose of this study was to determine if the treated compost would leach explosives over a three-year period when exposed to natural weather conditions. This portion of the project is described in another report.[Ref. 2]

- Analytical Methods Development (Phase IV). During this phase, and in conjunction with several other projects, analytical methods were developed for extracting explosives and explosive by-products from compost, compost leachate, and plant materials. Phase IV was conducted concurrently with Phases II and III.

- Plant Uptake Study (Phase V). During this phase, plants were grown in mature compost/soil mixtures, harvested, and analyzed for explosives and explosive by-products. Compost/soil samples were also collected and analyzed during this period. The purpose of this study was to determine if specific plants would take up and store explosive compounds.

- Write Plant Uptake Report (Phase VI). During this phase, the final report for the plant uptake portion of the project was written.

Project operations took place in the main ERC building, the Chemical Engineering building (CEB), the ERC composting facility, and the ERC greenhouses and growth chamber.

On July 17, 1996, compost amendments and uncontaminated soil obtained from UMADA arrived at TVA's facility in Muscle Shoals, Alabama (Figure 1-1). These materials were used to produce the control compost pile which was established at the ERC on July 22-24, 1996. Monitoring of the control compost pile began on July 28, 1996.

The treated compost was produced at UMADA by Bioremediation Services, Inc. (BSI), in late June 1996. At that time, BSI was under contract with the DoD to remediate explosives- (TNT, RDX, and HMX) contaminated soil at UMADA. The treated compost was produced by thoroughly mixing a volume ratio of approximately 70% organic amendment to 30% contaminated soil using method and amendment formulations developed by BSI. The treated compost was then stored in bulk bags and transported by truck to TVA's ERC in Muscle Shoals, Alabama, around July 31, 1996. The treated compost arrived at TVA on August 12, 1996, and was stored at the ERC composting facility. On August 13, 1996, a treated compost pile was constructed at the ERC composting facility and monitoring of the treated compost pile began that day. The treated compost was estimated to be approximately 30 days older than the control compost. On September 15, 1996, a portion of the immature treated compost was taken from the treated compost pile for use in the Microbial Weathering Study. Both the treated and control composts were allowed to mature until January 20, 1997, when they were used in the Plant Uptake Study. To determine whether the composts were mature, they were analyzed for:

- Respiration rate,
- Nitrate and ammonium concentrations,
- Carbon-to-nitrogen ratio, and
- Odor.

Once the composts' maturities were established, the Plant Uptake Study was begun.

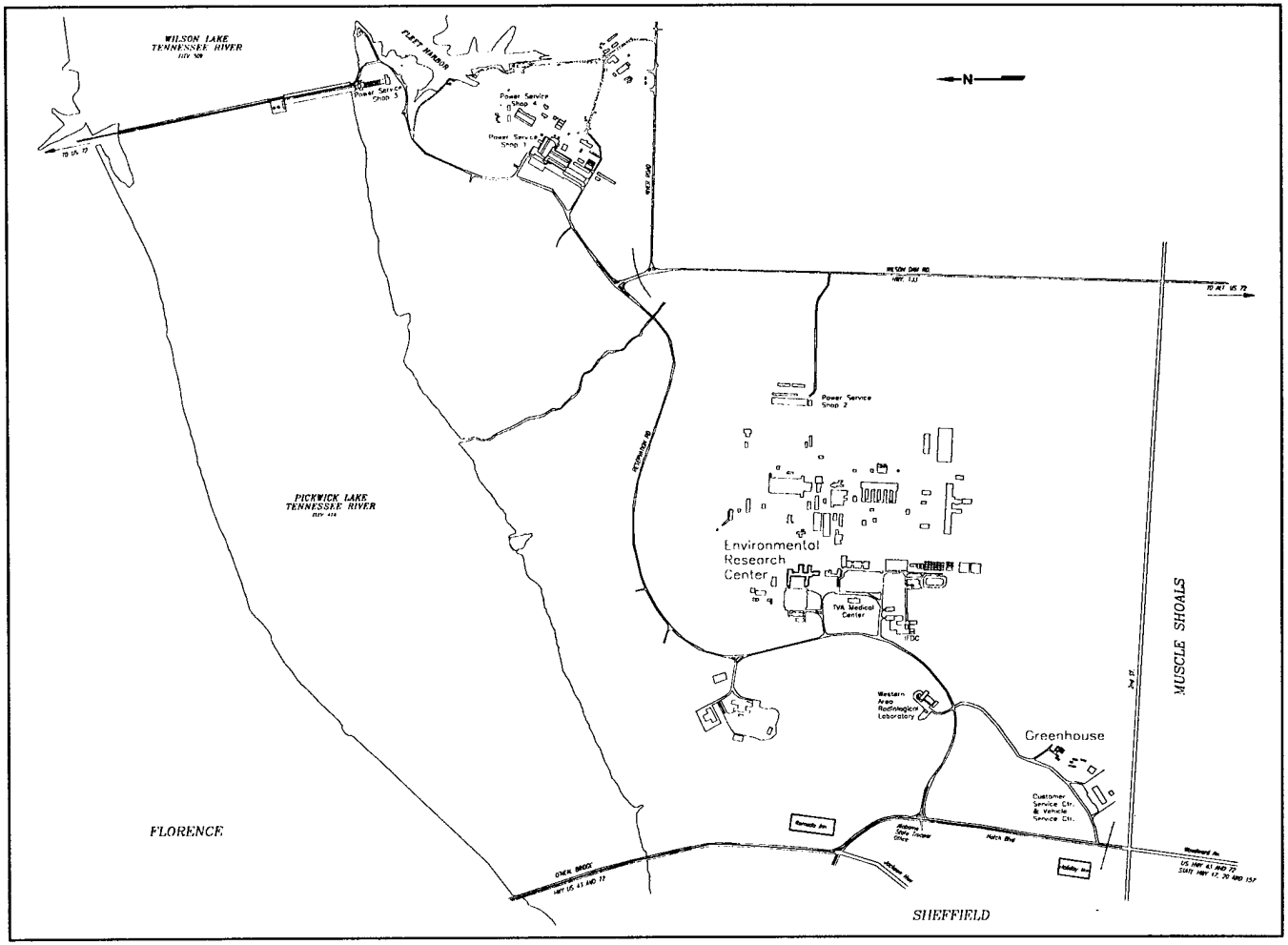


Figure 1-1

Location of TVA Environmental Research Center

The Plant Uptake Study consisted of three sub-studies:

- A Seed Germination Study to determine conditions necessary to grow the plants (Study 1),

- A Preliminary Plant Uptake Study (Study 2) conducted to quantify the appropriate ratio of soil-to-compost to be used, and

- The Main Plant Uptake Study (Study 3) conducted to determine if the plants would take up explosives or explosive by-products.

During Study 1, treated and control composts that were mature (based on compost maturity tests) were diluted with soil at four ratios: 100% compost, 75% compost, 50% compost, and 25% compost, and seeded with radish, kale, and lettuce to determine if the seed would germinate and grow. Results of this study were used to select ratios of compost and soil for use in the Preliminary Plant Uptake Study. Details of the Seed Germination Study are given in Section 3.2.4.1 - Study 1: Seed Germination Study.

In Study 2, radish and kale were grown in uncontaminated UMADA soil and mature control and treated compost mixed with soil at soil-to-compost ratios of 5:1, 3:1, and 1:1. In addition to providing a basis for the selection of the compost dilution for the Main Plant Uptake Study (Study 3), Study 2 provided information on the effect of compost dilution on plant growth. If explosives or explosive degradation by-products had been taken up by the radish or kale, the study would have shown the effect of compost dilution on uptake of explosives and explosive degradation by-products.

During Study 3, eight plant varieties (seven plant species) were grown in a mixture of mature compost and uncontaminated soil and then harvested. During plant growth, the plants were examined for height, morphology, and color. Once mature, the plants were harvested and analyzed for explosives and explosive by-products. In addition, samples of the soil/compost mixture were collected both before planting and after harvest. These samples were analyzed for explosives and explosive by-products.

1.5 Schedule

A GANTT chart showing project-related activities is provided in Figure 1-2. The quarter designations in the chart refer to fiscal year quarters. As indicated in the section above, there were six phases to this project. The timelines for these phases were:

- Planning, Permitting, and Materials Acquisition (Phase I). Began on June 17, 1996, and completed on September 18, 1996.

- Compost Production and Maturity Test (Phase II). Began on July 19, 1996, and completed on February 13, 1997.

- Microbial Weathering Study (Phase III). Began on July 17, 1996, and scheduled to continue through fiscal year 1999.

- Analytical Methods Development (Phase IV). Began on June 17, 1996, and completed on July 23, 1997.

- Plant Uptake Study (Phase V). Began on December 12, 1997, and completed on May 9, 1998.

- Write Plant Uptake Report (Phase VI). Began on August 17, 1998, and scheduled to be completed on January 1, 1999.

ID	Task Name
1	**Receive Funding**
2	**Phase I - Planning, Permits, and Matl. Acquisition**
3	**Task 1 -- Develop Detailed Project Plan**
4	Write Draft Test Plan
5	Consolidate/Format/Edit Draft Test Plan
6	AEC Reviews Test Plan
7	Write Final Test Plan
8	Final Test Plan Issued -- Task 1 complete
9	**Task 2 -- Environmental Permit Acquisition**
10	Permit Acquisition Started
11	Analysis of Cont. Soil and Treated Compost Received
12	Acquire Project Information & Regulatory Reqd.
13	EDR Submitted to TVA Envir. Compliance Staff
14	TVA OGC Office Reviews ERC Approach
15	State Contacted for First Time
16	State Notification Written and Received
17	All Permits Acquired - Task 2 Complete
18	**Task 3 -- Materials Acquisition**
19	Feedstocks Acquisition Starts
20	Obtain Soil Amendments and Clean Soil
21	Clean Soil and Amendments Arrive at Muscle Shoals
22	Obtain Treated Compost
23	Treated Compost Arrives at Muscle Shoals
24	All Study Feedstocks Obtained - Task 3 Complete
25	Phase I Complete

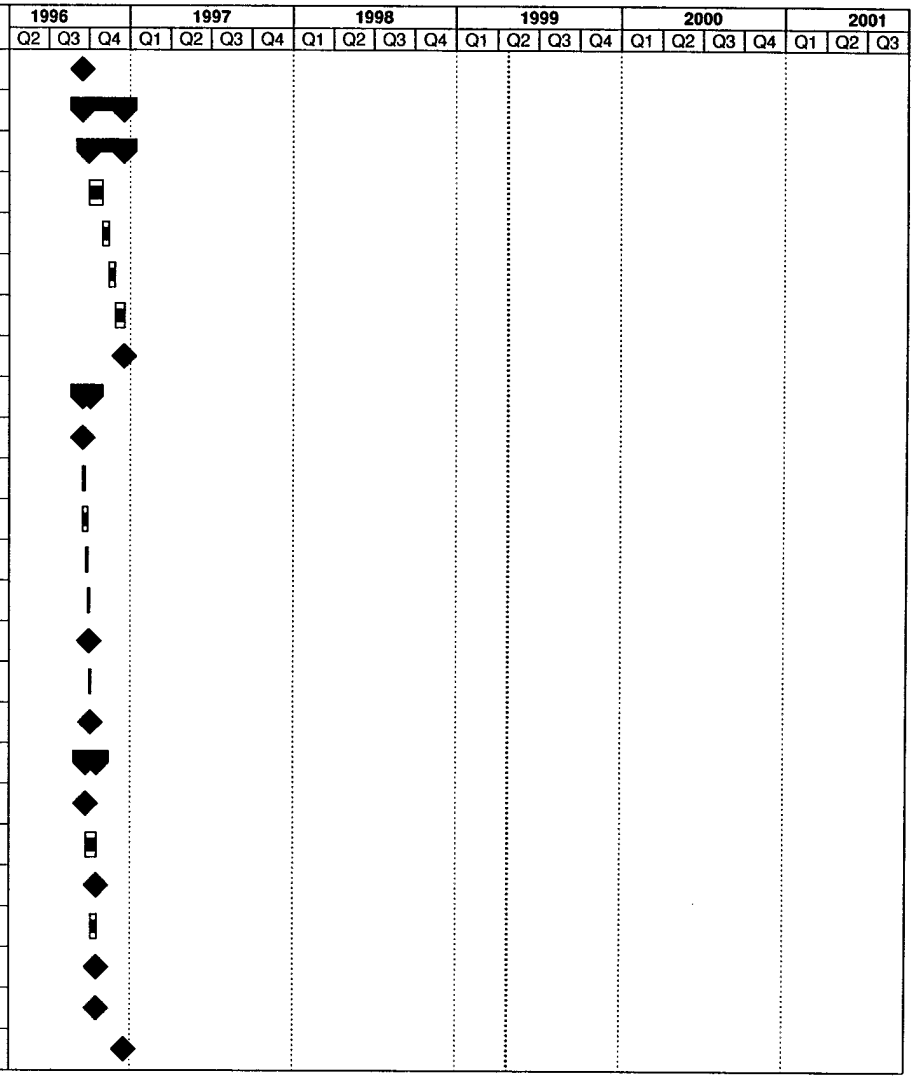


Figure 1-2

Gantt Chart for Plant Uptake Study

ID	Task Name
26	**Phase II - Compost Production & Maturity Test**
27	Earliest Maturity Period
28	Additional Curing
29	Sample and Monitor Control Compost
30	Data Analysis
31	Phase II Complete
32	**Phase III - Microbial Weathering Test**
33	Prepare Bins
34	Compost Placed in Bins and Exposed to the Elements
35	Data Collection and Sampling
36	Data Analysis
37	Write Draft Interim Weathering Report
38	Format/Draft Interim Weathering Report
39	AEC Reviews Interim Weathering Report
40	Finalize Interim Weathering Report
41	Develop Scope of Work for 98/99 Extension
42	Data Collection for 98/99 Extension
43	Data Analysis for 98/99 Extension
44	Write Draft Final Weathering Report
45	Format/Draft Final Weathering Report
46	AEC Reviews Final Weathering Report
47	Finalize Final Weathering Report
48	Phase III Complete

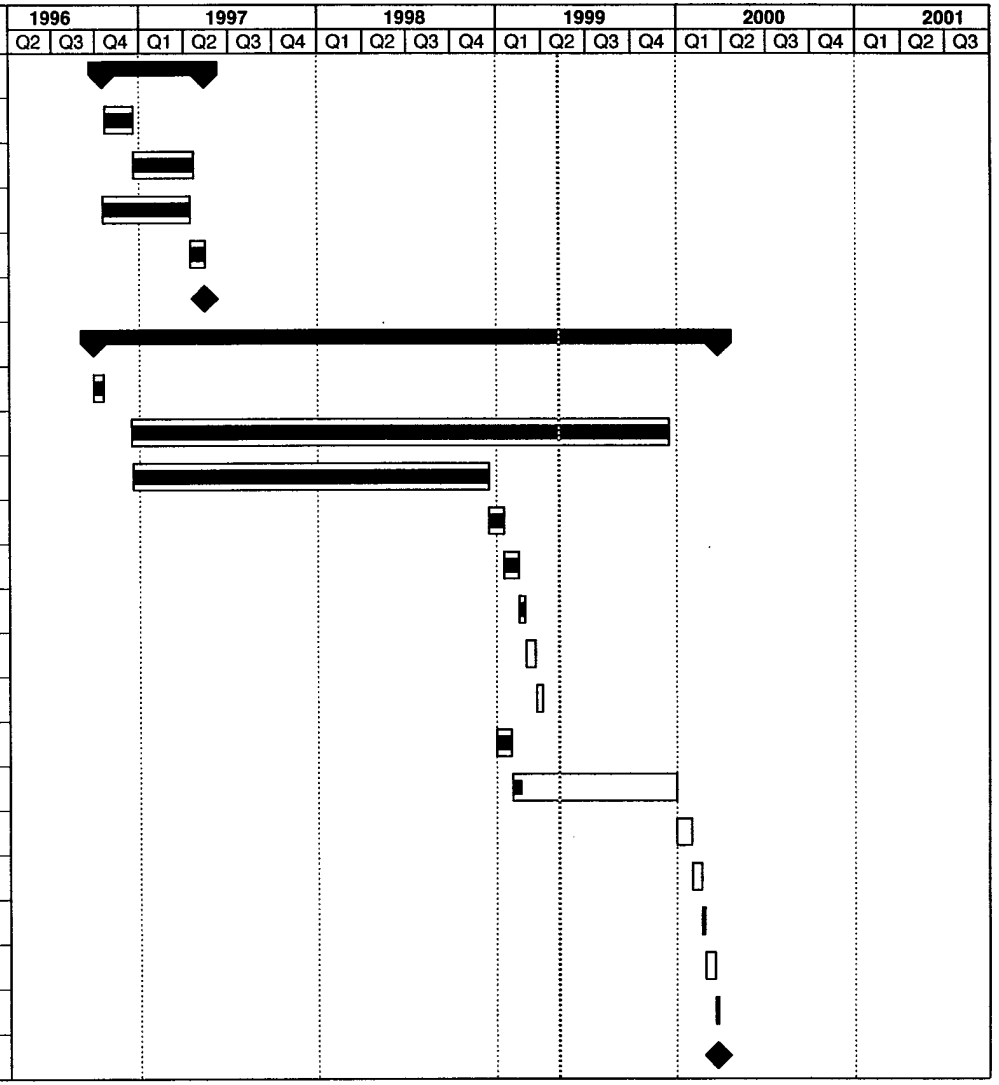


Figure 1-2 (Continued)

Gantt Chart for Plant Uptake Study

ID	Task Name
49	**Phase IV - Analytical Methods Development**
50	Methods Development
51	Phase IV Complete
52	**Phase V - Plant Uptake Test**
53	Start Plant Uptake Study 1
54	Obtain Materials (Plants, Pots, etc.)
55	Grow Plants & Harvest - Plant Uptake - Study 1
56	Data Acquisition and Analysis - Plant Uptake - Study 1
57	Data Analysis - Plant Uptake - Study 1
58	Plant Uptake Study 1 Complete
59	Start Plant Uptake Study 2
60	Design Experiment - Plant Uptake - Studies 2 & 3
61	Grow Plants & Harvest - Plant Uptake - Studies 2 & 3
62	Data Acquisition and Analysis - Plant Uptake - Studies 2 &
63	All Final Reports Complete - Phase V Complete
64	**Phase VI - Write Plant Uptake Report**
65	Write Draft of Plant Uptake Report
66	Format/Edit Draft of Plant Uptake Report
67	AEC Reviews Plant Uptake Report
68	Plant Uptake Report Issued - Phase VI Complete
69	End of Study -- PROJECT COMPLETE

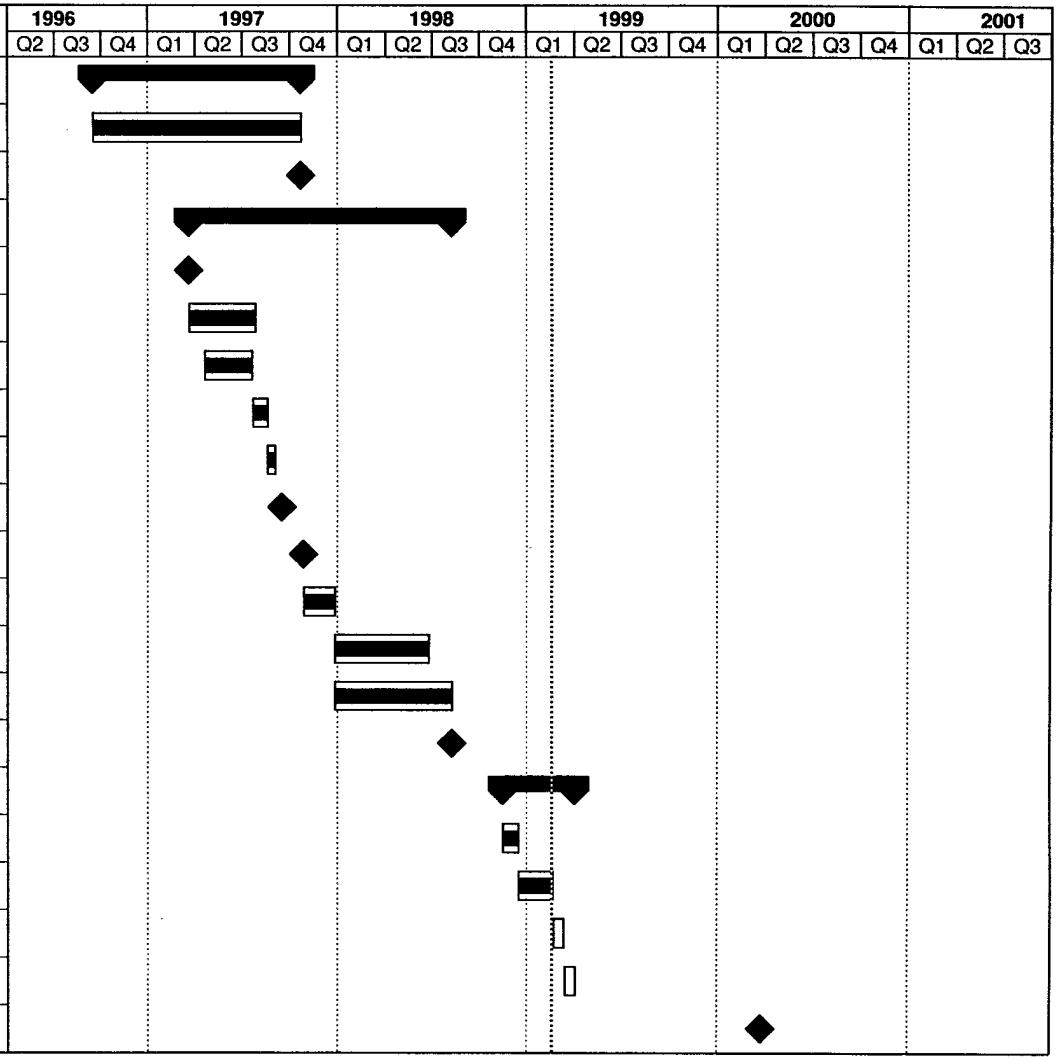


Figure 1-2 (Continued)

Gantt Chart for Plant Uptake Study

SECTION 2.0
COMPOST DESCRIPTION

The compost used during this project was obtained from UMADA in 1996. At that time, the Army was remediating explosives-contaminated soil at UMADA using windrow composting. The soil at UMADA had become contaminated with explosives when wastewater from an ammunition unloading facility was routed to two lagoons resulting in contamination of the lagoon's sediment and surrounding soil. The level of explosives in the soil and sediments ranged from 6,000-8,000 ppm total explosives. Further analysis revealed the mix of explosives in the soil to be approximately 90% TNT, 8% RDX, and 2% other explosives.

To identify an effective remediation method for the site, the USAEC conducted field-scale pilot demonstrations of several composting technologies.[Ref. 1] The goal was to remove TNT and RDX to levels below the state of Oregon's action limit of 30 ppm for each explosive. Of the methods examined, windrow composting was found to be the most effective and economical. The treatability studies also indicated that the compost was non-leaching.

BSI was employed to remediate the UMADA site using the windrow composting method. During the remediation process, excavated soil contaminated with explosives was screened to remove large rocks and debris and then transported to soil storage buildings. In the treatment building, the contaminated soil was combined with:

- Biodegradable carbon sources such as alfalfa, potato culls, chicken manure, and cow manure;
- Bulking agents, such as wood chips and sawdust, to increase porosity and oxygen content; and
- Water.

The final volume of the mixture contained 30% soil and 70% organic amendments. The composition of the mixture was based on a formulation developed by BSI. The mixture was placed in windrows (long piles) within a treatment building to prevent windblown contaminant

migration and to protect the piles from the weather. The windrows were periodically mixed and aerated with a compost turner. Moisture, temperature, and explosive concentrations were monitored throughout the composting process. The biodegradable contaminants were degraded by microorganisms which produce organic and inorganic transformation products and heat during the process. Following treatment, the compost was tested and certified to contain less than the action levels of 30 ppm TNT and 30 ppm RDX. Once the concentration of contaminants dropped below the action levels, the compost was removed from the treatment building and stored in a pile. This compost was later used as part of the vegetative cover on the installation's solid waste landfill.

The treated compost used in this project was produced at UMADA by BSI in late June 1996. It was then loaded into bulk bags and transported by truck to TVA's ERC in Muscle Shoals, Alabama.

Upon arriving at TVA, a treated compost pile was constructed and monitored for oxygen content and temperature. The pile was conical with a base diameter of 3 m and a height of about 1.8 m. The total volume of the treated compost pile was about 4,500 liters (160 cubic feet). All composting took place inside an enclosed building for control of weather-related variables. Monitoring of the treated compost pile began on August 13, 1996. Whenever oxygen levels fell below 5%, the pile was aerated (turned) with a compost skid steer loader. Initially, the pile had to be aerated daily to maintain oxygen levels; however, as the pile matured, it was aerated less frequently. The treated compost pile was aerated a total of ten times prior to September 15, 1996, when 700 liters of the compost was diverted from the pile for use in the microbial weathering study. When the treated compost was received, a sample of compost was taken to identify the amount of water that should be added to adequately wet the compost prior to aeration. Based on these measurements, it was established that the compost should contain approximately 20% moisture on a dry weight basis. Therefore, prior to aerating the pile, water was added to bring the moisture up to this level while taking care not to exceed the compost's saturation level. Moisture determinations were made weekly to ensure that the optimum moisture level was being maintained. During the 33-day interval between receipt of the treated compost and beginning of the microbial weathering study, 98 gallons of water were added to the treated compost. A detailed discussion of the composting activities is given in

Section 3.3.2 (Production of Control Compost) and in Section 3.3.3 (Production of Treated Compost).

In order to develop meaningful greenhouse and laboratory comparisons and to clearly evaluate compost effects on crop growth, it was necessary to produce a control compost under conditions similar to those used to produce the treated compost. Under ideal conditions, both the treated and control composts would have been produced and stored at the same site. However, this was not possible due to the logistical complexity involved. Instead, the control compost was produced by TVA at its composting facility at the ERC in Muscle Shoals, Alabama.

The control compost was produced using procedures which attempted to duplicate the conditions (e.g., moisture content, mixing frequency, temperature control, and oxygen concentration) being used at UMADA. TVA used amendments (e.g., sawdust, alfalfa hay, potato culls, chicken manure, and cow manure) and uncontaminated soil from UMADA.

During the production of the control compost, daily oxygen and temperature readings (except on weekends at the UMADA site) were taken at three locations and three defined depths into the pile. During the composting period, weekly samples were taken for moisture analysis. Moisture measurements were used to maintain the control compost at a level consistent with that used at UMADA. Details describing the production of the control compost are provided in Section 3.2.2 (Production of Control Compost).

The control compost was used for maturity testing and the plant uptake studies. To minimize any age differences between the two composts, the control and treated compost were produced in as close a time frame as possible (within 30 days of each other).

SECTION 3.0
EXPERIMENTAL DESIGN

3.1 Introduction

The primary objective of this study was to determine if vegetable or range plants can take up explosive residues from treated compost. A treated compost is defined here to mean the compost resulting from the remediation of explosives-contaminated soil when using composting techniques. In the study, a control compost was used to distinguish those phenomena occurring as a result of explosives contamination. The control compost consisted of uncontaminated soil and compost amendments from UMADA.

In order to achieve the primary goal, a project was undertaken utilizing nine plant species (two in the Preliminary Plant Uptake Study and seven in the Main Plant Uptake Study) grown in treated compost and control compost. The plants were chosen to cover a broad range of plant types such as fruit-bearing crops, root crops, leafy vegetable crops, nodulating plants, monocots, and dicots.

To support the project, analytical methods were developed for analyzing the explosives content of compost, plant tissues, and compost-derived leachate. The goal of this segment of the project was to improve the efficiency of the extraction techniques used.

3.2 Description of Experimental Project Phases

3.2.1 Analytical Methods Development (Phase IV)

3.2.1.1 Method Development for Compost Analysis

The procedure for analyzing the compost for explosives and explosive by-products was based on a method developed by Phil Thorne at the U.S. Army Cold Regions Research and Engineering Laboratory (CRREL)[Ref. 5] The compost was sonicated with acetonitrile for 18 hours. The acetonitrile was filtered off and analyzed using HPLC. The remaining solids were then digested successively with a strong acid and a strong base. The purpose of the digestion

in the acid and base was to release any explosives or explosive by-products bound to the compost matrix. The liquid from the digestions were then analyzed using HPLC.

After evaluating CRREL's method, TVA, in consultation with CRREL and USAEC, decided to conduct the acetonitrile extraction and the acid digestion only. The base digestion was not conducted. The purpose of the base digestion was to release the amino and di-amino compounds from the humic part of the compost matrix. The base digestion was omitted for a number of reasons including:

- Explosives such as TNT, RDX, and HMX are degraded during base digestion.
- The base extraction required a third analysis in which no analyte of interest was ever detected by TVA.
- Due to the compost's high sand content, the compost acted more like a soil than compost for extraction purposes.
- Based on spiking tests conducted by TVA, it was concluded that the base digestion of the compost residue provided minimal additional information about the content of explosives and their by-products in the compost, especially when compared to the additional cost of conducting the base digestion.

The details of the procedure used to analyze the UMADA compost are provided in Appendix B-16.

3.2.1.2 Method Development for Plant Analysis

When this project was initiated, a satisfactory method for extracting explosives and explosive by-products from plant tissues had not been developed. Consequently, a method had to be developed to meet the needs of this and other USAEC projects. The goal of the development work was to develop an extraction procedure that would remove both explosives and explosive by-products into one sample which could be analyzed by HPLC. This was an ambitious goal because explosives such as TNT, RDX, and HMX are easily extracted with polar solvents such

as acetonitrile while the amino and diamino explosive by-products are as effectively extracted with acetonitrile. TVA overcame most of these problems by developing a method in which the plant tissues were freeze dried, extracted with acetonitrile (twice), and then digested with acid. The resulting liquids from the extraction and digestion were then purified utilizing solid phase extraction techniques to remove the plant's chlorophyll and pigments that were extracted, along with the explosives and their by-products. The purified extract was then analyzed by HPLC. For each plant type, the procedure provided consistent recoveries for all of the analytes of interest. It was deemed sufficient for determining if explosives were being taken into the plant matrix. However, it should be noted that each plant type behaves differently during the extraction process, so there are recovery variations between plant types.

The details of the procedure are provided in Appendix B-16.

3.2.1.3 Methods Development for Compost Leachate Analysis

Procedures were developed for the Microbial Weathering Study (Phase III) to enable the analysis of explosives and explosive by-products in leachate from piles of compost which were exposed to the environment. The results of the Microbial Weathering Study are presented in a separate report.[Ref. 2] The leachate collected during the Microbial Weathering Study was initially a very rich brown color due to the presence of humic compounds, tannin resins, and other organic acids. These compounds represented the water-soluble fraction of the compost being treated in the weathering study. When the leachates were analyzed using a standard EPA method developed for extracting explosive from water (Method 8330), these organic compounds were carried over to the extract. As a result, they tended to contaminate the HPLC column during analysis causing stability problems. Furthermore, some of the compounds had retention times very similar to those of the explosives and explosive by-products being analyzed. To eliminate these interferences, several other extraction techniques were attempted to find a process that would provide acceptable recoveries of the explosive analytes while also removing the interfering humic compounds.

The first experiments were designed to remove interferences and qualitatively determine if humic compounds could be satisfactorily removed from the leachate samples so explosive analysis could be conducted. Solid phase extraction was first attempted to remove the humic

compounds from the leachate samples. The following solid phase extraction procedures were attempted.

Solid Phase Porapak-RDX Extraction

Initially, a Porapak-RDX solid phase extraction cartridge (1.0 g) was used. A leachate sample was filtered through the cartridge directly. However, on the first attempt, the humic compounds broke through the cartridge. A second experiment was tried with a larger (5.0 g) Porapak-RDX cartridge to prevent breakthrough. After the sample was concentrated on the cartridge, it was eluted using acetonitrile following the solid phase extraction procedures outlined in Method AP-0062 (Appendix B-16). The resulting extract was light brown in color. When analyzed using HPLC, the extract was found to have a number of unknown peaks that masked the peaks associated with the explosives and their by-products.

Solid Phase Alumina/Porapak-RDX Extraction

Since it appeared that some of the soluble humic compounds appeared to elute from the Porapak-RDX cartridge with acetonitrile, it was decided to try to remove them from the sample before it was concentrated on the Porapak-RDX cartridge. A leachate sample was first filtered through an Alumina-A, Alumina-B, or Alumina-N cartridge. The sample was then concentrated onto a Porapak-RDX cartridge and then extracted using acetonitrile. In all three cases, the resulting elute had a light brown color and, when analyzed, had peaks that masked the peaks associated with the explosives.

Based on these initial experiments, it was determined that solid phase extraction of the leachates was going to be unsatisfactory for quantitatively analyzing explosives and explosive by-products in the sample.

Liquid/Liquid Extraction

Since solid phase extraction was unsuitable for these samples, liquid/liquid extraction was attempted. The first experiment used the liquid/liquid extraction procedure as outlined in EPA Method 8330. In this procedure, the sample was extracted using acetonitrile and salt water. In the process, the explosive compounds are extracted from the water phase into the acetonitrile phase in a quantitative way based on the dielectric strength of the solutions. The acetonitrile layer is then analyzed for explosives and explosive by-products. The procedure, as outlined in

EPA Method 8330, utilizes volumetric flasks and shakers to conduct the extraction. It was found that this was cumbersome and difficult to get reproducible data due to variations in the mixing and extraction of the samples. To eliminate this variability, separatory funnels were utilized for the extraction instead of the volumetric flasks. Samples of leachate that were extracted utilizing the liquid/liquid extraction process appeared to have a lighter color than those extracted by solid phase extraction. When they were analyzed with HPLC, the resulting chromatograms had fewer unknown peaks and these peaks did not appreciably interfere with the peaks associated with the explosives or explosive by-products.

Based on these qualitative results, it appeared that liquid/liquid extraction would be adequate for these samples. To determine quantitatively the efficiency of the liquid/liquid extraction, spiking experiments were performed. Four types of samples were prepared for this experiment. They consisted of: 1) a sample of the leachate that was extracted directly and analyzed; 2) a sample of the leachate spiked with a known amount of explosives that was extracted and analyzed; 3) a water (HPLC purity) sample spiked with a known amount of explosives that was extracted and analyzed; and 4) an extracted leachate sample that was spiked with a known amount of explosives and analyzed.

By analyzing the water sample spiked with explosives, the efficiency of the extraction process in an ideal matrix was determined. By analyzing the extracted leachate sample spiked with explosives, the efficiency of the HPLC detection could be determined in a realistic sample matrix. The leachate sample was spiked with explosives and then extracted to allow a determination of the efficiency of the extraction in a realistic matrix. Analyzing the leachate sample itself showed the amount of explosives present in the natural matrix. There were a number of duplicates run to determine the reproducibility of the process. The results of these experiments are presented in Tables 3-1, 3-2, and 3-3.

As Table 3-1 shows, the spiked water sample gave good quantitative recovery when liquid/liquid extraction was used on the leachate samples. When the leachate sample was spiked, then extracted and analyzed, the recoveries for all of the analytes except 2,4-DANT and 2,6-DANT were good (Table 3-2). This is expected since this extraction process relies

Table 3-1

Summary of Water (HPLC Purity) Samples Spiked Before Extraction

Analyte	n	Mean Recovery	Standard Deviation
TNT	15	89.4	6.4
RDX	15	88.2	4.7
HMX	15	91.6	8.9
2,6-DNT	15	86.7	6.5
2,4-DNT	15	88.5	4.8
2A-DNT	15	89.8	5.8
4A-DNT	15	87	7.2
2,4-DANT	15	100.3	10.3
2,6-DANT	15	95.3	6.7
TNB	15	96.8	4.9

Table 3-2

Summary of Leachate Samples Spiked Before Extraction

Analyte	n	Mean Recovery	Standard Deviation
TNT	18	81.8	7
RDX	18	76	5.2
HMX	18	86	7.6
2,6-DNT	18	84.9	7.6
2,4-DNT	18	82.1	6.3
2A-DNT	18	84	5.2
4A-DNT	18	81.4	5.2
2,4-DANT	18	60.3	5.3
2,6-DANT	18	59.2	4.1
TNB	18	83.8	6.4

Table 3-3

Summary of Leachate Samples Spiked After Extraction, But Before Analysis

Analyte	n	Mean Recovery	Standard Deviation
TNT	4	85.6	3.7
RDX	4	79.1	1.3
HMX	4	87.9	7.7
2,6-DNT	4	87.4	6.7
2,4-DNT	4	83.6	3.1
2A-DNT	4	86	2.9
4A-DNT	4	83.9	2.2
2,4-DANT	4	62.5	4.2
2,6-DANT	4	60.9	2
TNB	4	87.9	2.6

on the dielectric strength and the polarity of the explosives. The leachate is highly concentrated with salts, humic compounds, and organic acids that are going to greatly affect the dielectric strength of the solution. Since both 2,4-DANT and 2,6-DANT are relatively polar, it is understandable that they would not be extracted as effectively as the other compounds.

However, in all cases, the values recovered were very reproducible. Part of the loss in the recovery of the explosive compounds in Table 3-3 is due to interferences in the instrument detection. This could have been caused by some of the humic compounds which were carried over in the extract. However, as the data from all three tables indicates, the extraction is good (80% range) for all of the analytes except 2,4-DANT and 2,6-DANT. The reproducibility of the extraction and analysis was within an acceptable range (±10%).

Based on these experiments, the liquid/liquid extraction was determined to be the best method for analyzing the compost leachate samples for explosives. Even though the recoveries of 2,4-DANT and 2,6-DANT were low, it was decided this was the best that could be done without running a completely separate analysis for these two analytes. The liquid/liquid extraction procedure was refined for routine analysis and is described in detail in Method AP-0062 (Appendix B-16).

For the first few months, each sample was run with an individual matrix spike to ensure recoveries stayed consistent as the dielectric properties of the leachates changed over time and sampling location. The results of those experiments indicated that the recoveries fell within the same range as the experiment above with all but one or two exceptions. After discussions with the USAEC, it was determined that a matrix spike would be run with only one sample per batch to save project funds.

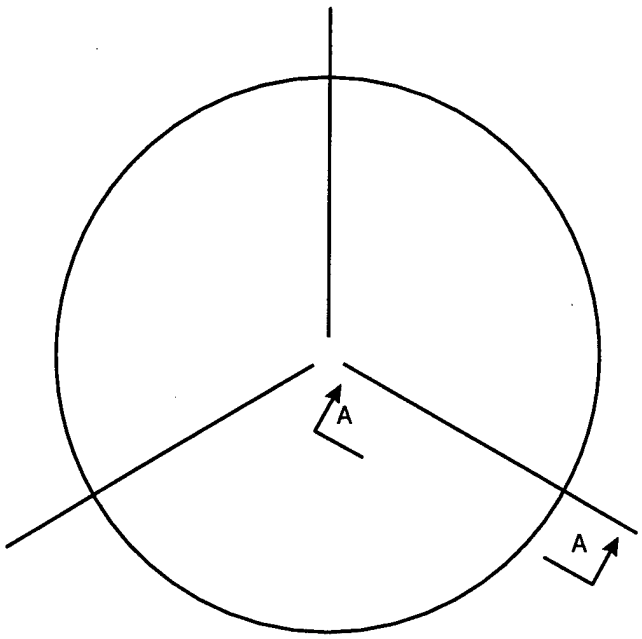
3.2.2 Production of Control Compost (Phase II)

The control compost was developed so that effects on plant growth attributable to the compost could be distinguished from those attributable to explosives or explosive by-products bound to the treated compost. The control compost was produced by TVA at its composting facility at the ERC in Muscle Shoals, Alabama.

To produce the control compost, TVA obtained uncontaminated soil, organic amendments (straw, alfalfa hay, chicken and cow manure, and potato wastes), and the organic formulation from BSI at UMADA. The organic amendments were combined and placed in bulk bags for shipping. The uncontaminated soil was placed in different bags for shipping. The organic amendments and uncontaminated soil were both transported by truck to TVA's ERC. The materials were shipped on July 9, 1996, and were received on July 18, 1996. On July 22-24, 1996, a 2-meter-high by 3.5-meter-wide control compost pile was constructed. The pile was cone-shaped with a flattened top and had a volume of about 8,300 liters (290 cubic feet). Approximately 2,500 liters (3.900 kg) of uncontaminated soil and 5,800 liters (4,900 kg) of amendments were used to construct a pile of sufficient mass to conserve heat and sustain thermophilic temperatures. Extra soil was obtained for use in other parts of the study.

Monitoring of the control compost pile began on July 28, 1996. Temperature and oxygen levels were measured daily (except on weekends as at the UMADA site). Oxygen levels were measured using an oxygen meter, Model OXOR II, made by Bacharach. The meter was calibrated using atmospheric air (approximately 20.9% oxygen). Oxygen measurements were taken as close to the center of the pile as possible and at a depth of 30 cm above the floor. This is normally the least aerobic area of the pile. Three oxygen levels were taken for each monitoring event with the probe inserted from three sides of the pile (at the 12 o'clock, 4 o'clock, and 8 o'clock positions). Oxygen levels plotted in Figure 4-6 are averages of three readings.

Temperature measurements were also taken daily using Type T (copper/constantan) thermocouple probes. Three sets of three probes were inserted into the pile as shown in Figure 3-1. Each set of three included a 183-cm-long, 91-cm-long, and 30-cm-long probe. The three in a set were placed in line vertically with the longest probe being about 30 cm above the floor, the 91-cm probe being 60 cm above the floor, and the 30-cm probe being 90 cm above the floor. The three sets of probes were placed similarly, but spaced 120 degrees around the pile (at 12 o'clock, 4 o'clock, and 8 o'clock). The thermocouples of the three 183-cm-long probes were, therefore, all located about 30 cm above the floor in the center of the pile. Unlike the oxygen probe, which had to be inserted for making measurements, the thermocouple probes



Plan View

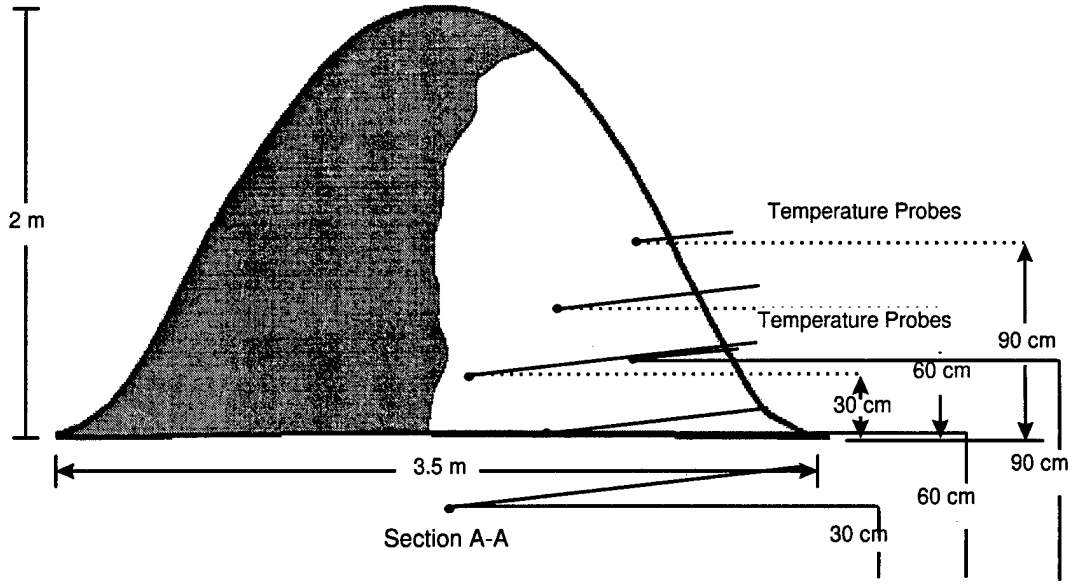


Figure 3-1

Diagram of Control Compost Pile

were left in place, but had to be removed from the pile before aeration and repositioned afterward.

The control compost was used for maturity testing and the plant uptake studies. To minimize any age differences between the two composts, the control and treated compost were produced in as close a time frame as possible (within 30 days of each other). The control compost pile was established July 22-24, 1996, and monitoring began on July 28, 1996. Sampling of the compost for maturity testing began on August 22, 1996, giving the control compost approximately a month of active composting prior to maturity testing. A single batch of both composts was used for all studies.

3.2.3 Production of the Treated Compost

The treated compost had been produced in late June 1996 at UMADA by BSI. At that time, BSI was under contract with the U.S. Army Engineer District, Seattle, Washington, to remediate explosive- (TNT, RDX, and HMX) contaminated soil at UMADA. The treated compost was produced by thoroughly mixing a volume ratio of approximately 70% organic amendment to 30% soil using method and amendment formulations developed by BSI. The treated compost was then loaded into bulk bags and transported by truck to TVA's ERC in Muscle Shoals, Alabama. The treated compost was shipped around July 31, 1996, and was received August 12, 1996. The treated compost pile was constructed on August 13, 1996, and monitoring of the pile began on the same day. As a means of characterizing the treated compost, it was sampled upon arrival at TVA's facility and analyzed for the characteristics listed in Table 3-4.

The treated compost pile was shaped similar to the control compost pile, but had smaller dimensions because it contained less material. The treated pile was a flattened cone about 1.8 m high and 3 m in diameter. It contained about 4,500 liters (160 cubic feet). The placement of the temperature probes was identical to that of the control compost pile, however, the probes were not inserted their full length so that they would be located in the same positions relative to the pile boundaries. Section 2 (Compost Description) gives details of the aeration and moisture control of the treated compost pile between the time the pile was formed

Table 3-4

Initial Analysis of Treated Compost

Analyte	Preparation Method[1]	Analytical Method[1]
Explosives and Explosive By-Products		
TNT	AP-0062	AP-0062
TNB	AP-0062	AP-0062
HMX	AP-0062	AP-0062
RDX	AP-0062	AP-0062
2A-DNT	AP-0062	AP-0062
4A-DNT	AP-0062	AP-0062
2,4-DANT	AP-0062	AP-0062
2,6-DANT	AP-0062	AP-0062
2,4-DNT	AP-0062	AP-0062
2,6-DNT	AP-0062	AP-0062
Nutrients and Moisture		
Moisture	Not Applicable	ASTM E 871
Total P	Method ASA 24-2.3	6010 B
Inorganic P	Method ASA 24-3.3	6010 B
Organic P	Not applicable	Calculated (Total P - Inorganic P)
Potassium	3050 B	6010 B
NH_4-N	Analyze 2N KCl extract	AP-0059
$(NO_3 + NO_2)$-N	Analyze 2N KCl extract	AP-0058
Total Kjeldahl Nitrogen	AP-0064	AP-0064
Metals		
Arsenic	3050 B	7060 A
Barium	3050 B	6010 B
Cadmium	3050 B	6010 B
Chromium	3050 B	6010 B
Lead	3050 B	6010 B
Mercury	7470/71A	7470/71A
Selenium	7740	7740
Silver	3050 B	6010 A

(1) See Appendix B for details on methods and procedures.

(August 13, 1996) and the time some material was diverted to the Microbial Weathering Study (September 15, 1996).

All composting activities took place inside an enclosed building for control of weather-related variables. The treated compost pile was established on August 13, 1996, and monitoring began on the same day. Sampling of treated compost for maturity testing also began on August 22, 1996.

3.2.4 Compost Maturity Testing (Phase II)

The stability and maturity of compost determines its suitability for different agricultural and horticultural applications. A mature compost is required to eliminate toxicity to plants from compost intermediates and to eliminate nutrient imbalances which might arise from excessive microbial activity. Without a mature compost, effects related to explosive by-products would be difficult to evaluate because of effects created by the composting process alone.

Two criteria were utilized to assess the suitability of the compost for use. These criteria were compost maturity and stability. Four indicators were used to evaluate these criteria. These indicators were:

1) The odor of the compost. Immature compost, particularly under moist, anaerobic conditions, produces undesirable odors which can be associated with the presence of toxic compounds inhibitory to seed germination and plant growth. Odor tests were performed at each scheduled sampling period by placing approximately 150 g (dry weight) of compost into plastic bags.[Ref 7] Three bags were prepared for both the control and treated composts (six total bags for each testing period) using the same material collected for chemical analyses and soda lime carbon dioxide absorption testing. The moisture content of the compost was adjusted to thoroughly moisten the compost and excess air was pressed from the bags before they were tightly sealed. Bags were placed in a 30°C incubator for a week before being evaluated. Evaluation consisted of four staff members subjectively estimating odor description, odor intensity, and odor acceptability for each sample. Ratings were tabulated and a consensus of opinion among the four

evaluators was determined. This consensus was used to evaluate odor acceptability for the two composts over time.

2) The ratio of nitrate to ammonium in compost. This indicator was used as a maturity criteria. When compost is curing, ammonium is microbially converted into nitrate. Increasing levels of nitrate relative to ammonium indicate increased compost stability and maturity because nitrate-producing organisms are inhibited by the temperatures present during active composting. Elevated ammonium levels can also influence compost toxicity to seed germination and plant growth. Three samples of control and treated compost were submitted for analysis every two weeks until compost maturity was achieved.

3) The carbon-to-nitrogen ratio of the compost. The carbon-to-nitrogen ratio was determined using total organic carbon and total nitrogen analyses. The stabilization of the carbon-to-nitrogen ratio can be used to estimate compost maturity, particularly for composts having an initial carbon-to-nitrogen ratio of 30:1 or higher. While both the control and treated composts used in this study had significantly lower carbon-to-nitrogen ratios, the test allowed comparisons of similarity and maturity between the control and treated composts.

4) The release of respiratory carbon dioxide. Respiration rates were estimated using soda lime absorption. This method, which has been used previously to measure the respiration from soil and forest litter,[Refs. 6,7] was suitable for both treated and untreated compost evaluation. To conduct the respiratory carbon dioxide test, between 100 and 200 g of compost (at least 3 replications per compost type) were sealed in a plastic container for 24 hours at 37°C. This sample size helped minimize problems with compost heterogeneity. Distilled water was added to the compost to ensure that moisture was not a limiting factor. The container held a glass petri dish with approximately 40 g of soda lime which had been previously dried at 100-105°C for at least 4 hours and weighed to the nearest 0.001 g. After the 24-hour incubation period, the soda lime was dried and weighed to estimate the amount of carbon dioxide evolved during the incubation period. The compost samples were removed from the plastic containers, weighed, and dried to determine both wet and dry weights. Results were expressed as

milligrams of carbon dioxide released per hour or day. Controls consisted of soda lime incubated for 24 hours at 37°C without compost, but containing a known quantity of water.

To compare the control and treated compost to a more conventional compost, an additional treatment was included in the test. The additional treatment was mature poultry litter compost adjusted to 50% moisture content 24 hours prior to testing the other compost materials and incubated at 30°C. For the control and treated composts, three replications were performed for each of three larger samples from each evaluation (nine samples for each compost). Similarly, nine poultry litter composts and nine water controls were evaluated during each measurement period.

For all maturity testing, samples of control and treated compost (3 replications each) were taken initially and at 14-day intervals for 170 days and analyzed as per Table 3-5. After approximately 30 days of active composting, samples for chemical analysis were obtained. These analyses were repeated at 14-day intervals until day 170. The specific analytical methods used are also described in Appendix B.

3.2.5 Plant Uptake Study (Phase V)

The Plant Uptake Study included three substudies: (1) a Seed Germination Study to determine if treated compost needed dilution or additional maturation, (2) Preliminary Plant Uptake Study where the plants were grown in a controlled environmental chamber, and (3) the Main Plant Uptake Study where the plants were grown in a greenhouse.

A total of ten plant species were used during the project (nine species were used in plant uptake studies and one was used only for germination tests). These plant species included a broad category of range or pasture crops and vegetable crops. The species were selected to include fruit-bearing vegetable crops, root crops, leafy vegetable crops, monocots, dicots, nodulating plants, organic acid root exuding plants, and forage crops. These representative plants included:

Table 3-5

Analysis of Treated Compost During Compost Maturity Testing

Analyte	Preparation Method[1]	Analytical Method[1]
NH_4-N	Analyze 2N KCl extract	AP-0059
(NO_3 + NO_2)-N	Analyze 2N KCl extract	AP-0058
Total Kjeldahl Nitrogen	AP-0064	AP-0064
Total Organic Carbon (TOC)	415 Series	415 Series
Odor Test	See Section 3.2.3	See Section 3.2.3
Respiration Rate	See Section 3.2.3	Soda Lime CO2 Absorption (see Section 3.2.3)
Ash	AP-0022	Method AP-0022
Moisture	ASTM E 871	ASTM E 871
pH	ASA 12-2.6	ASA 12-2.6
Electrical Conductivity	Series 120	Series 120

(1) See Appendix B for details on methods and procedures.

Vegetable Crops:

- Radish (*Raphanus sativus* L. variety Cherry Belle) - A quick-growing root crop which can be grown to maturity within 40 days. A cool season crop.

- Kale (*Brassica oleracea* L. variety Siberian) - Roots exude large amounts of organic acids which may affect the release of explosive residuals from soil. A cool season crop.

- Bush snapbean (*Phaseolus vulgaris* L. variety Habichulas) - A pod-producing plant with a nitrogen-fixing, nodulating root system (legume). A warm season crop.

- Tomato (*Lycopersicon esculentum* Mill. variety Patio) - A popular fruit crop that is widely grown throughout the U.S. For convenience in greenhouse culture, a short-season small bush type of tomato plant was used. A warm season crop.

- Lettuce (*Lactuca sativa* variety Bibb) - A small-seeded, cool season salad crop. This plant was used in the Seed Germination Study only.

Range and Pasture Crops:

- Alfalfa (*Medicago sativa* variety Vernal) - A cool season, perennial legume with a nitrogen-fixing nodulating root system. Requires soils of high fertility, pH range of 6-8, and good drainage. Widely grown throughout the U.S. as a hay and pasture crop.

- Chives (*Allium schoenoprasum* variety Cebolletas) - A plant which has been shown to take up significant amounts of TNT from soil. A cool season crop.

- Redtop (*Agrostis alba*) - A cool season perennial grass which spreads vigorously by rhizomes and has a wide tolerance to soil pH, soil fertility, and moisture conditions. Its large system of fine fibrous roots will provide maximum opportunity for root-soil contact and root exudate effect on TNT bound residues. It is often used for land reclamation on radically altered landscapes.

- Sorghum (*Sorghum bicolor* variety FFR 321DR) - A warm season grass widely grown throughout the U.S. for grain and herbage.

- Winter Barley (*Hordeum vulgare* varieties Parmunky and Starling) - A cool season annual, small grain cereal crop. Widely grown throughout the U.S. as a hay and pasture crop, as well as a winter cover crop. It is representative of cereal crops with demonstrated salt tolerance.

3.2.5.1 Study 1: Seed Germination Study

Before starting the Preliminary and Main Plant Uptake Studies, a Seed Germination Study was conducted to determine the conditions necessary to grow the plants. The Seed Germination Study was conducted using three plant species: radish, kale, and lettuce. Radish was chosen for its rapid maturity and because it has an edible root and a hard seed coat. Kale was chosen for its tendency to increase nutrient uptake by producing plant root exudates, and lettuce was chosen because it is a common small seed plant without a hard seed coat.

The main objectives for the Seed Germination Study were to ensure adequate plant growth by determining:

- If the compost needed to be diluted or amended, and
- If additional maturation of the compost was needed.

Tests conducted during the Seed Germination Study indicated that several compost dilutions would need to be evaluated to measure the effect of compost on seed germination. Poor germination occurred in both undiluted control and undiluted treated compost. Several preliminary tests using sand or soil were used to help identify the required test conditions and duration prior to conducting the main germination test. After these conditions were established, the control and treated composts were tested after diluting with uncontaminated UMADA soil to achieve compost concentrations of 100%, 75%, 50%, and 25%, and were compared to seed germination in UMADA soil. An outline of the experimental design is provided in Table 3-6. Ten seeds of radish (*Raphanus sativus* variety Cherry Belle), lettuce

Table 3-6

Experimental Design for the Seed Germination Study

Treatment	% Compost in Soil Compost Mixture	Plant Species	Replicates
With Treated Compost	100%	Radish	4
		Kale	4
		Lettuce	4
	75%	Radish	4
		Kale	4
		Lettuce	4
	50%	Radish	4
		Kale	4
		Lettuce	4
	25%	Radish	4
		Kale	4
		Lettuce	4
With Control Compost	100%	Radish	4
		Kale	4
		Lettuce	4
	75%	Radish	4
		Kale	4
		Lettuce	4
	50%	Radish	4
		Kale	4
		Lettuce	4
	25%	Radish	4
		Kale	4
		Lettuce	4
UMADA Soil	Undiluted	Radish	4
		Kale	4
		Lettuce	4

(*Lactuca sativa* variety Bibb), and kale (*Brassica oleracea* variety Siberian) were used in each replicate of the four replicate tests.

In each replicate, ten seeds of the same variety of plant were sown onto the surface of the soil/compost mixtures (100 g/dish) in glass petri dishes (100 mm by 15 mm high). The seeds were then covered with a small amount of the soil/compost mixture and kept moist by adding small amounts of water, as needed, and allowed to germinate at room temperature (22°C). Germination was judged to be initiated when approximately 1 mm of radicle was visible. Germination was judged to be successfully completed when evidence of a green epicotyl was detected after two weeks. This test was done three times for a total of 120 seeds per plant species (10 seeds, 4 replications, and 3 tests) and 12 replications per compost dilution.

3.2.5.2 Study 2: Preliminary Plant Uptake Study

A Preliminary Plant Uptake Study (Study 2) was conducted in a controlled environmental chamber to further quantify the appropriate soil-to-compost mixtures prior to starting the Main Plant Uptake Study. Two plant species were used during Study 2, radish (*Raphanus sativus* L. variety Cherry Belle) and kale (*Brassica oleracea* L. variety Siberian). An outline of the experimental design is provided in Table 3-7.

To conduct Study 2, radish and kale were planted into 3 kg of potted mixtures of UMADA soil, treated compost, or control compost. Three treatments consisted of uncontaminated UMADA soil mixed with control compost or treated compost at soil-to-compost ratios of 1:1, 3:1, and 5:1. The growth in these mixtures was compared with growth in undiluted UMADA soil. Nitrogen, phosphorus, and potassium were added prior to planting as mixtures of KNO_3 (100 mg-N/kg-soil), $NH_4(H_2PO_4)$ (25 mg-P/kg-soil), and K_2SO_4 (100 mg-K/kg-soil). The plant nutrients were mixed throughout each pot. In addition, an essential micronutrient mix was added at a rate of 300 mg/pot to ensure micronutrient adequacy. The micronutrient mix contained 4.6% Mg, 4.6% Fe, 3.7% Mn, 4.2% Zn, 1.3% Cu, and 0.5% B and supplied the following amounts of nutrients on the basis of mg-nutrient/kg-soil: 4.7 Mg, 4.7 Fe, 3.7 Mn, 4.0 Zn, 1.3 Cu, 0.5 B, and 12.6 S. Plastic 8-inch pots without drain holes were filled with approximately 3 kg of dry weight of soil/compost mixture. The soil/compost mixture was

Table 3-7

Experimental Design for the Preliminary Plant Uptake Study 2 (for Explosive Analytes)

Treatment	Soil to Compost Dilution Ratio	Plant Species	Plant Parts Analyzed	Replicates	Samples
With Treated Compost	1:1	Radish	Shoots	4	4
		Kale	Shoots	4	4
	3:1	Radish	Shoots	4	4
		Kale	Shoots	4	4
	5:1	Radish	Shoot and Roots	4	8
		Kale	Shoots and Roots	4	8
	Not diluted (Soil Only)	Radish	Shoot and Roots	4	8
		Kale	Shoots	4	4
With Control Compost	1:1	Radish	Shoots	4	4
		Kale	Shoots	4	4
	3:1	Radish	Shoots	4	4
		Kale	Shoots	4	4
	5:1	Radish	Shoot and Roots	4	8
		Kale	Shoots and Roots	4	8
				Total	76

wetted and then ten seeds of radish or kale were planted (on July 8, 1997) per pot and covered with the appropriate dry soil/compost mixture. The practice of placing seeds on top of wetted growth media is done to keep from disturbing the seeds after they are planted. The dry growth media placed on top of the seeds acts as a wick and pulls the moisture upward around the seed. The seeds were allowed to germinate at room temperature.

Germinated pots of radish and kale were placed in a walk-in, 9.29 m^2 environmental growth chamber and kept watered with deionized water to maintain 16.5% moisture. The radish was placed in the growth chamber 4 days after planting while the kale was placed 7 days after planting. Relative humidity was maintained at 80% because this is a desirable level for growing plants and because it could be easily maintained by the humidity control system. Illumination of 500-700 μ Einsteins of light for 14 hours per day was provided by a mixture of incandescent and fluorescent lighting. Temperatures were maintained at 68°F (20°C) during the night and 78°F (25.6°C) during the day. Shoots of both radish and kale grown in all mixtures were analyzed for explosives and explosive by-products listed in Table 3-8. Radish roots (fruit) grown in the 1:5 dilution and in the soil were also analyzed for explosives and explosive by-products, as were Kale roots grown in the 1:5 dilution.

3.2.5.3 Study 3: The Main Plant Uptake Study

The Main Plant Uptake Study (Study 3) was conducted to determine if a broad range of cool and warm season plants would take up explosives or explosive by-products that are bound to the compost of explosive-contaminated soil. The plants were chosen to cover a broad range of plant types such as fruit-bearing crops, root crops, leafy vegetable crops, nodulating plants, monocots, and dicots. A total of seven plant species (eight plant varieties) were used: four cool season species (five varieties) and three warm season species. An outline of the experimental design is provided in Table 3-9. The study was divided into two sub-studies: 1) Study 3A in which cool season species were grown and 2) Study 3B in which warm season species were grown.

Based on the results of Study 2, a soil-to-compost ratio of 5:1 was used during Study 3. The crops were grown in TVA's greenhouse. The cool season species (Study 3A) were grown first.

Table 3-8

Chemical Analysis of Plants for Plant Uptake Study 2

Parameter Measured	Analytical Method[1]
TNT	AP-0062
TNB	AP-0062
RDX	AP-0062
HMX	AP-0062
2,4 DNT	AP-0062
2,6 DNT	AP-0062
2A-DNT	AP-0062
4A-DNT	AP-0062
2,6 DANT	AP-0062
2,4 DANT	AP-0062

(1) See Appendix B for details on methods and procedures.

Table 3-9

Experimental Design for the Main Plant Uptake Study 3 (for Explosive Analytes)

Crop Type	Soil-to-Compost Dilution Ratio	Compost	Plant Species	Plant Parts Analyzed	Replicates	Samples
Cool Season (Study 3A)	5:1	Treated	Chives	Shoots	4	4
			Redtop	Shoots	4	4[1]
			Alfalfa	Shoots and Roots	4	8[1]
			Barley (Parmunky)	Seed	4	4
			Barley (Starling)	Not Analyzed[2]	4	0[2]
		Control	Chives	Shoots	4	4
			Redtop	Shoots	4	4[1]
			Alfalfa	Shoots and Roots	4	8[1]
			Barley (Parmunky)	Seed	4	4
			Barley (Starling)	Not Analyzed[2]	4	0[2]
Warm Season (Study 3B)	5:1	Treated	Sorghum	Seed	4	4
			Bush snapbeans	Bean pods	4	4
			Tomato	Fruit	4	4
		Control	Sorghum	Seed	4	4
			Bush snapbeans	Bean pods	4	4
			Tomato	Fruit	4	4
					Total	64

(1) Shoot tissues from multiple harvests were combined for analyses.

(2) Crop yields were obtained, but the plant parts were not analyzed for explosives or explosive by-product content.

These plant species included:

- Chives (*Allium schoenoprasum* variety Cebolletas), Redtop (*Agrostis alba*),
- Alfalfa (*Medicago sativa* variety Vernal), and
- Two winter barley species (*Hordeum vulgare* variety Parmunky and variety Starling).

Two varieties of barley were grown to ensure flower set on at least one of the plants. The cool season crops were planted on November 14, 1997. The composts were approximately 18 months old when the cool season crops were planted.

The Study 3B (warm season) crops were planted on February 17-18, 1998, when the composts were approximately 21 months old. The warmer season plant species included:

- Sorghum (*Sorghum bicolor* variety FFR 321 DR),
- Bush snapbeans (*Phaseolus vulgaris* variety Habichulas), and
- Tomato (*Lycopersicion esculentum* variety Patio).

In both Study 3A and 3B, batch loads of dry soil and compost (treated or control) were mixed in bulk using a cement mixer equipped with a plastic tub. Each batch weighed 210 kg. A fertilizer, Nursery Specialty (12-6-6) with micronutrients, was added to each pot at a rate of 7 g/6 kg of mix per pot. This rate of fertilizer supplied N at 140 mg/kg, P at 31 mg/kg, and K at 39 mg/kg. Plastic pots without holes were filled with 6 kg of dry mix and brought to 16% moisture before planting. The potted mix used in growing warm season crops (3B) was stored dry until used. The dry compost mixtures were analyzed for explosives, explosive by-products, and moisture before and after the plant growth (Table 3-10).

Seeding rates were as follows: chives, 250 mg of seeds per pot; redtop, 70 mg of seeds per pot; alfalfa, 1.3 g of seeds per pot; and barley, 16 seeds per pot. Alfalfa root inoculate was added to the soil surface of each pot at a rate of 5 cc/pot. After plant emergence (4-7 days), pots were moved to the greenhouse and temperature controls were adjusted to give 65-68°F at night and 80-90°F daytime. Barley was thinned to 8 plants per pot.

Table 3-10

Chemical Analysis of the Soil/Compost Mixture During Plant Uptake Study 3

Analyte	Preparation Method[1]	Analytical Method[1]
Explosives and Explosive By-Products		
TNT	AP-0062	AP-0062
TNB	AP-0062	AP-0062
HMX	AP-0062	AP-0062
RDX	AP-0062	AP-0062
2A-DNT	AP-0062	AP-0062
4A-DNT	AP-0062	AP-0062
2,4-DANT	AP-0062	AP-0062
2,6-DANT	AP-0062	AP-0062
2,4-DNT	AP-0062	AP-0062
2,6-DNT	AP-0062	AP-0062
Moisture (%)	ASTM 871	ASTM 871

(1) See Appendix B for details on methods and procedures.

Supplemental light was supplied during cloudy days during the natural photoperiod. After 6 weeks of growth, the photoperiod was increased to 16 hours to induce the barley to flower. After 8 weeks, the barley was put into an unheated greenhouse to vernalize the plants to induce flowering. Evidence of flowering was noted on March 11, 1998 (116 days after planting), and seeds were harvested 188 days after planting.

Two clippings of alfalfa were combined for yield and analyses and two clippings of redtop were combined for yield and chemical analyses. The clippings were double rinsed to remove dust and debris. The two clippings were made 53 and 116 days after planting. One clipping of chives was taken 116 days after planting for yield and chemical analysis. Roots were washed free of the soil and compost mixture and weighed.

Tomatoes were seeded onto a commercial potting mix (Promix) on January 6, 1998, and transplanted to the test compost mixtures 42 days after planting. Sorghum and snapbeans were seeded into the compost treatments on February 18, 1998. Three tomato harvests and two bean harvests were pooled for total yield and tissue analyses for explosive analytes. Bean pods, tomato fruit, alfalfa shoots, sorghum seed, redtop shoots, barley seed, and chive shoots were analyzed for explosives and explosive by-products. The only plant in the main plant uptake study for which root tissue was analyzed was alfalfa and this was done because alfalfa roots could be ingested by livestock-grazing alfalfa (Table 3-9).

After harvesting, the plant parts were analyzed for explosives and explosive by-products in accordance with Table 3-11.

3.3 Sampling Procedures

3.3.1 Overview of Sampling Operations

Sampling during this study was done for the following purposes:

- Determine maturity of compost by sampling compost every two weeks after an initial 30-day composting period.

Table 3-11

Chemical Analysis of the Plant Parts During Plant Uptake Study 3

Analyte	Preparation Method[1]	Analytical Method[1]
Explosives and Explosive By-Products		
TNT	AP-0062	AP-0062
TNB	AP-0062	AP-0062
HMX	AP-0062	AP-0062
RDX	AP-0062	AP-0062
2A-DNT	AP-0062	AP-0062
4A-DNT	AP-0062	AP-0062
2,4-DANT	AP-0062	AP-0062
2,6-DANT	AP-0062	AP-0062
2,4-DNT	AP-0062	AP-0062
2,6-DNT	AP-0062	AP-0062

(1) See Appendix B for details on methods and procedures.

- Determine if the chemistry and structure of the compost changed over time by taking samples annually of the compost exposed to the elements in the Microbial Weathering Study.

- Determine if plants grown in the compost of explosive-contaminated soil will take up explosives or explosive by-products.

Optimal plant and compost sample preparation techniques were developed in concert with analytical methods development. Studies determined the amount of sample necessary for quantitative analysis of explosives and explosive by-products.

3.3.2 Sample Collection and Laboratory Procedures

3.3.2.1 Sampling Procedures for Compost Production and Maturity Testing

During compost production, samples of the treated compost were taken shortly after the treated compost arrived at TVA using the sampling procedure outlined below for the maturity testing. The treated compost sample was then analyzed for the parameters outlined in Table 3-4.

For the maturity tests, samples of control and treated compost were taken initially and at two-week intervals after 30 days of composting. During the sampling periods, three replications were collected from each compost pile. Samples were generally taken during compost turning. Scoops of compost from throughout the compost pile were composited in 18.9-L (5-gallon) buckets. This procedure was repeated three times each for the control and treated compost. The contents of the buckets were thoroughly mixed and sufficient samples for further testing were sealed in labeled plastic bags. If appropriate for the particular analytical test, samples were frozen or stored at 4°C prior to analysis. Compost remaining in the buckets after sample collection was returned to the compost piles. All samples were analyzed for the parameters outlined in Table 3-5.

3.3.2.2 Plant Uptake Sampling Procedures for Studies 2 and 3

Plants were sampled at maturity as determined by the production of edible fruits, roots, or foliage. Snap beans, tomatoes, and radishes were harvested when they were of marketable size. Kale was also harvested when it was of marketable size. Redtop and alfalfa were clipped when the foliage was well established and the grain crops, barley and sorghum, were harvested after seeds were developed. During Study 3, compost samples were also obtained. At maturity, the plants were removed from the pots onto brown paper and the plants were separated from the compost. Surfactant (Tween 20-polysorbitol) was added to water used for washing soil and compost from plants. The wash water was also analyzed by liquid chromatography to verify that no interferences would be caused by the surfactant or residues of fungicide and insecticide used to treat some of the plant parts.

During Study 3, the soil/compost mixtures in each pot were sampled with a 25-mm core stainless steel soil sampler. Three cores, the full depth of the pot, were taken from each pot and were composited for analysis. The cores were taken from areas in the pot which contained the least amount of roots. For some plants, root tissue invariably was included in the growth media samples. Representative samples were placed in a foil-wrapped, amber glass bottle and stored in the freezer.

During both Studies 2 and 3, the plant tissue was separated into fruit, leaves, stems, and roots (where applicable) for further analysis. Plant parts were stored in a plastic Ziploc™ bag or brown paper bag in the freezer until analyzed. Each bag was labeled with the project, date, sample number, plant species, and sample plant part. All bags were placed into a large plastic bag or enclosed cardboard box. All bags were transported frozen into custody of the TVA's analytical laboratory. Analyses conducted on the plants during Study 2 are outlined in Table 3-8. The type of analyses conducted during Study 3 on the soil/compost mixtures and plant parts are outlined in Tables 3-10 and 3-11, respectively.

All samples were handled in accordance with TVA's chain of custody procedures (Appendix B-1).

3.3.3 Laboratory Procedures

Standard analytical procedures for data collected in the laboratory, including those for determining the explosive content of sediment and plant samples, are provided in Appendices B-2 through B-16.

3.3.4 Sample Storage, Packaging, and Shipping

All samples received from the experimental operations were handled in accordance with analytical laboratory procedure SP-0001, "Sample Chain of Custody" (Appendix B-1).

After sampling, samples were refrigerated or stored on ice or a commercial substitute until delivery to the analytical laboratory. Samples were delivered to the analytical laboratory on ice or a commercial substitute. Samples were refrigerated upon receipt.

No attempt was made to store samples or sample extracts beyond that period of time required for initial assessment and review of laboratory data.

3.3.5 Laboratory Analytical Equipment

The equipment used for laboratory analysis is outlined in Table 3-12. Explosives and explosive by-product content were determined in plant tissues and compost collected from the tests via high performance liquid chromatography. Total Kjeldahl Nitrogen (TKN), NH_4-N, Total P, and NO_3-N were determined colorimetrically via an automatic analyzer. The pH was measured with a glass electrode and pH meter. Conductivity was measured with a conductivity meter and cell. Total carbon was measured with a combustion analyzer. Metals were measured with Varian Cold Vapor Atomic Absorption (AA), Varian Graphite Furnace AA, or Thermo Jarrel Ash Inductively Coupled Plasma (ICP).

3.4 Quality Assurance

Details of the Quality Assurance Program used during this project are provided in Appendix A.

Table 3-12

Equipment Used for Data Collection

Laboratory Data	Equipment
Explosives and Related By-Products	Varian HPLC
TKN, NH_4, NO_3, and Phosphorus	Lachat Quick Chem 8000 or Technicon AutoAnalyzer II
Total Organic Carbon	Dohrmann DC 190
pH	Orion Meter
Electrical Conductivity	Orion
Metals	Varian Cold Vapor AA, Varian Graphite Furnace AA, and Perkin Elmer or Thermo Jarrel Ash ICP

3.5 Advantages and Limitations of the Experimental Design

There were three advantages to this experimental design:

1. The media used for the plant uptake study was taken directly from a full-scale composting of explosives-contaminated soil.

2. A variety of plant species were used that included fruit-bearing vegetable crops, grasses, root crops, legumes, and forage crops.

3. The maturation of compost and its suitability for growing crops were studied using compost from the soil remediation activity and a control compost made with similar organic amendments and uncontaminated soil.

The experimental design had two limitations:

1. The UMADA soil used for plant uptake studies is a mineral, desert soil with a high ash (silica) content, low cation exchange capacity, low buffering capacity, and slightly basic pH (7.2). This is typical for soils in the UMADA area, but is not representative of soils at many installations. Since the Umatilla soil is low in organic matter and high in silica, it would generally be improved by the addition of compost. For soil enhancement purposes, compost application rates must be tailored to the soil and crop on which the compost is used.

2. The treated compost and amendments for the control compost were transported by truck to TVA's ERC and were not aerated during transit. This caused oxygen levels in these materials to become depleted which may have altered their biological activity. Also, despite following the same volumetric recipe for compost production, the actual composition of the control compost may have been slightly different than that of the treated compost due to variations in bulk density of ingredients.

SECTION 4.0
STUDY RESULTS

4.1 Initial Composition of Treated Compost

After the treated compost arrived at TVA's facility, a pile was constructed and three core samples were taken and analyzed for explosives, explosive by-products, nutrients, moisture, and metals. Of the explosives and explosive by-products, TNT, TNB, RDX, and 2A-DNT were detected in the compost (Table 4-1). TNT was found in all three samples, TNB was found in two samples, and RDX and 2A-DNT were found in three samples. Of the metals, arsenic, barium, cadmium, and chromium were found, but not at levels high enough to warrant leachate analyses.

4.3 Compost Monitoring

4.3.1 Temperature Monitoring

Temperature data for the treated and control composts are shown in Figures 4-1 to 4-5. Temperatures were measured daily except on weekends. Temperature profiles for the control (Figure 4-1) and treated (Figure 4-2) composts at the three thermocouple depths show that for both composts, the highest temperatures were recorded at the 30-cm depth during the early part of the study. As composting proceeded, there was less variation in temperature related to thermocouple depth. Because of its younger age, the control compost was warmer when temperature monitoring began and had a wider range in temperature within the pile than did the treated compost. The treated compost never achieved a temperature greater than 60°C while the control compost showed several days of temperatures above 60°C at the 30-cm depth (Figure 4-3).

Comparisons between the two compost piles at the three temperature measurement depths (Figures 4-3, 4-4, 4-5) further illustrate the control compost's lag in temperature decline during the majority of the test period and the similarities in temperature profiles during the last three months of measurement. These figures also show a stair-step temperature pattern at the 91-cm

Table 4-1

Initial Analysis of Treated Compost

Analyte	Sample 1	Sample 2	Sample 3	Average
Explosives				
HMX (mg/kg)	ND[1] (0.0589)	ND (0.0608)	ND (0.0593)	ND
RDX (mg/kg)	0.0704	ND (0.0041)	ND (0.0040)	0.0248[2]
TNT (mg/kg)	0.0442	0.0500	0.0974	0.064
TNB (mg/kg)	0.1820	ND (0.0485)	0.5520	0.252[2]
2,6-DANT (mg/kg)	ND (0.0272)	ND (0.0281)	ND (0.0274)	ND
2,4-DANT (mg/kg)	ND (0.00495)	ND (0.00511)	ND (0.00498)	ND
2,6-DNT (mg/kg)	ND (0.0243)	ND (0.0250)	ND (0.0244)	ND
2,4-DNT (mg/kg)	ND (0.0114)	ND (0.0118)	ND (0.0115)	ND
2A-DNT (mg/kg)	ND (0.0104)	ND (0.0107)	0.0245	0.012[2]
4A-DNT (mg/kg)	ND (0.0193)	ND (0.0199)	ND (0.0194)	ND
Nutrients and Moisture				
Moisture (%)	21.9			
Phosphorus (%)	0.31			
Potassium (%)	0.78			
Total Nitrogen (%)	0.55			
Metals				
Arsenic (ug/kg)	981	1,140	1,330	1,150
Barium (mg/kg)	59	57.1	57.7	57.9
Cadmium (mg/kg)	1.2	1.39	1.22	1.27
Chromium (mg/kg)	5.09	5.52	6.08	5.56
Lead (mg/kg)	ND (1.5)	ND (1.5)	ND (1.5)	ND
Mercury (ug/kg)	ND (115)	ND (115)	ND (115)	ND
Selenium (ug/kg)	ND (600)	ND (600)	ND (600)	ND
Silver (mg/kg)	ND (0.1)	ND (0.1)	ND (0.1)	ND

(1) ND = Not detected above the detection limit shown in parentheses.
(2) 0.5 times the detection limit used for ND when averaging.

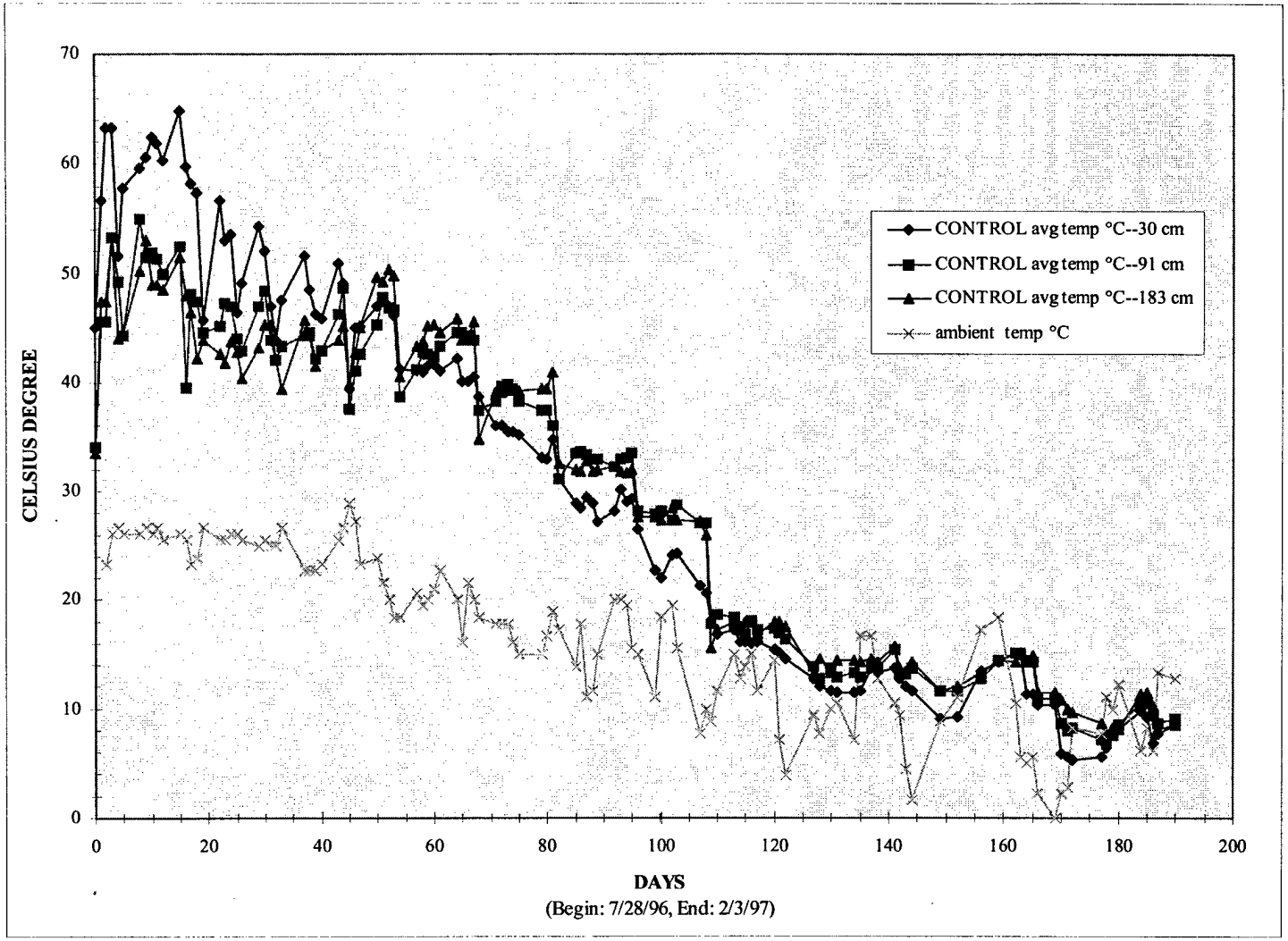


Figure 4-1

Control Compost Temperature Profile

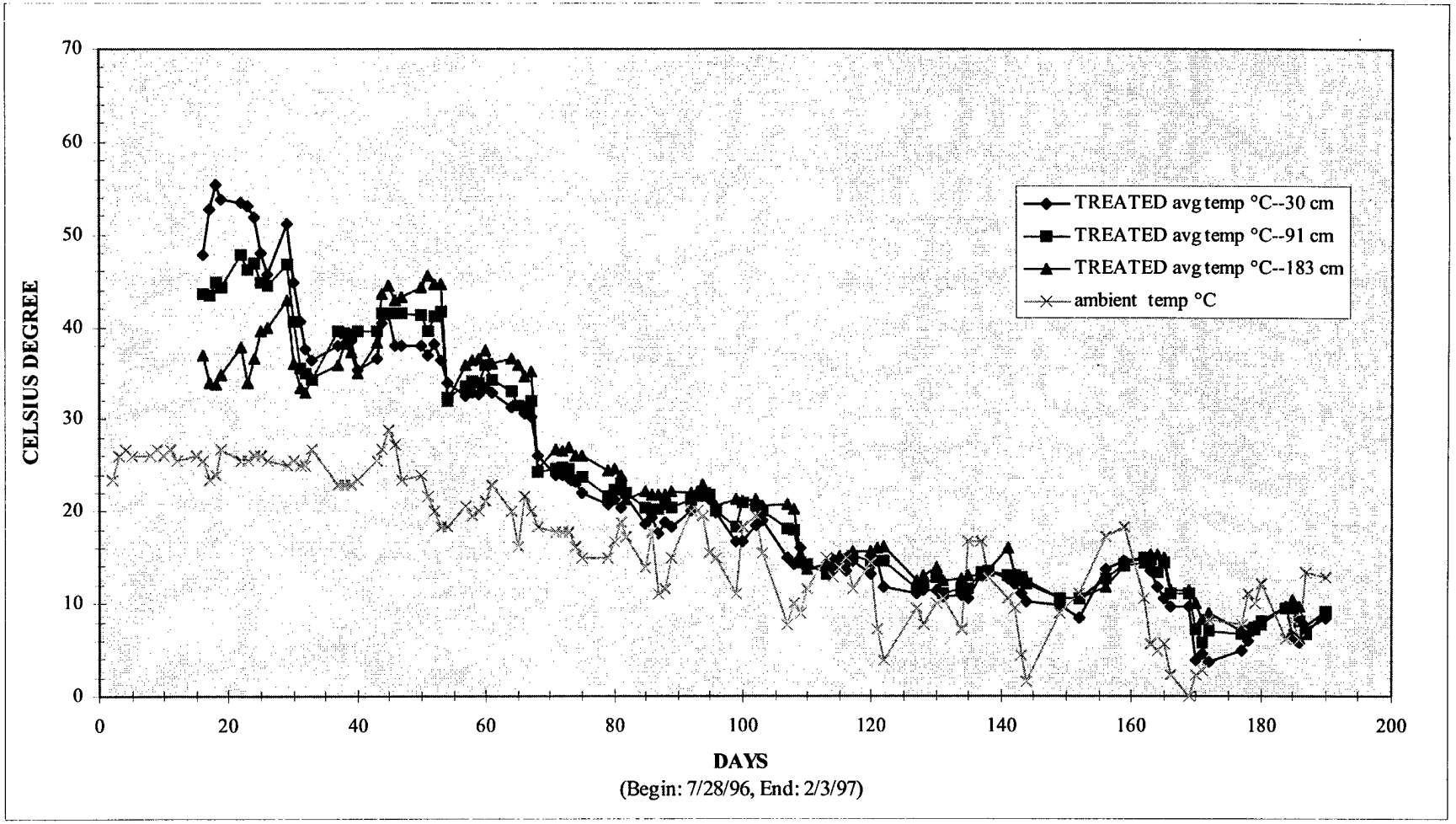


Figure 4-2

Treated Compost Temperature Profile

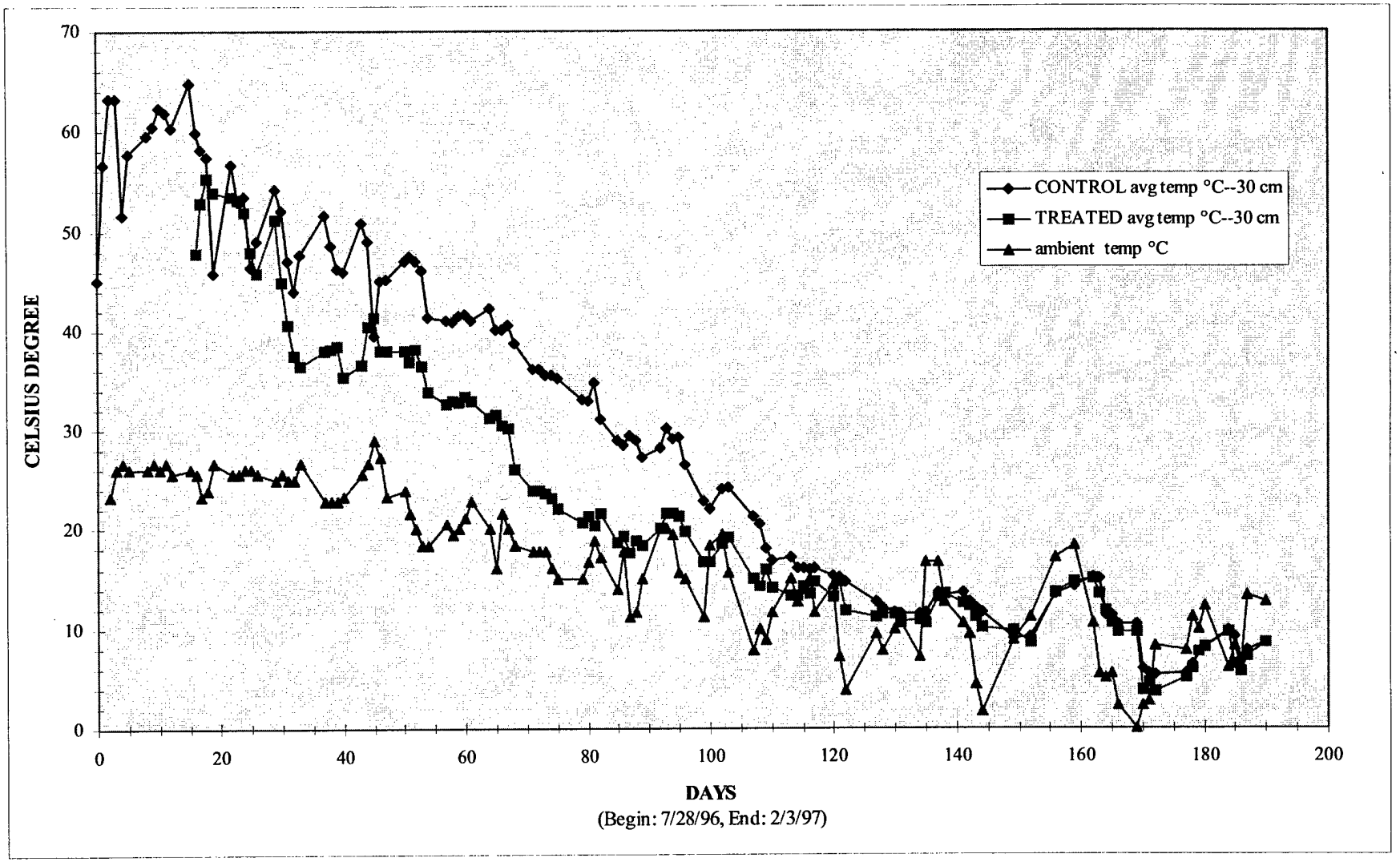


Figure 4-3

Temperature Profile - 30 cm

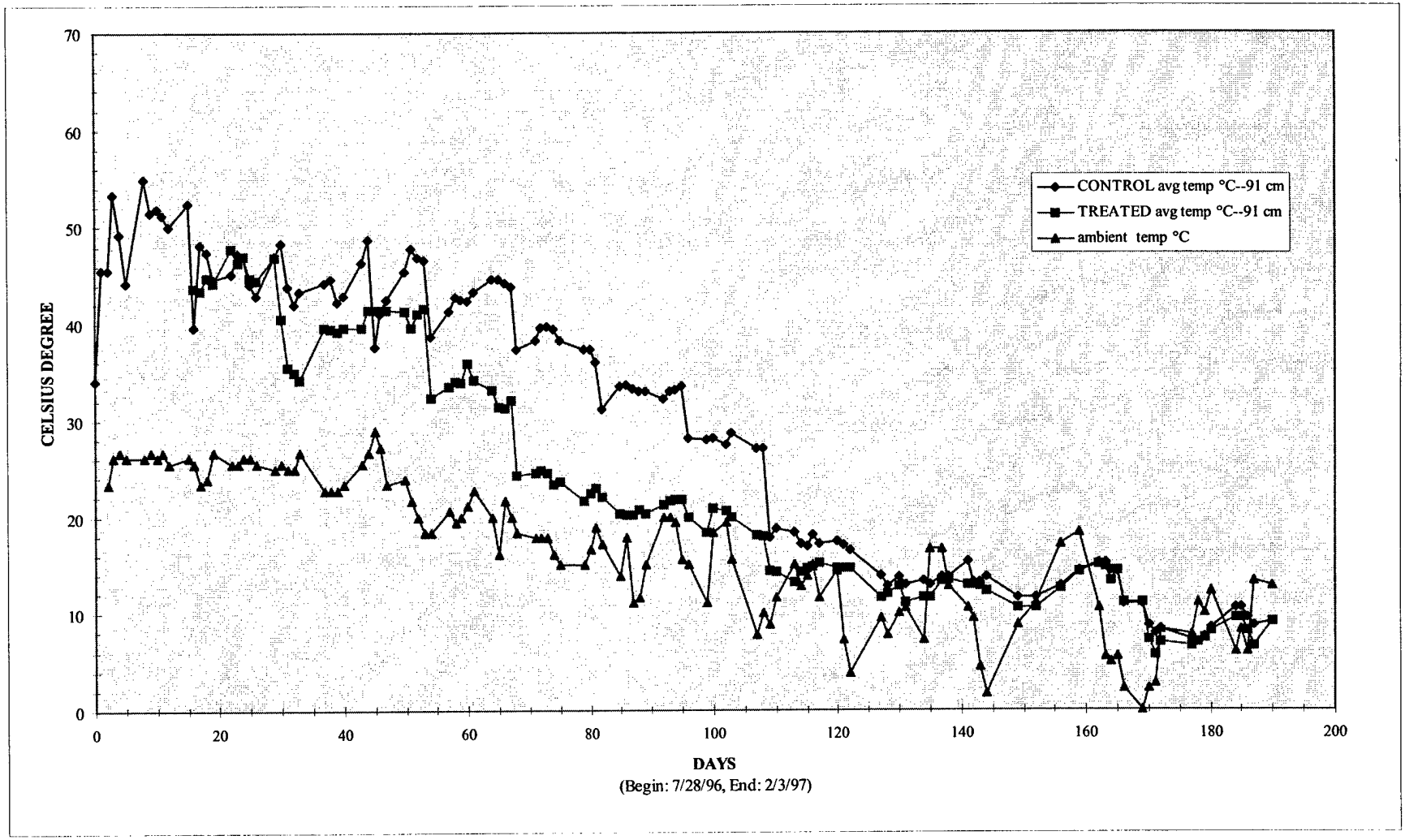


Figure 4-4

Temperature Profile - 91 cm

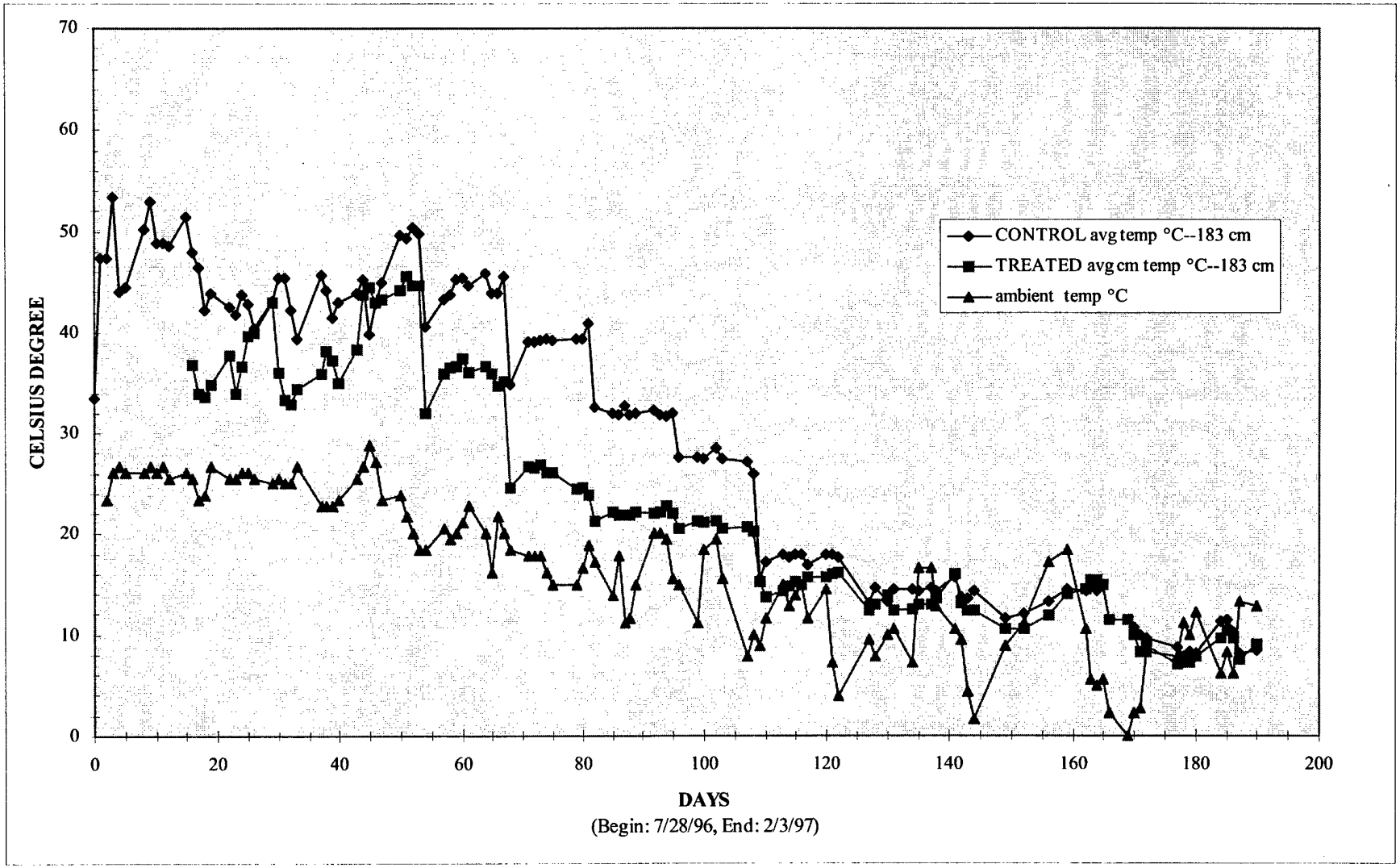


Figure 4-5

Temperature Profile - 183 cm

and 183-cm depths. This pattern is the result of turning and adding moisture to the compost piles. The temperature at the 30-cm level appeared to equilibrate quickly and continue to decline steadily after mixing. However, temperatures at 91 cm and 183 cm tended to be more stable between turnings and show a greater change in temperature after mixing. The stability of the temperature readings at the two deeper depths is related to the insulating capacity of the piles. The stair-step pattern is associated with heat loss during turning, the cooling effect of water addition, and declining microbial activity during this part of the period.

4.3.2 Oxygen Measurements

During the early stages of the composting process, both compost piles consumed oxygen at a rate which required frequent turning (Figure 4-6). Efforts were made to maintain the oxygen concentration above 5% to minimize the creation of anaerobic conditions and promote optimum aerobic microbial activity. After about a month for the control compost and two weeks for the treated compost (because it had already experienced a period of active composting at UMADA), oxygen demand declined and less frequent turning was required to maintain aerobic conditions. Until the compost temperatures moderated, the control compost had lower oxygen levels than the treated compost, indicating that it consumed more oxygen than the treated compost. As both piles cooled to 30°C, oxygen levels approached atmospheric levels indicating that either residual oxygen or passive oxygen movement was sufficient to satisfy the oxygen demand during compost curing.

4.3.3 Chemical Analysis of Compost

The summary of analytical results for the control and treated composts (three samples for each compost for each sampling time) is shown in Tables 4-2 and 4-3. Sample heterogeneity and perhaps leaching effects from moisture additions contributed to variability in the data from both the control and treated compost. This variability made it difficult to make meaningful

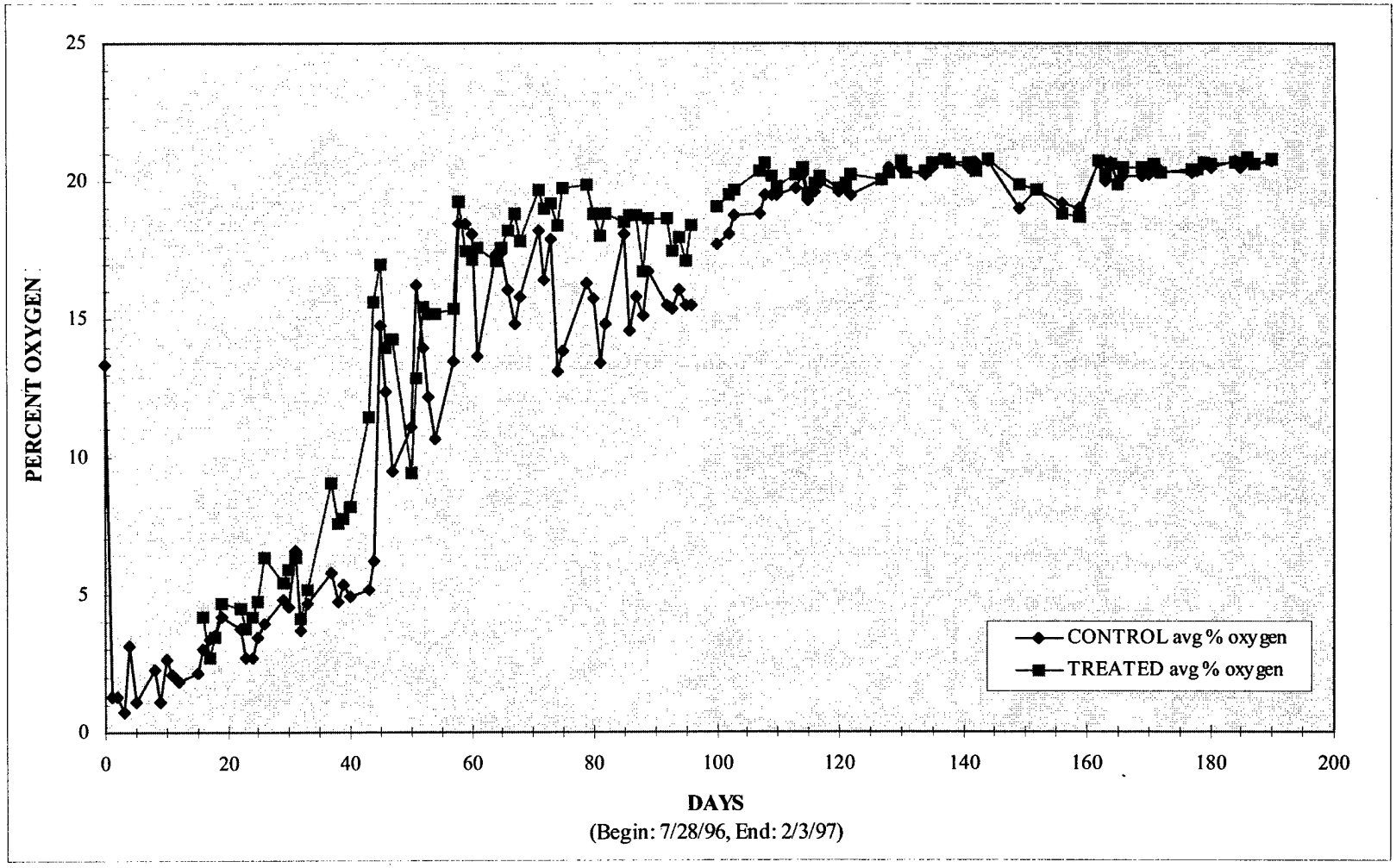


Figure 4-6

Compost Pile Oxygen Levels

Table 4-2

Chemical Analysis of Control Compost

CONTROL Averages									
DATE	**Total Nitrogen wt%**	**Ammonium Nitrogen, wt%**	**Nitrate Nitrogen, wt%**	**Total Organic Carbon, wt%**	**Ash, wt%**	**Moisture, wt%**	**pH**	**Electrical Conductivity, µsiemens/cm**	**C:N Wt Ratio**
8/12/96									
8/22/96	0.349	0.0573	0.0001	6.80	85.8	16.8	9.34	10,500	19.5
9/5/96	0.413	0.0374	0.0003	6.44	86.8	13.0	9.33		15.6
9/19/96	0.420	0.0153	0.0153	6.03	87.6	13.3	8.87	4,870	14.4
10/3/97	0.330	0.0034	0.0181	5.97	87.6	12.9	8.66	3,730	18.1
10/17/96	0.343	0.0009	0.0218	6.31	87.3	20.3	8.49	7,300	18.4
10/31/96	0.413	0.0005	0.0277	6.33	86.9	18.6	8.62	4,120	15.3
11/14/96	0.483	0.0004	0.0299	6.40	87.0	21.4	8.81	5,120	13.2
11/27/96	0.417	0.0002	0.0119	6.93	85.9	22.7	8.45	6,640	16.6
12/19/96	0.400	0.0002	0.0359	6.42	87.6	18.2	8.37	5,640	16.0
1/9/97	0.278	0.0004	0.0387	5.86	87.9	20.3	8.05	4,860	21.2
1/30/97	0.350	0.0005	0.0422	5.50	88.8	19.6	8.30	6,670	15.7

Table 4-3

Chemical Analysis of Treated Compost

TREATED Averages									
DATE	**Total Nitrogen, wt%**	**Ammonium Nitrogen, wt%**	**Nitrate Nitrogen, wt%**	**Total Organic Carbon, wt%**	**Ash, wt%**	**Moisture, wt%**	**pH**	**Electrical Conductivity, µsiemens/cm**	**C:N Wt Ratio**
8/12/96	0.407			5.49		10.5	8.64		13.8
8/22/96	0.239	0.0495	0.0002	4.51	90.4	7.08	9.11	10,370	18.8
9/5/96	0.253	0.0006	0.0268	4.12	90.4	8.27	9.10		16.3
9/19/96	0.277	0.0024	0.0143	3.85	91.5	9.14	8.10	5,800	14.0
10/3/97	0.153	0.0005	0.0137	4.03	91.1	10.0	8.06	2,550	26.3
10/17/96	0.150	0.0004	0.0162	3.95	91.8	15.9	7.88	4,660	26.4
10/31/96	0.210	0.0004	0.0192	4.14	90.7	13.4	8.01	3,200	19.7
11/14/96	0.280	0.0007	0.0218	4.12	91.4	13.4	8.07	3,250	14.8
11/27/96	0.160	0.0002	0.0201	4.23	91.1	14.8	7.92	3,850	26.6
12/19/96	0.183	0.0002	0.0233	4.00	91.7	13.7	7.83	4,380	21.8
1/9/97	0.090	0.0006	0.0261	3.92	91.6	15.8	7.60	3,390	44.7
1/30/97	0.197	0.0005	0.0270	3.90	91.7	14.1	7.67	3,890	19.9

comparisons between composts at different sample times. Significant aspects of the data for each analysis are:

- Total-N—Total nitrogen in the treated compost was consistently lower than in the control compost except for the first sample collected after constructing the treated pile. The variability in total nitrogen among the different sample times reduced the reliability of the carbon-to-nitrogen calculations as a measure of compost maturity.

- Ammonium-N—Ammonium nitrogen levels for both composts were low compared to the amount of total nitrogen. The decline in ammonium nitrogen over time was partially attributable to the moderation of compost temperatures which allowed the establishment of nitrifying bacteria and their subsequent conversion of ammonium to nitrate.

- Nitrate-N—Nitrate nitrogen levels reflect the comparatively low compost temperatures. Nitrate-N first increased significantly in the treated compost. This was attributable to the older age of the treated compost and the fact that its temperature decreased sooner than that of the control compost. Because of the higher total nitrogen levels in the control compost (Tables 4-2 and 4-3), it would be expected that its nitrate nitrogen levels would be higher than that of the treated compost.

- Total organic carbon—Total organic carbon was consistently higher in the control compost than in the treated compost (Tables 4-2 and 4-3). However, both composts showed a general decline in total organic carbon over the study period due to loss of respiratory carbon dioxide. There is no indication that the age difference between the two composts attributed to the differences in total organic carbon concentration. It is more likely that the disparity in total organic carbon content for the two composts was a result of differences in mixing. Although both composts contained approximately 70% amendment on a volume basis, there is a possibility that more organic matter was added to the control compost than would have been the case had the amendments been added on a weight basis.

- Ash—The control compost had lower ash content than the treated compost due to its higher organic matter content. Both composts had much higher ash levels than are

typically found in composts produced from manures, biosolids, yard wastes, and food processing wastes. The high ash content of the treated and control composts could have influenced the maturity test results. Both composts used in the study were more similar to "organically enriched" soils than many other composted materials.

- Moisture—The control compost consistently held a higher moisture content than the treated compost. This difference was due to its higher organic content. Variation in moisture content during different sampling periods could be the result of non-uniform moisture distribution in the piles. Because of the sandy nature of the UMADA soil and the high ash content of the two composts, their moisture-holding capacity was less than typical composts having higher levels of organic matter.

- pH—Both composts had alkaline pH's which tended to decrease over time. At the end of the study period, the treated compost was less alkaline than the control compost, an attribute which could influence seed germination and plant growth. The high pH of both composts may have influenced nitrogen loss during composting and curing through ammonia volatilization.[Ref. 8,9]

- Electrical conductivity—The soluble salt content of both composts, as measured by electrical conductivity, was highest during the first sampling period. This was perhaps due to salts or organic acids which were later either incorporated into microbial biomass or bound to soil or organic matter. Variation in the electrical conductivity data could be associated with leaching during water addition. The electrical conductivity for both composts was high enough to influence seed germination and plant growth through salt effects (Tables 4-2 and 4-3). Generally, an electrical conductivity reading of less than 2,500 microsiemens/cm is desired to minimize detrimental plant effects.[Ref. 9] Because of these effects, electrical conductivity is considered a major indicator of the suitability of compost for different uses (e.g., potting media or soil amendment). Assuming that compost materials are mature, electrical conductivity is a valuable indicator for the need to either leach or dilute compost prior to use. To leach refers to the passing of water through the compost to remove salts and other soluble compounds that may inhibit seed germination.

4.3.4 Maturity Testing

To evaluate the suitability of the control and treated composts for use in the plant uptake studies, it was considered prudent to evaluate compost maturity. Four methods were used to assess maturity: compost odor, carbon-to-nitrogen ratio, the relationship between ammonium and nitrate nitrogen, and the liberation of respiratory carbon dioxide. The results of the maturity tests are as follows.

4.3.4.1 Odor

Results from the compost odor testing are summarized in Table 4-4. Although the evaluation was subjective, there was generally excellent agreement among the four evaluators. However, one of the evaluators tended to detect more odor components and the intensity of the odor than the other three. Acceptability was based on the use of the compost for general home and garden use where objectionable odors would affect use.

During the first two sampling periods, both the control and treated composts had a greater mixture of odors than during the remaining test periods. Ammonia odor was detected only during the first sampling period. Odors described as foul, musty, and manure-like were mixed with an earthy description for the first two sampling periods for the control compost. For the treated compost, a musty, earthy combination was used to describe the treated compost by several evaluators. The ammonia and foul odors detected in the control compost were likely related to its younger age. A factor in odor production for the control compost was the initial large particle size of the potato waste, straw, and alfalfa used in the amendment mixture. These materials would tend to create pockets of readily degradable substrates which could encourage odor production. If treated compost samples had been available for evaluation from the actual start of treated compost production, the initial odor descriptions for the treated compost would likely have been different. By the third sampling period, the majority of the evaluations showed both composts to have a predominantly earthy odor.

The consensus for odor intensity ranged from slight to very slight throughout the entire evaluation period. Odor intensity decreased over time with the control compost having slightly

Table 4-4

Odor Testing Summary[1,2]

			Description		Intensity		Acceptability	
Date	Day[3]	Sample	Control	Treated	Control	Treated	Control	Treated
8/30/96	33	1	- earthy	- earthy	slight	slight	acceptable	acceptable
		2	- earthy	earthy	slight	slight	acceptable	acceptable
		3	- earthy	- earthy	slight	slight	acceptable	acceptable
9/13/96	47	1	- earthy	earthy	slight	slight	acceptable	acceptable
		2	- earthy	earthy	slight	slight	acceptable	acceptable
		3	earthy	mixed	slight	- distinct	acceptable	acceptable
9/27/96	61	1	- earthy	earthy	slight	slight	acceptable	acceptable
		2	earthy	earthy	slight	slight	acceptable	acceptable
		3	earthy	earthy	slight	slight	acceptable	acceptable
10/11/96	75	1	earthy	earthy	- slight	very slight	acceptable	acceptable
		2	earthy	earthy	- slight	very slight	acceptable	acceptable
		3	earthy	earthy	- slight	very slight	acceptable	acceptable
10/25/96	89	1	earthy	earthy	- slight	- slight	acceptable	acceptable
		2	earthy	earthy	very slight	very slight	acceptable	acceptable
		3	earthy	earthy	- slight	- slight	acceptable	acceptable
11/8/96	103	1	earthy	earthy	- slight	- slight	acceptable	acceptable
		2	earthy	earthy	- slight	very slight	acceptable	acceptable
		3	earthy	earthy	- slight	- slight	acceptable	acceptable
11/22/96	117	1	earthy	earthy	- slight	- slight	acceptable	acceptable
		2	earthy	earthy	very slight	- slight	acceptable	acceptable
		3	earthy	earthy	very slight	- slight	acceptable	acceptable
12/5/96	130	1	earthy	earthy	very slight	- slight	acceptable	acceptable
		2	earthy	earthy	very slight	very slight	acceptable	acceptable
		3	earthy	earthy	very slight	very slight	acceptable	acceptable
12/27/96	152	1	earthy	earthy	very slight	- slight	acceptable	acceptable
		2	earthy	earthy	very slight	very slight	acceptable	acceptable
		3	earthy	earthy	very slight	- slight	acceptable	acceptable
1/17/97	173	1	earthy	earthy	very slight	- slight	acceptable	acceptable
		2	earthy	earthy	very slight	- slight	acceptable	acceptable
		3	earthy	earthy	very slight	- slight	acceptable	acceptable
2/7/97	194	1	earthy	earthy	very slight	- slight	acceptable	acceptable
		2	earthy	earthy	very slight	- slight	acceptable	acceptable
		3	earthy	earthy	very slight	very slight	acceptable	acceptable

(1) Consensus is based on four independent observations.
(2) A minus sign in front of an evaluation indicates the value is less than the recorded evaluation.
(3) Day refers to the number of days since monitoring began, July 28, 1996.

less odor intensity than the treated compost. Individual evaluators judged odor intensity on the same sample from "none" to "distinct," with the control compost having more distinct odor ratings during the first month of composting. There were only two "distinct" ratings for the treated compost. Both ratings were given to a sample collected during the second sampling period. Odor intensity was not a strong characteristic of either compost even before active composting was completed. Similarly, there were only two "unacceptable" ratings during the entire study period. One unacceptable rating was for the control compost and the other was for the treated compost, and both ratings resulted from "foul" odors detected on samples collected early in the test. Surprisingly, the other two evaluators found these samples very objectionable. The faint odor of both composts coupled with a predominately "earthy" odor were the main factors leading to overall acceptability of both composts based on odor characteristics.

4.3.4.2 Carbon-to-Nitrogen Ratio

The carbon-to-nitrogen ratio for the control compost ranged from approximately 15:1 to 20:1 throughout the testing period (Figure 4-7). The treated compost was much more variable and ranged from less than 15:1 to about 45:1 during the next to the last sampling period. The variability in the treated compost data is difficult to explain because the treated compost had been exposed to greater particle size reduction than the control compost from the equipment used for compost mixing and turning at UMADA. The 45:1 carbon-to-nitrogen ratio is considered an anomaly in the data primarily due to lower total nitrogen values obtained during the sampling period. Regardless, the carbon-to-nitrogen ratio was not an effective indicator of maturity for the composts used in this study. The treated compost showed a wider range of carbon-to-nitrogen ratios during the measurement period than the control compost.

4.3.4.3 Ammonium-to-Nitrate Relationships

Ammonium nitrogen levels were higher in the control compost than in the treated compost in the first four sampling periods (Figure 4-8). This is a reflection of both the greater age of the treated compost and its lower total nitrogen content. Throughout the entire test period, the amounts of ammonium and nitrate nitrogen were relatively small (less than 15%) compared to

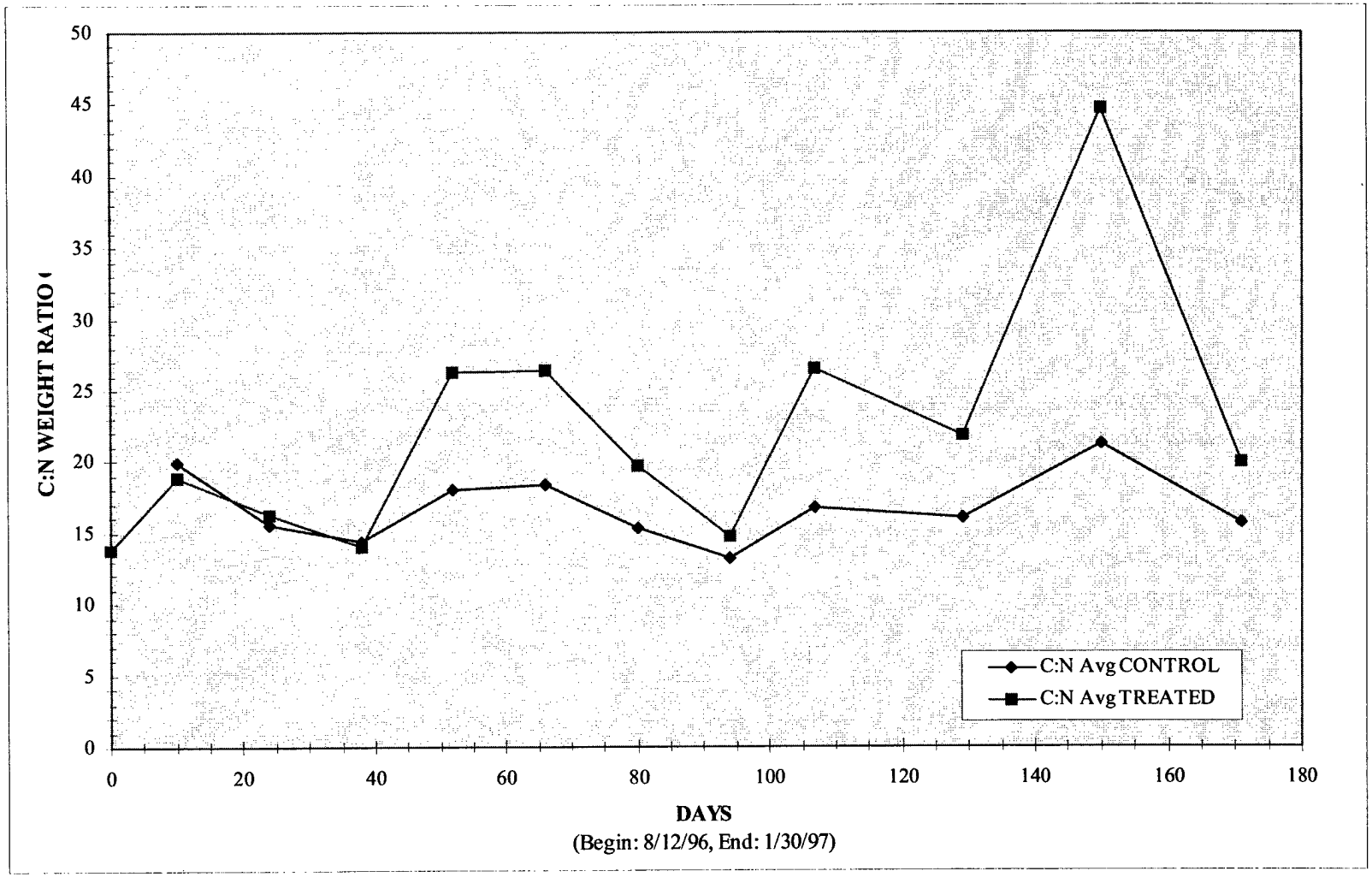


Figure 4-7

Carbon-to-Nitrogen Ratio

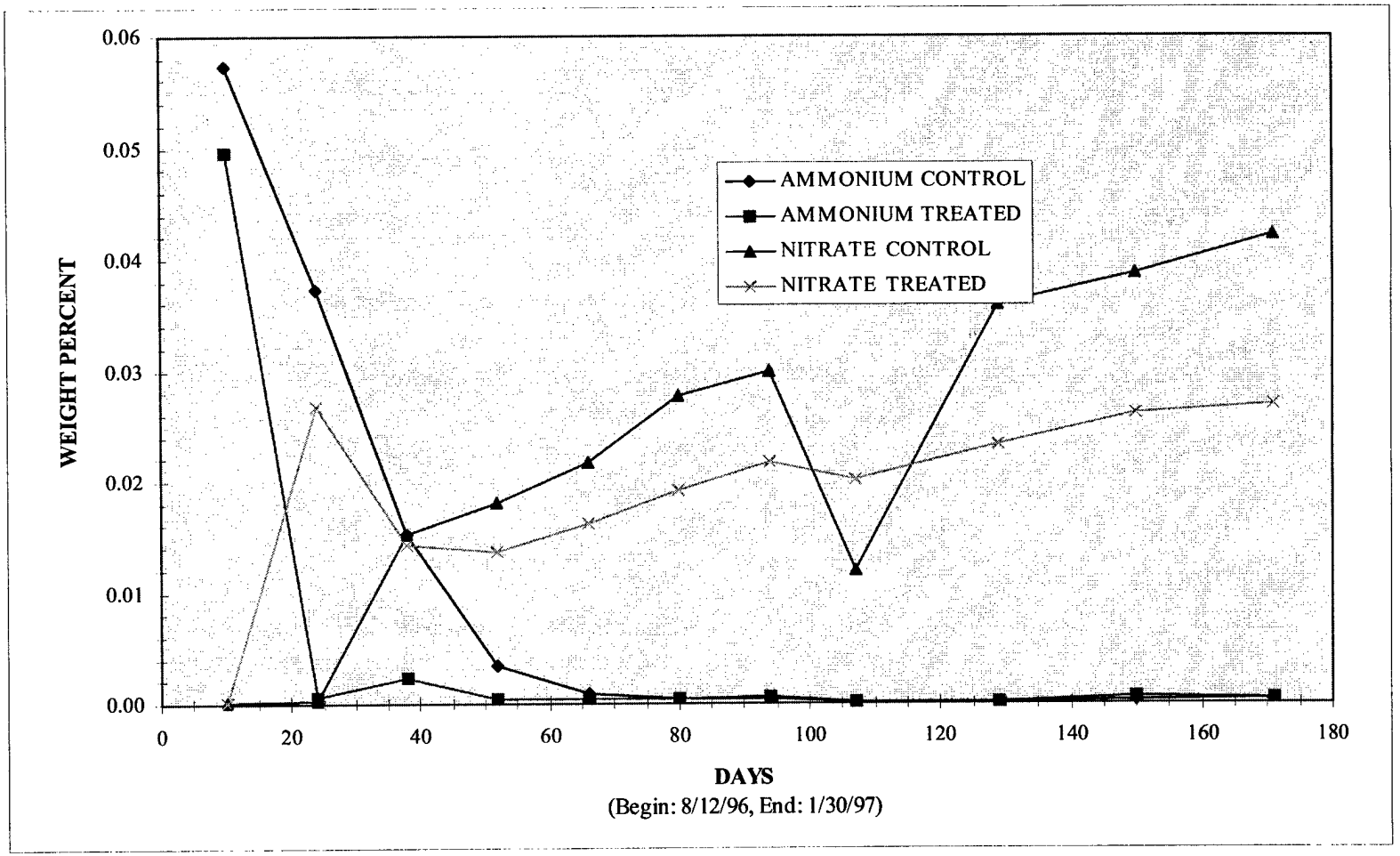


Figure 4-8

Ammonium-Nitrate Relationships

the amount of total nitrogen. Nitrate nitrogen first appeared in the treated compost as temperatures moderated and nitrifying organisms were able to become established. By the middle of October 1996, ammonium nitrogen was essentially zero and the temperature in the compost piles was 30°C or less. Similarly, nitrate nitrogen increased steadily as the ammonium nitrogen concentrations decreased and reached the highest levels at the end of the study period. Higher nitrate nitrogen levels in the control compost are associated with its higher total nitrogen concentration. While low levels of ammonium nitrogen and the appearance of nitrate nitrogen are indicative of a maturing compost, in this study, the relationship did not sufficiently define a mature compost since germination rates for lettuce, radish, and kale were quite low despite the absence of ammonium. Perhaps a longer curing period would have shown the stabilization of nitrate nitrogen concentrations which could be a better indicator of maturity.

4.3.4.4 Carbon Dioxide Liberation

Compost microbial activity was estimated by the absorption of liberated carbon dioxide using soda lime. The results summarized in Figure 4-9 are gravimetric measurements of soda lime. Values are corrected for weight gain by a set of water controls and are expressed on a dry weight basis. Previous uses of this method[Refs. 6,7] had been in the field where it was difficult or impossible to have suitable controls. In this study, the water controls were included to ensure that soda lime weight gains could be clearly associated with carbon dioxide absorption from the compost samples and not residual water in the soda lime or carbon dioxide absorbed during petri dish weighing and handling. The relatively short incubation time for the composts made the water controls particularly important because of the small amounts of carbon dioxide expected to be produced. Also, soda lime tests were conducted with a mature poultry litter compost, which had been cured for over two years, to help evaluate the method.

The aged poultry litter compost liberated considerably more carbon dioxide than the control and treated composts. Despite its maturity, the poultry litter compost contained between 7 and 10 times the amount of total organic carbon found in the control and treated composts, respectively. This was undoubtedly a contributing factor in the comparatively high levels of carbon dioxide liberated from the poultry litter compost. While the control compost tended to

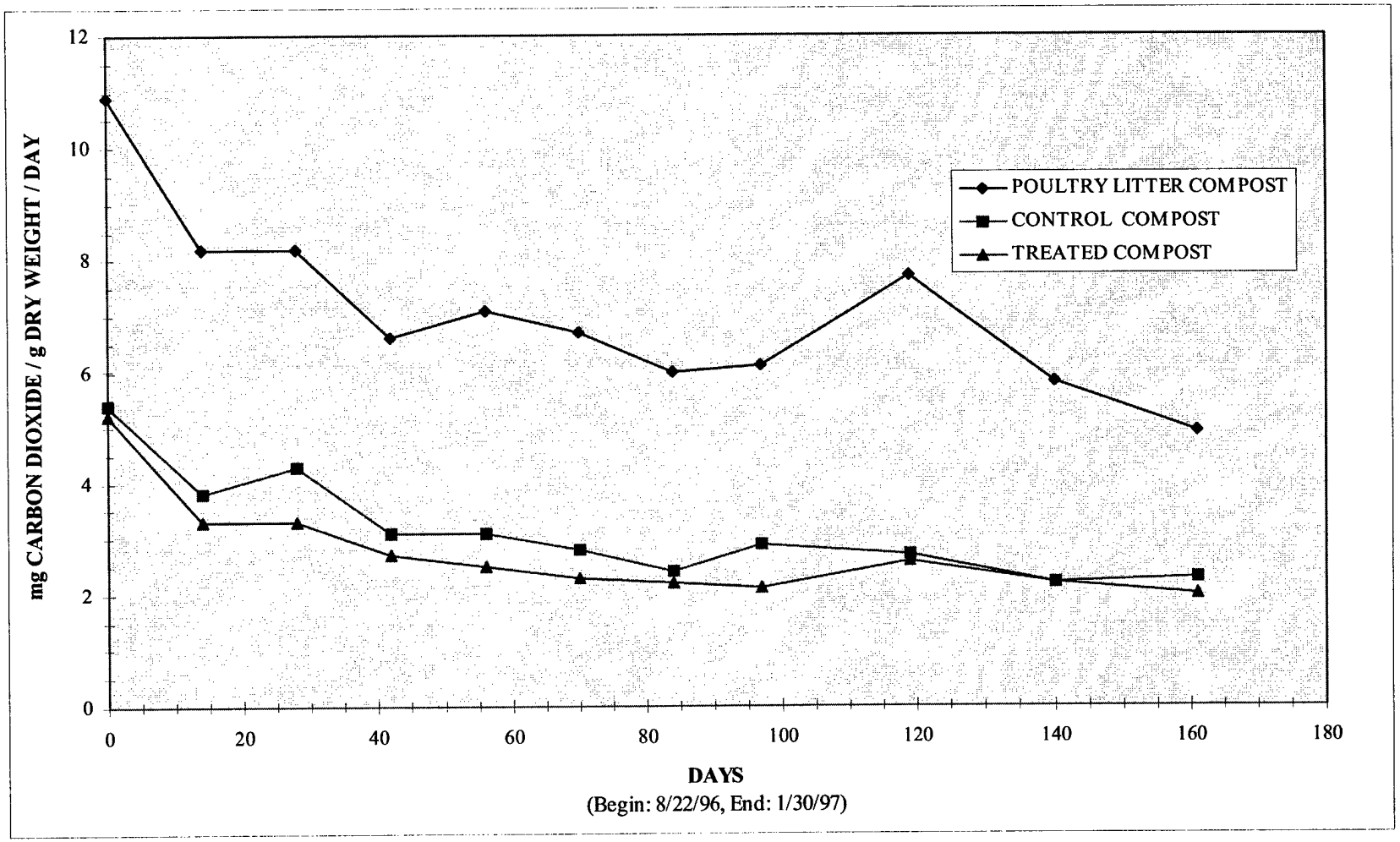


Figure 4-9

Carbon Dioxide Absorption

show higher amounts of carbon dioxide liberation than the treated compost, the difference was not significant enough to support the conclusion that the two composts were different. While carbon dioxide liberated from the treated and control composts decreased during the study period, the poultry litter compost values also decreased. Because of this trend, there is uncertainty about how accurately the soda lime absorption method describes maturity development in the control and treated composts.

4.4 Plant Uptake Studies

4.4.1 Germination Study (Study 1)

The Seed Germination Study results are shown in Figure 4-10. Analysis of variance (ANOVA), t-test, and the appropriate least significant difference (LSD 0.05) were used to statistically separate the means at the 95% confidence level of probability using the general linear models procedure. To determine the differences in germination among plants or among compost mixtures, the length of the vertical bars labeled Plant or Mixture LSD $_{(0.05)}$ can be used. For example, by looking at the first set of bars labeled UMADA soil, it can be concluded that radish germination was significantly higher than lettuce or kale germination. For the plants grown in 50% control compost, however, radish germination was significantly higher than kale, but not significantly higher than lettuce. In other words, differences that are no greater than the length of the bars (about 9% for plants and 12% for mixtures) are not significant based on a 95% confidence level. Differences in germination due to the mixtures of soil and compost must be based on a single plant (i.e., radish, lettuce, or kale).

Kale and lettuce seed germinated poorly during this study in comparison to the radish seed. Differences due to plant species were significant (germination of radish>lettuce>kale). Best germination was achieved in the dilutions containing 25% or 50% compost. Germination in 25% treated compost was not significantly different from germination in soil. Germination of the larger and harder seeded radish was affected least by the compost mixtures. Maximum germination was achieved in the 75% treated compost and 25% control compost for radish. Lettuce seed germination exhibited a threshold response for satisfactory germination for both treated and control compost. Maximum germination for lettuce occurred in 25% control and

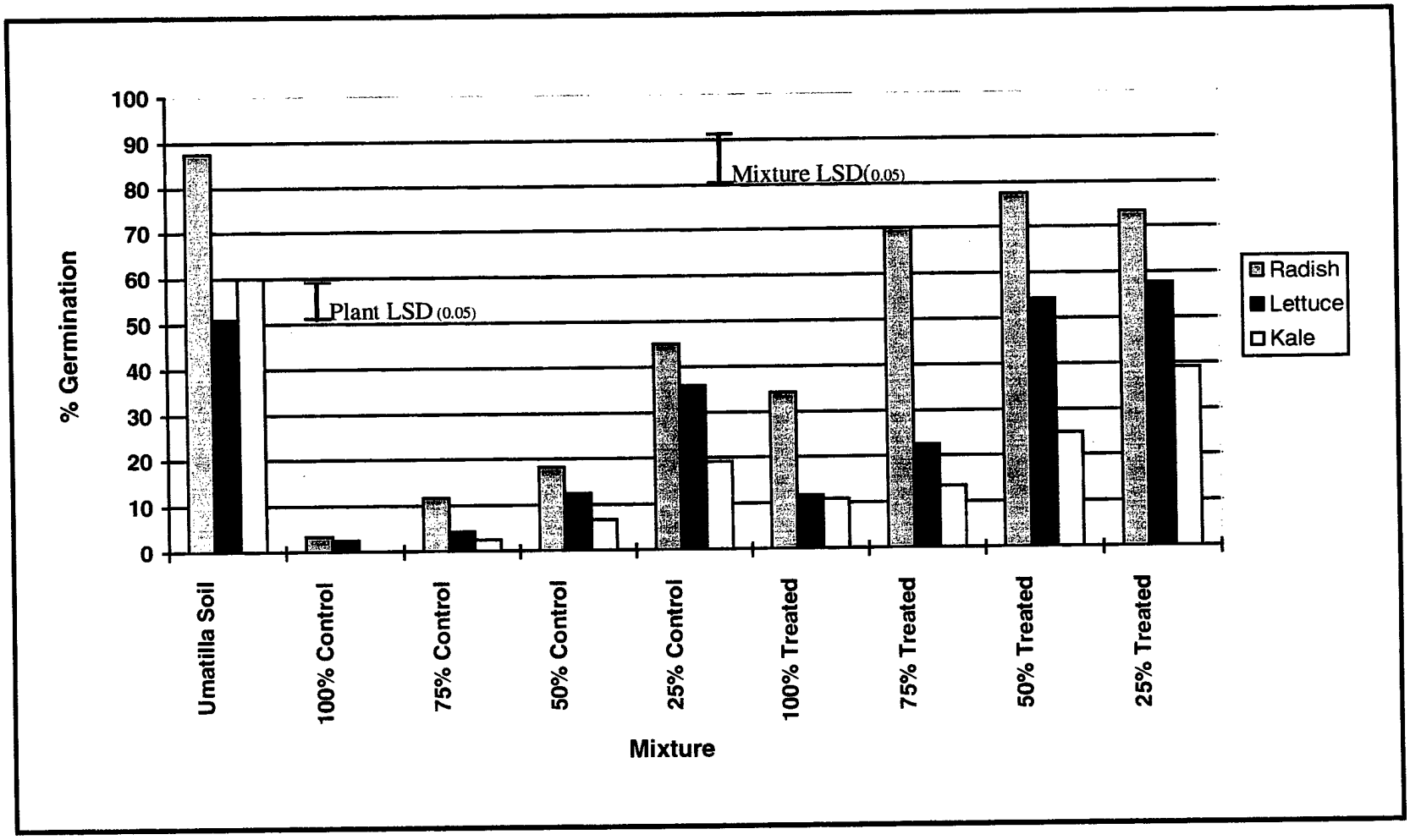


Figure 4-10

Germination of Radish, Lettuce, and Kale in Soil or Soil Mixed with Control or Treated Composts

50% treated compost. Kale seed germination increased gradually with decreasing concentrations of compost to a maximum of approximately 20% germination in control compost diluted to 25% with UMADA soil and approximately 40% germination in treated compost diluted to 25% with UMADA soil.

4.4.2 Preliminary Plant Uptake Study (Study 2)

4.4.2.1 Uptake of Explosives and Explosive By-Products

During Study 2, radish and kale shoots and roots were analyzed for explosives and explosive by-products as indicated in Table 3-7. These analyses indicate that no explosives and explosive by-products were detected above the analytical method detection limits (MDL). The median of the MDL's for the explosives and explosive by-products are shown in Table 4-5. A list of the analytical results for the radish and kale tissues are provided in Tables 4-6 and 4-7, respectively. For each crop, a fifth pot was planted as a backup in case one of the four pots labeled A, B, C, or D failed to produce enough tissue for analysis. These pots were denoted "extra" and were only used when one of the other four pots failed to produce enough tissue for analysis.

4.4.2.2 Growth Characteristics

Both radish and kale were grown in Study 2. The radishes grown in only soil were harvested 31 days after planting. All other radishes were harvested as maturation was achieved (Table 4-8) as judged by size, color, and uniformity in a treatment. Radishes grown in soil only matured in the shortest time and the days to maturity (harvest) increased with increased percentages of compost in the growing media. The longer times to harvest (slower growth) was more noticeable in the control compost than in the treated compost. Physiologically, radishes matured faster in the treated compost than in the control compost. Harvest of the radishes from treated compost was delayed to improve the yield comparisons with the control compost at the same chronological age.

Some pear-shaped radishes were apparent in both the control and treated compost at soil-to-compost ratios of 1:1. All fruit was judged to be good quality in terms of color,

Table 4-5

Median Minimum Detection Limits (MDLs) for Explosive Residues in Radish and Kale Plant Tissues and in Soil/Compost Mixtures

Explosive Residues	Plant Tissue (µg/g)	Soil/Compost Mixtures (mg/kg)
HMX	31	0.15
RDX	25	0.1
TNT	25	0.1
TNB	25	0.1
2,6-DANT	25	1.5
2,4-DANT	25	1.0
2,6-DNT	25	0.1
2,4-DNT	25	0.1
2A-DNT	27	0.1
4A-DNT	25	0.1
1,3-DNB	25	0.1
3,5-DNA	25	0.1
DN-4,4-AZT	25	0.1

Table 4-6

Preliminary Plant Uptake Study - Explosives and Explosive By-Products in Radish

RADISH Explosives Analyses (ug/g dry weight)													
Sample ID	Soil Mixture	Plant Part	Rep	HMX	RDX	TNT	TNB	2,6-DANT	2,4-DANT	2,6-DNT	2,4-DNT	2A-DNT	4A-DNT
101	soil only	top	A	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra										
		root	A	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra										
102	1:1 soil:control	top	A	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (39)	ND (31)	ND (31)	ND (31)	ND (31)	ND (31)	ND (31)	ND (31)	ND (34)	ND (31)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra										
		root	A	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra										
103	1:1 soil:treated	top	A	INSUFFICIENT QUANTITY FOR ANALYSIS									
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		root	A	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra										

ND = Not detected above the detection limit shown in parentheses.

Table 4-6

Preliminary Plant Uptake Study - Explosives and Explosive By-Products in Radish (Continued)

RADISH Explosives Analyses (ug/g dry weight)													
Sample ID	Soil Mixture	Plant Part	Rep	HMX	RDX	TNT	TNB	2,6-DANT	2,4-DANT	2,6-DNT	2,4-DNT	2A-DNT	4A-DNT
104	3:1 soil:control	top	A	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra										
		root	A	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra										
105	3:1 soil:treated	top	A	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra										
		root	A	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra										
106	5:1 soil:control	top	A	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra										
		root	A	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra										

ND = Not detected above the detection limit shown in parentheses.

Table 4-6

Preliminary Plant Uptake Study - Explosives and Explosive By-Products in Radish (Continued)

RADISH Explosives Analyses (ug/g dry weight)													
Sample ID	Soil Mixture	Plant Part	Rep	HMX	RDX	TNT	TNB	2,6-DANT	2,4-DANT	2,6-DNT	2,4-DNT	2A-DNT	4A-DNT
107	5:1 soil:treated	top	A	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra										
		root	A	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			B	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			C	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			D	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
			Extra										

ND = Not detected above the detection limit shown in parentheses.

Table 4-7

Preliminary Plant Uptake Study - Explosives and Explosive By-Products in Kale

KALE Explosives Analyses (ug/g dry weight)													
Sample ID	Soil Mixture	Plant Part	Rep	HMX	RDX	TNT	TNB	2,6-DANT	2,4-DANT	2,6-DNT	2,4-DNT	2A-DNT	4A-DNT
201	soil only	top	A	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			B	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			C	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			D	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			Extra										
202	1:1 soil:control	top	A	INSUFFICIENT QUANTITY FOR ANALYSIS									
			B	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			C	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			D	INSUFFICIENT QUANTITY FOR ANALYSIS									
			Extra	ND(52)	ND(42)	ND(42)	ND(42)	ND(42)	ND(42)	ND(42)	ND(42)	ND(45)	ND(42)
203	1:1 soil:treated	top	A	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			B	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			C	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			D	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			Extra										
204	3:1 soil:control	top	A	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			B	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			C	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			D	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			Extra										
205	3:1 soil:treated	top	A	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			B	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			C	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			D	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			Extra										
206	5:1 soil:control	top	A	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			B	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			C	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			D	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			Extra										
		root	A	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			B	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			C	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			D	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			Extra										
207	5:1 soil:treated	top	A	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			B	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			C	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			D	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			Extra										
		root	A	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			B	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			C	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			D	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
			Extra										

ND = Not detected above the detection limit shown in parentheses.

Table 4-8

Fresh Yield and Harvest Days After Planting of Radish and Kale From Cool Season Growth Chamber Study in Soil and Compost Mixtures

Radish					
Ratio of Soil to Compost	Harvest	Yield (fresh weight g/pot)			
		Control Compost		Treated Compost	
	Days after Planting	Top	Root	Top	Root
1:1	62-63	8.0	18.2	44.8	104.6
3:1	43-45	28.0	60.1	42.3	114.0
5:1	38-45	39.4	112.6	48.0	114.3
Soil only	31	36.7	69.8	36.7	69.8
	LSD (0.05)	12.5	26.8	NS	39.0
Kale					
Ratio of Soil to Compost	Harvest	Yield (FW g/pot)			
		Control Compost		Treated Compost	
	Days After Planting	Top	Root	Top	Root
1:1	66	61.7	NA	102.5	NA
3:1	66	130.1	NA	226.9	NA
5:1	66	167.8	NA	187.7	NA
Soil only	66	141.5	NA	141.5	NA
	LSD (0.05)	63.4		33.8	
Control vs Treated Compost					
Control		64.6			
Treated		109.8			
LSD (0.05)		21.3			

NA = Not Analyzed
NS = nonsignificant at the p=0.05

texture, and juiciness compared to those grown in clean soil. Slow growth characteristics can be partly attributed to high salinity. Electrical conductivity in undiluted control compost was 6.7 dS/m and 3.9 dS/m in undiluted treated compost (Table 4-9). These salinity values approach reported values of 50% yield for these crop tolerances. For optimal yields, these composts should be diluted with soils with low salinity values or subjected to leaching to remove unwanted salts.[Ref. 10]

Yield of radish root (fruit) was significantly increased in the 5:1 soil-to-compost mixture (63% yield) of the treated compost and in the 3:1 mixture (61% yield) of the control compost mixture when compared to the yields in soil (Table 4-8). Radish yields of both top growth and root growth were reduced by the 1:1 soil to control compost mixture compared to those grown in the soil. Radish root yields from the higher concentration of treated compost were not different from the other mixtures of treated compost.

Leafy vegetable yield of kale was not significantly affected by the mixtures of the control compost (Table 4-8). Leaf yields of kale were increased 60% and 33% when grown in 5:1 and 3:1 mixtures of treated compost, respectively, and reduced 28% by the 1:1 treated compost mixture compared to yields from soil. Treated compost was significantly more beneficial than the control compost for growing kale. Kale was notably more green and fleshy when grown in the dilute concentrations of treated compost. Kale root yields were not determined, but the shoots were analyzed for the presence of explosives and explosive by-products. Kale root exudates may have improved the nutrient value of the treated compost. The growth of the *Brassica* crop appeared to have been improved by the use of treated compost to amend the UMADA soil.

4.4.3 Main Plant Uptake Study (Study 3)

The Main Plant Uptake Study involved seven different plants grown in treated compost and control compost. Cool season crops of chives, redtop, alfalfa, and barley (varieties Parmunky and Starling) were grown first (Study 3A) and the warm season crops of sorghum, bush snapbeans, and tomato were planted in the spring (Study 3B). Based on the growth study with radish and kale in Study 2, it was decided to use the 5:1 soil:compost mixture with cool season and warm season crops.

Table 4-9

Chemical Analysis of Soil and Mature Compost

Sample	Total Nitrogen (%)	Total Organic Carbon (%)	Ash (%)	pH	EC (dS/m)	P (%)	K (%)
Control Compost[1]	0.35	5.50	88.8	8.3	6.67	0.23	0.59
Treated Compost[1]	0.20	3.90	91.7	7.7	3.90	0.23	0.59
Soil only	0.04	0.32	98.3	7.2	0.27	0.09	0.03

(1) Last analysis of compost during maturity testing.

4.4.3.1 Cool Season Test (Study 3A)

4.4.3.1.1 Uptake of Explosives and Explosive By-Products by Cool Season Plants

During Study 3A, no explosives or explosive by-products in cool season plant tissues or the compost mixture were found to exceed the analytical method detection limits (Table 4-10).

4.4.3.1.2 Growth Characteristics of the Cool Season Plants

Plant emergence was 1-3 days slower in the mixture containing the control compost. Germination and survival of the chives in the control was about half of the treated compost during the first ten days after planting. No unusual growth characteristics were noted. Additional nutrients were provided to barley after two months because of its longer growing season. These nutrients consisted of 100 mg of potassium and nitrogen provided as potassium chloride and ammonium nitrate. Aphids and white flies were controlled with the insecticides Kicker (pyrethrin) and Orethene (acephate). Molds were controlled with the fungicides Benomyl and Maneb. A comparison of plant yields in the treated and control compost mixtures are provided (Table 4-11). Barley root yield was significantly higher in the treated compost mixture than in the control mixture, but grain yield and shoot yield were not different. The root-to-shoot ratio was higher in the control compost mixture and there was a trend to produce more grain than the treated compost mixture. Barley is known to be tolerant to the higher salinity and the control compost mixture may have supplied more nitrogen during the season to improve the yield at the expense of root growth in the control compost mixture. The grain of the Parmunkey variety was analyzed for explosives and explosive by-products. The shoots and roots of the Starling variety were used for yield comparisons in different soil/compost mixtures. Two barleys were grown to ensure that at least one of them would flower and produce grain. The Starling variety did not produce flowers or grain due to a lack of cold temperatures necessary to induce grain production.

Alfalfa and redtop shoot yields in the treated and control compost mixtures were about the same. Chive shoot growth was noticeably stunted in the control compost mixture relative to the treated compost mixture. Root yield of the chives in the control compost mixture was only 40% of the root yield from the treated compost mixture. Similarly, the root yield of redtop in

Table 4-10

Main Plant Uptake Study - Explosives and Explosive By-Products in Cool Season Plants

Cool Season	Mixture[1]	Rep	Plant Part	HMX	RDX	TNT	TNB	2,6-DANT	2,4-DA-6-NT	2,6-DNT	2,4-DNT	2A-DNT	4A-DNT
Chives	T	A	Top	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		B		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		C		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		D		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
	C	A	Top	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		B		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		C		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		D		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
Redtop	T	A	Top	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		B		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		C		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		D		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
	C	A	Top	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		B		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		C		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		D		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
Alfalfa	T	A	Top	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		B		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		C		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		D		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		A	Roots	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		B		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		C		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		D		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
	C	A	Top	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		B		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		C		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		D		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		A	Roots	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		B		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		C		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		D		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
Barley (Parmunkey)	T	A	Grain	ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		B		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		C		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		D		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
	C	A		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		B		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		C		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)
		D		ND (31)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (25)	ND (27)	ND (25)

ND = Not detected above the detection limit shown in parentheses.

T = Treated

C = Control

(1) All mixtures had a soil-to-compost ratio of 5:1.

Table 4-11

Plant Biomass Yields of Cool Season Greenhouse Crops Grown in the Treated Compost Compared With the Control at a Soil-to-Compost Ratio of 5:1

Crop	Compost Mixture	Yield (fresh weight g/pot)		
		Shoot	Root	Grain
Alfalfa	Control	295.5	235.0	--
	Treated	299.5	237.5	--
	LSD (0.05)	NS	NS	--
Barley	Control	232.3	148.8	15.0
	Treated	201.2	211.1	10.5
	LSD (0.05)	NS	60.1	NS
Redtop	Control	197.5	103.0	--
	Treated	209.4	146.9	--
	LSD (0.05)	NS	39.9	--
Chives	Control	65.6	143.5	--
	Treated	115.8	357.1	--
	LSD (0.05)	38.0	73.7	--

NS = nonsignificant at the p=0.05

the control compost mixture was only 69% of that in the treated compost mixture. The response of these crops to the compost mixtures can be ranked in the following order: from most tolerant to least tolerant, alfalfa ≥ barley ≥ redtop > chives.

4.4.3.2 Warm Season Test (Study 3B)

4.4.3.2.1 Plant Uptake of Explosives and Explosive By-Products by Warm Season Plants

Tomato fruit, edible bean pods, and grain sorghum were analyzed for explosives and explosive by-products in the warm season plant tissues. No explosives or explosive by-products in the warm season plant tissues were found to exceed the method detection limits (Table 4-12).

4.4.3.2.2 Growth Characteristics of the Warm Season Plants

Sorghum and snapbeans grew well in this study and no relative differences were found due to the type of compost (Table 4-13). The tomatoes developed some blossom end rot and had to be sprayed with 1% calcium chloride to improve calcium nutrition. Blossom end rot is often aggravated by calcium deficiency in tomatoes. This problem was common among tomatoes grown in both types of compost. Plant uptake of calcium can be reduced by cool root temperatures and calcium soil deficiency. High pH of the compost should help prevent calcium deficiency unless non-calcium salts are too high relative to the plant-available calcium. Less variation in growth by plant type and among the replicates for a given plant was observed in the warm season crops than in the cool season crops.

Table 4-12

Main Plant Uptake Study - Explosives and Explosive By-Products in Warm Season Plants

Cool Season	Mixture[1]	Rep	Plant Part	HMX	RDX	TNT	TNB	2,6-DANT	2,4-DA-6-NT	2,6-DNT	2,4-DNT	2A-DNT	4A-DNT
Tomatoes	T	A	Fruit	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		B		ND(26)	ND(21)	ND(21)	ND(21)	ND(21)	ND(21)	ND(21)	ND(21)	ND(23)	ND(21)
		C		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		D		ND(27)	ND(22)	ND(22)	ND(22)	ND(22)	ND(22)	ND(22)	ND(22)	ND(24)	ND(22)
	C	A	Fruit	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		B		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		C		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		D		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
Snapbeans	T	A	Fruit	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		B		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		C		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		D		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
	C	A	Fruit	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		B		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		C		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		D		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
Sorghum	T	A	Seed	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		B		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		C		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		D		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
	C	A	Seed	ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		B		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		C		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)
		D		ND(31)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(25)	ND(27)	ND(25)

ND = Not detected above the detection limit shown in parentheses.

T = Treated

C = Control

(1) All mixtures had a soil-to-compost ratio of 5:1.

Table 4-13

Plant Biomass Yields of Warm Season Greenhouse Crops Grown in the Treated Compost Compared With Control Compost at a Soil-to-Compost Ratio of 5:1

Crop	Compost Mixture	Yield (Fresh weight g/pot)		
Sorghum		**Shoot**	**Root**	**Grain**
	Control	269.8	323.7	64.3
	Treated	293.8	394.8	47.1
	LSD (0.05)	NS	NS	NS
Snapbeans		**Shoot**	**Root**	**Beans**
	Control	186.5	44.9	268.5
	Treated	181.0	41.2	268.5
	LSD (0.05)	NS	NS	NS
Tomatoes		**Shoots**	**Root**	**Fruit**
	Control	---	55.1	805.2
	Treated	---	59.3	701.3
	LSD (0.05)	---	NS	NS

NS = nonsignificant at the p=0.05

SECTION 5.0
CONCLUSIONS

5.1 Study Results

5.1.1 Compost Monitoring and Maturity Testing

Control and treated composts were monitored for temperature and oxygen levels, sampled for chemical analyses, and tested for compost maturity prior to starting plant growth studies. Temperature and oxygen measurements are important indicators of the composting process because they provide information on the need for aeration and moisture adjustment, as well as compost stabilization as microbial activity decreases with the age of the compost. Because of age differences, the younger control compost experienced larger temperature changes than did the treated compost. However, during the latter part of the study, the temperatures measured for both composts became similar and approached ambient temperature. Low oxygen levels were a transient problem only in the early stages of study.

Differences in chemical parameters between the two composts included: total organic carbon, total nitrogen, nitrate nitrogen, ash, pH, and conductivity. The control compost generally had higher values for all the characteristics except ash. The very high ash content of the composts distinguishes them from composts produced from other, more highly organic materials. The high salt content of both composts is a characteristic which could influence their use for some applications. Despite having the organic amendments for the control compost mixed at the UMADA treatment site and despite closely following the composting formulation used for explosives remediation, changes in the organic amendments during shipping could have caused the differences in chemical parameters found between the two composts. The treated compost and materials for the control compost were in transit, respectively, 9 and 12 days. The oxygen demand of the organisms in the organic amendments was such that anaerobic conditions appeared to have occurred. The organic amendments had a strong odor when unpacked in Muscle Shoals. The treated compost, on the other hand, emitted little odor indicating that it was less anaerobic upon arrival. These differences in characteristics could have influenced seed germination and could have influenced plant growth.

The maturity tests evaluated—odor, carbon-to-nitrogen ratio, ammonium and nitrate nitrogen relationships, or carbon dioxide absorption with soda lime—were accurate indicators of compost maturity; however, they were no better than the monitoring of temperature and oxygen levels for determining when biological activity in the compost had stabilized. While changes over time were seen in odor, carbon-to-nitrogen ratio, and ammonium and nitrate nitrogen relationships which indicated that the compost was mature, these changes were not correlated with seed germination tests performed to assess the suitability of the composts for plant growth studies. In the germination tests conducted using radish, kale, and lettuce seeds, germination percentage was consistently higher in the treated compost than in the control compost despite the fact that the maturity indicators showed the two composts to be stable (Figure 4-10). So there were clear differences between the effects of the two composts on seed germination. The absorption of carbon dioxide by soda lime was a time-consuming procedure that showed no clear differences between the treated and control composts which could be associated with maturity and compost stability. If additional compost maturity testing is needed in the future, the tests should be performed less frequently, the compost should be given sufficient time to cure before testing begins, the compost maturity should be based on temperature and oxygen levels, and the suitability of the compost for growing plants should be directly tied to seed germination tests.

5.1.2 Plant Uptake Studies

5.1.2.1 Germination Study (Study 1)

During the Seed Germination Study, it was concluded that:

- Both treated and control compost must be diluted with soil to achieve optimum germination. Kale required the highest dilution of compost, followed by lettuce, then radish.

- Treated compost supported seed germination and seedling emergence better than control compost.

- Plants with soft seed coats and smaller seed may be more likely to have poor germination in compost.

- Differences in the seed germination and seedling emergence were found between the treated compost and the control compost. When properly diluted, germination in the treated compost was approximately twice as great for these small seeds than the control compost.

5.1.2.2 Preliminary Plant Uptake Study (Study 2)

The conclusions from the Preliminary Uptake Study are:

- No explosives and explosive by-products were found in the plant tissues analyzed.

- Mixing UMADA soil and compost in ratios of 5:1 to 3:1 may allow some types of smaller seeded plants to be grown immediately after compost maturity.

- The salinity of these composts can limit compost use if they are not diluted with soil and/or leached prior to use.

5.1.2.3 Main Plant Uptake Study (Study 3)

The conclusions from the Main Plant Uptake Study are:

- No explosives or explosive by-products were found in the cool or warm season plants' roots, fruit, stems, leaves, or grain.

- At a soil-to-compost ratio of 5:1, the treated compost is sufficiently dilute to initiate seed germination in most cases. The two exceptions were for chives and kale.

- Germination could be inhibited by high concentrations of compost with some plants. Kale benefited in growth with the same mixture. Germination of more sensitive plants can result in a poor plant stand and poor growth (chives).

- The treated compost mix should be leached to remove salts if it is used as an ingredient in horticultural plant mixes for small seeded bedding plants.

- Growth and yield from the UMADA soil for many crops would probably benefit if amended no higher than 25% compost in the plow zone (6-inch depth) with the 1- to 2-year-old treated compost.

5.2 Summary

The results of this study indicate:

- Plants grown in UMADA treated compost did not cause a measurable increase in the level of explosives or explosive degradation by-products found in the compost.

- No explosives or explosive by-products were found in the plant tissues examined.

- Plants with smaller seeds may be more likely to have poor germination in unleached treated soil/compost mixtures.

- With some types of plants, the treated compost should be diluted with the appropriate amount of soil to achieve satisfactory seed germination and plant growth.

- Soil/compost dilutions of 3:1 or 5:1 were satisfactory for plants grown in this study.

- Some plant yields in some soils will be enhanced when properly amended with the treated compost.

SECTION 6.0
REFERENCES

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APPENDIX A

QUALITY ASSURANCE

APPENDIX A
QUALITY ASSURANCE PLAN

A.1 Purpose and Scope of the Plan

The purpose of the quality assurance plan is to outline processes to ensure that:

- Sufficient measurements were made to assess the effectiveness of the proposed treatment methods.
- Samples taken were representative of the conditions in the experimental setup.
- Samples were delivered to the laboratory for analysis without deterioration.
- Measurement techniques were sufficiently specific to measure the target compounds.
- Data taken were reliable.

The quality assurance plan applied to all activities including performing experiments, sampling, and laboratory analysis of samples.

TVA's Analytical Laboratory provided analytical chemistry support for the project by performing analyses for explosives and degradation products. New procedures for extraction of explosives and explosive by-products from plant tissues and compost were developed and tested for this project.

A.2 Quality Assurance Responsibilities

The attached organizational chart (Figure A-1) shows the TVA organizations providing support to the project. Responsibilities of staff members were as follows:

- The Project Manager provided overall direction for the project, ensured staffing was adequate to meet project goals and schedules, and provided progress reports to the USAEC.

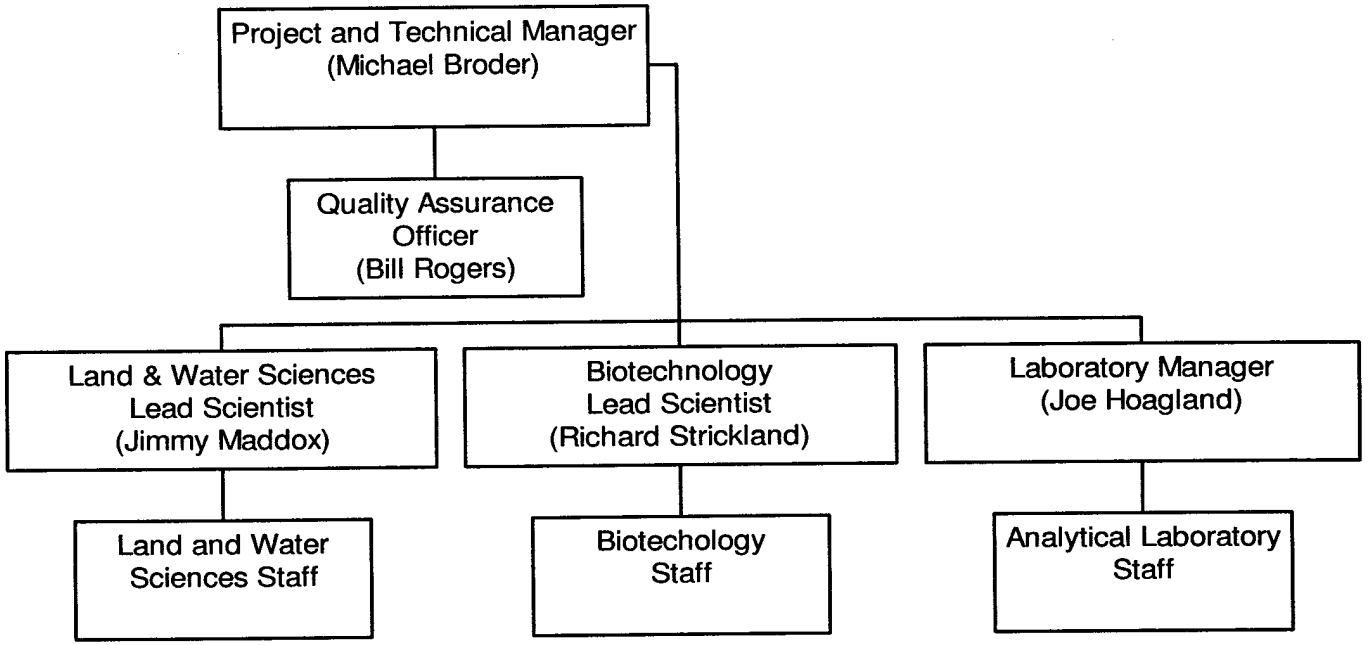


Figure A-1

TVA Organizations Providing Project Support

- The Technical Manager was responsible for providing technical direction and staff for development of processes and experimental design. The Technical Manager also provided oversight of experimental design, assisted in resolution of technical questions, and coordinated technical activities.

- The Quality Assurance (QA) Officer reported to the Project Manager and had no direct responsibilities in testing or analysis of the samples. The QA Officer was responsible for auditing actions and documentation to ensure adherence to this plan. The QA Officer was responsible for providing quarterly quality control data reports to the Laboratory Manager.

- Land and Water Sciences Lead Scientist reported to the Project Manager and Technical Manager and was responsible for providing technical direction and staff for development of processes and experimental design.

- Land and Water Sciences staff reported to the Land and Water Sciences Lead Scientist and was responsible for performing experiments and experimental operations. Staff members were responsible for planning, design, testing, and documentation of the various sub-projects assigned to them. They were responsible for review of data falling under their areas of responsibility.

- Biotechnology Lead Scientist reported to the Project Manager and Technical Manager and was responsible for providing technical direction and staff for development of processes and experimental design.

- Biotechnology staff reported to the Biotechnology Lead Scientist and was responsible for performing experiments and experimental operations. Staff members were responsible for planning, design, testing, and documentation of the various sub-projects assigned to them. They were responsible for review of data falling under their areas of responsibility.

- The Laboratory Manager reported to the Project Manager and Technical Manager and was responsible for providing project analytical oversight and for final analytical data integrity. The Laboratory Manager was responsible for providing monthly project reports to the Project Manager.

- Research Chemists and Research Scientists in the Analytical Laboratory reported to the Laboratory Manager and were responsible for planning, design, testing, and documentation of the various sub-projects assigned to them. They were responsible for review of data falling under their areas of responsibility.

- Chemical Laboratory Analysts and Technicians in the Analytical Laboratory report to the Laboratory Manager and were responsible for following procedures and instructions to provide analytical measurements required in the course of the project. They were responsible for review of the data they produce, documentation of analytical runs, and equipment maintenance.

A.3 Quality Program Procedures and Documents

The Analytical Laboratory activities conducted during this project were carried out in accordance with the laboratory's Quality Assurance Manual which contains the following documents:

QAPLAN - "Quality Assurance Plan"
GLP-0001 - "Procedure Format and Style"
GLP-0002 - "Quality Assurance Records Control"
GLP-0003 - "Procedure Preparation and Distribution"
GLP-0004 - "Training"
GLP-0005 - "Nonconformances and Corrective Actions"
GLP-0006 - "Control of Reagents and Standards"
GLP-0007 - "Analysis Work Plan Preparation"
GLP-0012 - "Treatment of Data"
GLP-0013 - "Instrument Logbook and Control Chart Maintenance"
GLP-0016 - "Sample Receipt, Log-in, and Data Handling"

GLP-0017 - "Control of Changes to Software"

CP-0001 - "Measurement and Test Equipment Control and Calibration"

SP-0001 - "Sample Chain of Custody"

Laboratory analyses were conducted in accordance with written procedures. Modifications to procedures found to be necessary to perform the analyses required in this test plan were noted in equipment operation logs or research notebooks until included in revisions to procedures. A revision to procedure AP-0062, "Extraction, Preparation, and Analysis of Explosives and Their Degradation Products by HPLC," was produced to cover extraction and analysis of plant and compost material.

The experimental portion of this plan was performed in accordance with the project plan. Data, observations, experimental conditions, and minor modifications to planned activities were recorded in research notebooks in a complete enough fashion that all actions, results, and conclusions could be reconstructed. Details of experiments involving method development were logged to facilitate production of the revision to AP-0062.

Sampling was conducted in accordance with written work plans, procedures, or instructions to ensure complete samples were taken at correct times and in a manner which did not invalidate conclusions. All actions in sampling were recorded in research notebooks or on forms designed to ensure complete documentation of all experimental parameters. Instructions were provided for proper preservation of samples.

A.4 Control of Purchased Items

Chemicals, equipment, materials, and other items purchased to conduct this project were of suitable quality to meet the project needs as specified in the written procedures. Purchased items were inspected upon receipt to ensure they met the requirements specified in purchase requests. Nonconforming items were not used. Suitable handling activities, storage conditions, and other controls were utilized to ensure quality of purchased items was not degraded after receipt.

A.5 Record Control

Records of analysis, records of calibration, research notebooks, chromatograms, sampling logs, custody records, work plans, machine printouts, chromatogram traces, logsheets, standard material use records, raw data calculation sheets, and copies of procedures were maintained as quality assurance records as specified in GLP-0003. Records were accumulated in logical arrangement to facilitate retention and review. In-process records and logbooks were stored in the work area in a safe manner to protect against loss, fire, spills, or other damage.

Records of experiments and analyses will be maintained for a three-year period after the end of the project. This includes machine printouts or chromatogram traces, logbooks, notebooks, logsheets, standard material use logs, and raw data calculation sheets. Due to the limited lifetime of computer storage media, any computer media utilized to store analytical file backups or raw data files will be stored for the lifetime of the project plus one year.

A.6 Data Quality Parameters

A.6.1 Accuracy and Precision

Percent recovery, relative percent difference, standard deviation, and other commonly used statistical indicators of accuracy and precision were calculated as defined in Chapter 1 of SW-846, 3rd Edition.

A.6.2 Method Detection Limit, Method, Quantitation Limit

Method Detection Limits were calculated as defined in Title 40, Code of Federal Regulations, Part 136, Appendix A, "Definition and Procedure for the Determination of the Method Detection Limit" - Revision 1.11.

Method Quantitation Limits were defined as five times the Method Detection Limit as in Chapter 1 of SW-846, 3rd Edition, or as the lowest point used in making the calibration curve, whichever was higher.

A.7 Calibration Procedures and Quality Control Checks

The precision and accuracy of new or revised analytical procedures were investigated before the procedures were used for analysis of samples. Since no standard reference material was available for plant material or compost, constructed test samples were used to estimate precision and accuracy.

A.7.1 Initial Calibration Procedures

A.7.1.1 Laboratory Instrumentation

The calibration frequencies and tests required in SW-846 for Method 8330 were used in the HPLC methods. Guidelines for calibration frequencies and tests as specified by the manufacturer were used for FIA methods.

A.8 Analytical Laboratory Calibration and Quality Control

A.8.1 General Quality Control Requirements

The Analytical Laboratory ran appropriate method blanks for the procedures used in this portion of the project. Method accuracy and precision were demonstrated by running quality control samples. Analysts demonstrated the ability to generate acceptable results with the methods by utilizing appropriate proficiency samples or standard reference materials. The Analytical Laboratory determined Method Detection Limits for target compounds.

A.8.2 Batch QC

With each batch of 20 samples or subset thereof, one method blank, one matrix spike, and one laboratory control sample were run. In addition, one sample duplicate or one matrix spike duplicate was run with each batch. Note: For some analytical techniques, matrix spikes were not possible.

A.8.3 Quality Control Requirements for HPLC

Retention time windows were determined and the device was calibrated during development of the procedure. Five calibration standards were used.

At the beginning of each day that analyses were conducted, the midpoint calibration standard was analyzed. Then, every ten samples and at the end of the run, a midpoint calibration standard was run again in accordance with the quality control requirements for HPLC devices. Any group of ten samples preceding and following a midpoint calibration check which fell outside the 15% limit was reanalyzed.

A laboratory control sample made from a separate stock than the calibration standards was run with each batch.

Samples exhibiting a signal above the linear range of the device were diluted and reanalyzed.

A.8.4 Quality Control for Automated Laboratory Instrumentation

Flow injection analyzers (FIA) were calibrated before each use following written procedures. For FIA, calibration was performed with standards of five concentrations at the beginning of each day. Concentrations bracketed the range of interest, but were limited to the range of linear response of the device.

For these devices, a midpoint calibration standard was run at least every ten samples and at the end of the run throughout the day. Any group of ten samples preceding and following a midpoint calibration check which fell outside the 15% limit was reanalyzed.

For these devices, a laboratory control sample made from a separate stock than the calibration standards was run with each batch.

For combustion analyzers (Total Nitrogen and Total Carbon), manufacturers' instructions were followed for single-point calibration on each day of use. Sample duplicates and quality control

check samples were usually run with each batch. For the carbon analyzer, a laboratory control sample made from a separate stock than the calibration standards was run with each batch.

For any of these devices, samples exhibiting a signal above the linear range of the device were diluted and reanalyzed.

A.8.5 Definitions

- **Batch** - Usually a group of no more than 20 samples of the same matrix prepared or extracted at the same time with the same reagents.

- **Method Blank** - A sample of clean reagent carried through preparation and extraction in the same manner as samples. One method blank was run with each batch.

- **Matrix Spike** - An aliquot of a sample spiked with a known concentration of all target analytes. Spike concentration was selected to read at five times the method quantitation limit in the sample or about the midpoint of the calibration curve. One matrix spike was run for each batch. Spiking occurred prior to sample preparation and analysis.

- **Matrix Spike Duplicate** - A second aliquot of the same sample treated in the same manner as the matrix spike.

- **Duplicate** - A second aliquot of a sample taken independently through extraction and preparation before analysis.

- **Quality Control Check Sample** - A quality control sample of the same type and matrix as calibration solutions, but made independently from the calibration solutions. This sample is also referred to as a **laboratory control sample**.

A.8.6 Data Reduction, Validation, and Reporting

A.8.6.1 Data Reduction

The project's analytical data were calculated and reduced on vendor-supplied chromatographic software for HPLC systems and on vendor-supplied analysis software for FIA systems. These systems typically calculate calibration curves automatically and apply the curves to sample measurements. However, a spreadsheet developed at TVA was used to fit curves and calculate data for the HPLC analysis. Other laboratory calculations were carried out on spreadsheets developed and tested at TVA or on hand-held calculators (e.g., soil moisture). Some devices, such as pH meters or combustion analyzers for total N or total C, give direct readout or printout of analytical data.

The Analytical Laboratory's Chemical Laboratory Analysts were responsible for calculation and reduction of data.

A.8.6.2 Data Validation

Analytical measurements were first reviewed by the chemist producing them and then by another chemist before being interfaced with the laboratory database. If quality control samples fell outside limits, the samples were usually scheduled for reanalysis. After questions were resolved, results were passed on to the Laboratory Manager for final review and validation. Group supervisors or team leaders were responsible for decisions concerning reanalysis of samples and coordinated with the Project Manager when significant problems were discovered or when resampling was required.

A.8.6.3 Data Reporting

Analytical data were reported in units of milligrams per liter for liquid samples. Solid sample results were reported as milligrams per kilogram dry weight unless other units such as percent were more appropriate.

Method Detection Limits and Instrument Detection Limits were reported for each run. Recovery of matrix spikes and recovery of quality control samples were calculated and reported as percentages.

A.8.6.4 Corrective Action

Corrective action in accordance with the requirements of GLP-0005 was not identified in the course of this project.

A.9 Performance and System Audits

A.9.1 Performance Audits

The Analytical Laboratory participated in EPA Water Pollution Studies twice yearly during this project. The Analytical Laboratory investigated any analyte falling outside control limits and reported its findings to the Quality Assurance Officer in writing. Participation in this cross-checking process provides information on Analytical Laboratory's performance as compared to other laboratories in the nation.

A.9.2 Onsite System Audits

The Analytical Laboratory Quality Assurance (QA) Officer periodically inspected logs, records, printouts, results of quality control checks, documentation, case narratives, research notebooks, and other quality-related aspects of the project to ensure detailed compliance.

A.10 Quality Assurance Reports

A.10.1 Status Reports

TVA's Project Manager provided periodic progress reports to USAEC which contained a summary of accomplishments and a discussion of significant problems and their resolution.

Quarterly quality control data reports were written by the QA Officer addressing:

- Changes in this QA project plan
- Changes in analytical procedures
- Summary of QC program results
- Summary of training
- Results of audits
- Results of performance sample evaluations
- Data quality assessment in terms of precision, accuracy, and MDLs
- Discussion of whether QA objectives were met

A.11 Data Management and Analysis

A.11.1 Analytical Data

Analytical data packages for the project included:

- Sample description or identification information
- Sample analytical results
- Quality control sample results with surrogate recoveries and percent recovery of known compounds

Sufficient data were maintained such that experimental and analytical results could be reconstructed.

Records of all attempts at analysis were maintained whether or not the analysis was successful. However, unusable data were not reported. Data were unusable when quality control samples or quality control checks failed; however, the records for these attempts at analysis were maintained with relevant documentation. Data Qualification Codes in use by the laboratory and which may have been encountered in review of this project's data were as follows:

NA - Compound Not Analyzed

<MDL - Compound not detected (value falls less than Method Detection Limit)

TR or Trace - Compound present at trace level, indicated but less than MDL

Q - "Qualified" - For a sample in which an analyte was quantified, but an associated quality control sample fell outside control limits

APPENDIX B
METHODS AND PROCEDURES

Appendix B-1 – Chain of Custody

Tennessee Valley Authority

Analytical Laboratory of Environmental Applications
Environmental Research Center
Muscle Shoals, AL 35662

Procedure Number : SP-0001

Title: Sample Chain of Custody

Signature	Title	Date
Prepared by: William J. Rogers	QA Officer	11/26/96
Concurred: Eugene A. Zarate	Laboratory Section Leader	11/26/96
Concurred:		
Concurred:		
Approved: Joseph J. Hoagland	Manager	11/27/96

Revision	R0	R1	R2			
Control Date	29-Sep-89	10-Jan-96	29-Nov-96			

Copy No: ___ has been issued to holder on _____

"Sample Chain of Custody"

1.0 PURPOSE

This procedure provides instructions for sample custody from collection to final disposition.

2.0 SCOPE

This procedure applies to all samples collected under a sampling plan which requires documentation of sample custody.

3.0 SUMMARY

Requirements for documentation of sample collection and sample custody are specified.

4.0 REFERENCES

4.1 U. S. Environmental Protection Agency, "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods," SW-846, 3rd Edition, Most Recent Update (September 1994)

4.2 "Preparation Aids for the Development of Category II Quality Assurance Project Plans," EPA/600/8-91/004, February 1991, Guy F. Simes, Risk Reduction Engineering Laboratory, Office of Research and Developent, U.S. Environmental Protection Agency, Cincinnati, OH 45268

4.3 "Preparation Aids for the Development of Category III Quality Assurance Project Plans," EPA/600/8-91/005, February 1991, Guy F. Simes, Risk Reduction Engineering Laboratory, Office of Research and Development, U.S. Enviromental Protection Agency, Cincinnati, OH 45268

4.4 "Sample Receipt, Log-in, and Data Handling", GLP-0016, Tennessee Valley Authority, Analytical Laboratory of Environmental Applications, Muscle Shoals, AL.

5.0 RESPONSIBILITIES

5.1 The laboratory team leader shall ensure that this procedure is followed.

5.2 The sampler shall follow this procedure to ensure sample integrity in the field.

5.3 The person transporting the samples shall follow the procedure to ensure sample integrity in transit.

5.4 The person receiving the samples shall follow this procedure to ensure sample integrity upon receipt and immediately following.

5.5 Laboratory analysts shall follow this procedure during sample analysis.

6.0 REQUIREMENTS

6.1 Prerequisites

6.1.1 Sample containers shall be cleaned to specifications of the sampling plan, or in their absence, to good commercial practice.

6.1.2 Sample containers shall have preservative added before sampling as required by the sampling plan.

6.2 Limitations and Actions

6.2.1 If the sampling organization has its own sampling procedure, sample custody procedure, labels, or custody forms, they may be substituted for the contents of this procedure as permitted by the sampling plan.

6.2.2 The number of persons handling samples from the time of sampling to receipt by the laboratory should be held to a minimum.

6.2.3 Sample containers shall be labeled by attaching tie-on tags, adhesive labels, or by writing on sample containers with indelible markers. Sample containers shall be labeled with sufficient information that they may be traced to sample collection logs, field sheets, or custody records. Choice of adhesive labels or indelible ink should take into consideration that samples may come into contact with melted ice or condensed moisture during shipment or storage.

"Sample Chain of Custody"

6.2.4 Individual samples shall be sealed or sample shipping containers shall be sealed with a tamper-proof seal when they will be relinquished by TVA to a common carrier or if the sampling plan requires it. If the samples will remain in the custody of TVA employees from the time of sampling through transport to the laboratory or under lock and key (as in a locked vehicle or storage container) during this time, use of seals is not required. However, even if seals are not required, their use is strongly urged on shipping containers if the sample is to change hands several times in transport.

6.3 Requirements

6.3.1 Apparatus/Equipment

This procedure specifies no additional apparatus or equipment in addition to any sampling plan.

6.3.2 Materials

6.3.2.1 Sample containers specified in the sampling plan shall be utilized.

6.3.2.2 Labels - Samples labels shall have an adhesive which does not readily release when containers become damp.

6.3.2.3 Custody Forms - Sample chain of custody forms shall be used to record custody of samples after sampling from relinquishment by the sampling organization through transport to receipt by the laboratory. The following information shall be supplied on the custody form:

a. Project identification
b. Sample collection date
c. Sample identification
d. Collection time
e. Number of containers per sample identification code
f. Requested analysis
g. Sampling location
h. Comments
i. Signature of sample collector.

In addition the form shall contain an area so that each relinquishment and receipt of samples may be documented.

Example custody forms are attached as appendices 10.1 and 10.2. Other forms specific to a given project may be developed as long as they contain the minimum information specified above.

Note: If sample collection time and location are already recorded on a field sheet or sampling log, that information need not be repeated on this form provided a copy of the sampling information is transmitted to the laboratory with the custody sheet.

6.3.2.4 Tamper-evident seals - These seals shall be individually numbered or otherwise marked so that they could not be removed and replaced without it being detected. Two styles have been useful for samples or sample containers.

6.3.2.4.1 Adhesive seals advertised as meeting forensic science requirements, such as Kapak brand seals.

6.3.2.4.2 Padlock-style plastic seals for hasps.

6.3.2.5 Field Logbooks or Field Sheets - Sampling activities may be documented in field logbooks or field sheets designed for that purpose. When these are used, they shall contain:

a. Project identification
b. Sample collection date
c. Sample identification
d. Collection time
e. Number of containers per sample identification code
f. Reference to the sampling procedure
g. Sampling location
h. Comments
i. Signature of sample collector.

7.0 PROCEDURE

7.1 Field Operations

7.1.1 Prior to sampling, label sample containers with an adhesive label or with indelible marker. (Note: If the sampling conditions require it, labels may be affixed after sampling and cleaning the outside of the container.)

"Sample Chain of Custody"

7.1.2 Document sample information in a field log, field sheet, or the custody sheet if the first two are not provided.

7.1.3 Seal the sample container with an adhesive seal if the sampling plan requires it.

7.1.4 Complete a "Sample Chain of Custody" form.

7.1.4.1 If field logs or field sheets contain collection time and location, these items may be omitted from the form. In that case, draw a diagonal line in that column and attach a copy of the field logs or sheet so that the laboratory may have pertinent sampling information.

7.1.4.2 If a numbered seal is to be used on the shipping container, note that number in the comments section of the custody form.

7.1.4.3 If the shipping container is to be sealed, sign and date the "relinquished" area of the form.

7.1.5 Place the original copy of the paperwork in a plastic bag inside the shipping container. Retain one copy for field files. Transmit a third copy by separate courier, mail or fax to the laboratory.

7.1.6 Place the samples in a shipping container. As required by the sampling plan, place ice (or commercial substitute) and a temperature test bottle in the container as well. Seal the shipping container if the sampling plan requires it. See also 6.2.4.

7.1.7 Deliver the container to be transported to the laboratory.

7.2 Laboratory Receipt (Reference also GLP-0016)

7.2.1 Inspect the seals. Open the shipping container. Inspect the sample custody form to ensure that it is correctly completed. Sign as receiver. Compare the shipping container contents to the information on the form.

7.2.2 If the "relinquished" blank is not completed and the person delivering the samples is present, have that person sign the "relinquished by." Otherwise write "Not completed", date and initial. If a person signs "relinquished by," provide that person a copy of the paperwork.

"Sample Chain of Custody"

7.2.2 As required by the sampling plan, measure the temperature of any samples or temperature blanks and record that information on the custody sheet.

7.2.3 Communicate any errors, broken seals, missing seals, broken samples, differing identification numbers, extra samples, missing samples or misidentification to field personnel. Document all discussions by memorandum or database sample comment file. Document all problems and their resolution by memorandum or database sample comment file. If seals show signs of tampering, bring this to the attention of the group leader or team leader.

7.2.4 Refer to GLP-0016 for further sample receipt and log-in instructions.

7.2.6 Following logging, store the samples in a locked, refrigerated storage area as required by the sampling plan or project plan.

7.3 Laboratory Custody

7.3.1 Samples in locked storage areas, being prepared, being processed, or in autosampler trays are considered to be in the custody of the laboratory. When sampling plans require it, laboratory work areas shall be locked when unattended.

7.4 Sample Disposal

7.4.1 When customers request it, samples shall be returned to them following analysis.

7.4.2 Otherwise, dispose of samples after the time period specified in the sampling plan or project plan. If these do not specify a date, samples should be kept no longer than three months after all analyses are complete.

7.4.3 If the sampling plan requires it, document sample disposal in the workorder file, or custody records.

8.0 SAFETY

8.1 Wear rubber gloves and protective eyewear when handling samples unless it is known that the samples are innocuous.

8.2 Avoid contact with samples. Be aware of broken containers, corrosives, irritants, biohazards, flammability, pyrophoricity, reactivity, radioactivity

and toxicity. Inspect labels and shipping information for warnings. When hazards are known, label samples with hazard information if that is not already provided by the customer.

8.3 In case of skin contact, wash thoroughly with soap and water.

8.4 In case of eye contact, hold the eyes open and wash for at least 15 minutes in an eyewash. Call for help.

8.5 Flammable liquids must be refrigerated only in explosion-proof refrigerators to avoid the risk of explosion caused by sparks in the electrical contacts of the compressor.

8.6 In handling samples, be aware of spills on outside of containers. Clean the exterior of containers as needed.

9.0 NOTES

None

"Sample Chain of Custody"

10.0 ATTACHMENTS AND APPENDICES

10.1 Chain of Custody Record - TVA 29203 B (RC-CTR 4-94)

CHAIN OF CUSTODY RECORD

Environmental Site Remediation • TVA Environmental Research Center • Muscle Shoals, Alabama 35660

SWMU NO.	SWMU SEQUENCE	SWMU NAME	SWMU LOCATION	PROJECT
SAMPLERS (SIGNATURE)				

SAMPLE NO.	DATE	TIME	COMP/GRAB (C/G)	SAMPLE LOCATION	NO. OF CONTAINERS	ANALYTES									REMARKS

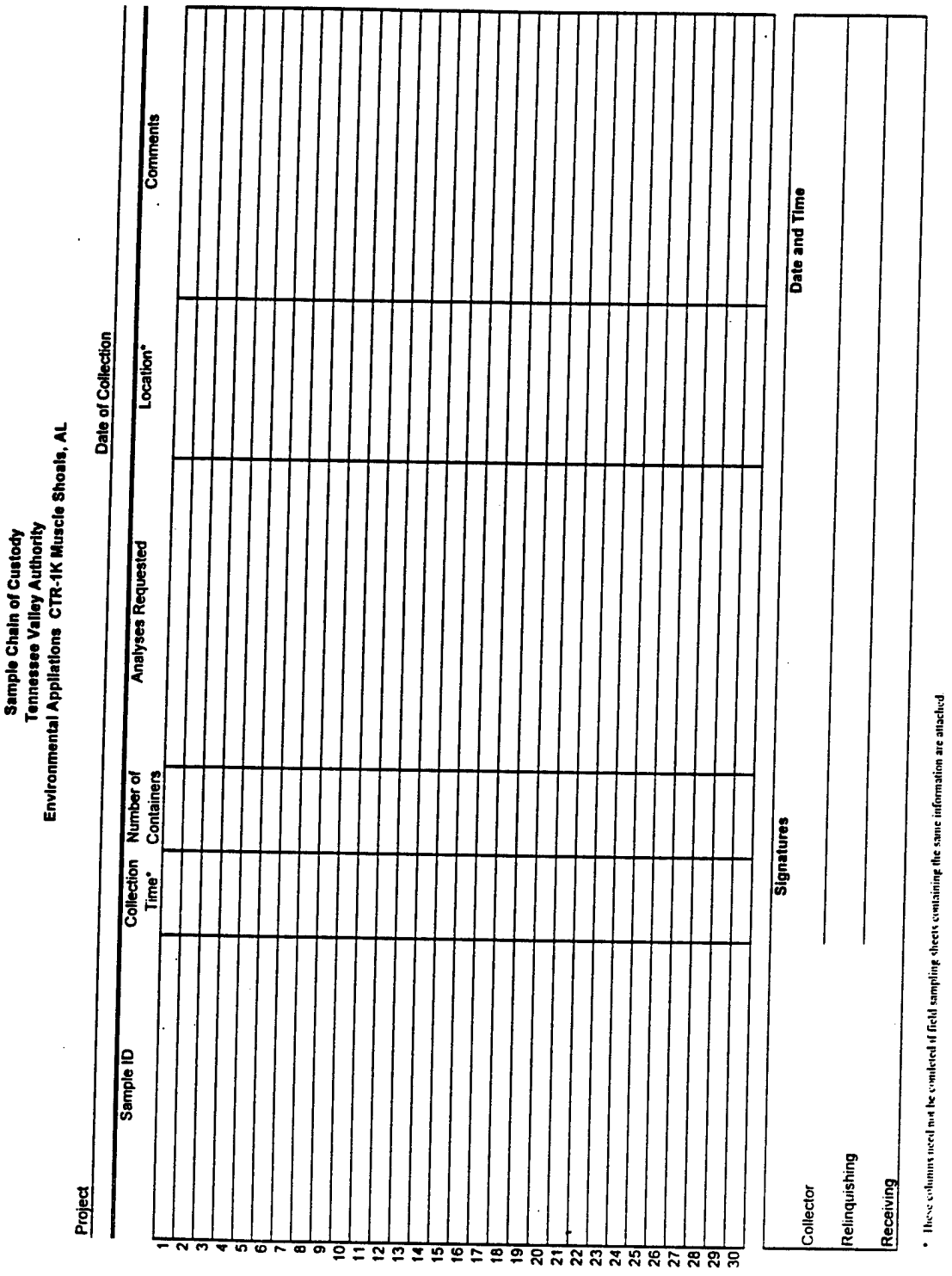
RELINQUISHED BY: (SIGNATURE)	DATE/TIME	RECEIVED BY: (SIGNATURE)	DATE/TIME	RELINQUISHED BY: (SIGNATURE)	DATE/TIME	RECEIVED BY: (SIGNATURE)	DATE/TIME
RELINQUISHED BY: (SIGNATURE)	DATE/TIME	RECEIVED BY: (SIGNATURE)	DATE/TIME	RELINQUISHED BY: (SIGNATURE)	DATE/TIME	RECEIVED BY: (SIGNATURE)	DATE/TIME
RELINQUISHED BY: (SIGNATURE)	DATE/TIME	RECEIVED FOR LABORATORY BY: (SIGNATURE)	DATE/TIME	DATE/TIME	LABORATORY (NAME, CITY, STATE)		

TVA 29203 B (RG-CTR 4-94)

•COPY 1 - FIELD INVESTIGATOR •COPY 2 - FIELD INVESTIGATOR •COPY 3 - RETAINED BY LABORATORY

"Sample Chain of Custody"

10.2 Sample custody form - General



END OF PROCEDURE

Appendix B-2 – Moisture Analysis: Method ASTM E 871

Standard Method for Moisture Analysis of Particulate Wood Fuels[1]

This standard is issued under the fixed designation E 871; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This method covers the determination of total weight basis moisture in the analysis sample of particulate wood fuel. The particulate wood fuel may be sanderdust, sawdust, pellets, green tree chips, hogged fuel, or other type particulate wood fuel having a maximum particle volume of 16.39 cm^3 (1 $in.^3$). It is used for calculating other analytical results to a dry basis. Moisture, when determined as herein described, may be used to indicate yields on processes, to provide the basis for purchasing and selling, or to establish burning characteristics.

1.2 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.3 *This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 346 Practice for Collection and Preparation of Coke Samples for Laboratory Analysis[2]

D 2013 Method of Preparing Coal Samples for Analysis[2]

3. Summary of Method

3.1 Moisture is determined by establishing the loss in weight of the sample when heated under rigidly controlled conditions of temperature, time and atmosphere, sample weight, and equipment specifications.

4. Significance and Use

4.1 The test procedures described in this method can be used to determine the total weight basis moisture of any particulate wood fuel meeting the requirements specified in this method.

5. Apparatus

5.1 *Drying Oven*—For determining the moisture of wood, an ordinary drying oven with openings for natural air circulation and capable of temperature regulation of 103 ± 1°C shall be used.

5.2 *Open Containers*, nonporous glass, metal, or ceramic and of a configuration so as to accommodate the test sample. The minimum volume shall be 32.18 cm^3 (2 $in.^3$).

5.3 *Desiccator*, of sufficient size to contain the open container.

6. Procedure

6.1 *Sampling:*

6.1.1 *Place of Sampling*—Take the sample where the wood is being loaded into or unloaded from means of transportation or when discharged from storage bins or conveyors.

NOTE—Samples collected from the surface of piles are, in general, unreliable because of the exposure to the environment. If necessary, collect nine increments from a foot or more below the surface at nine points covering the pile.

6.1.2 *Collection of Gross Sample:*

6.1.2.1 Collect increments regularly, systematically, and with such frequency that the entire quantity of wood sampled will be represented proportionally in the gross sample.

6.1.2.2 The quantity of the sample shall be large enough to be representative but not less than 10 kg (22 lb).

6.1.2.3 Place the samples in an airtight container immediately after collection. Maintain the samples in the airtight container whenever possible to prevent gains or losses in moisture from the atmosphere.

6.1.3 Sample reduction may be done by two methods, a coning and dividing process, or by using a riffle. The operations of mixing, coning, and quartering are described in Practice D 346.

6.1.3.1 Accomplish coning and dividing reduction by placing the gross sample on a sheet of rubber or oil cloth. Thoroughly mix it by raising first one corner of the cloth and then the other. After mixing cone and quarter sample, continue the operations until the sample is reduced sufficiently so that one quarter weighs about 50 g (0.11 lb). This shall constitute a laboratory sample.

6.1.3.2 Accomplish riffle reduction using a standard coal riffle. Riffle the gross sample repeatedly until one half of the riffle sample equals about 50 g (0.11 lb), which will constitute a laboratory sample. Riffles and procedures are described in Method D 2013.

6.2 Dry sample container for 30 min at 103 ± 1°C in the oven, then cool in desiccator to room temperature. Weigh to the nearest 0.02 g and record as container weight, W_c. Place a minimum of 50 g of sample in the container, weigh the sample and container to the nearest 0.01 g, and record as initial weight, W_i.

6.3 Place the sample and container in the oven for 16 h at 103 ± 1°C.

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[1] This method is under the jurisdiction of ASTM Committee E-48 on Biotechnology and is the direct responsibility of Subcommittee E48.05 on Biomass Conversion Systems.

Current edition approved May 28, 1982. Published December 1982.

[2] *Annual Book of ASTM Standards*, Vol 05.05.

6.4 Remove the sample and the container from the oven and cool in the desiccator to room temperature. Remove the sample and container from the desiccator, weigh immediately to the nearest 0.01 g, and record the weight.

6.5 Return the sample and container to the oven at 103 ± 1°C for 2 h. Repeat 6.4.

6.6 Continue 6.4 until the total weight change between weighings varies less than 0.2 % and record as the final weight, W_f.

7. Calculation

7.1 Calculate the percent moisture in the analysis sample as follows:

$$\text{Moisture in analysis sample, \%} = [(W_i - W_f)/(W_i - W_c)] \times 100$$

where:

W_c = container weight, g,
W_i = initial weight, g, and
W_f = final weight, g.

8. Precision and Bias

8.1 The following criteria should be used for judging the acceptability of results:

8.1.1 *Repeatability*—Duplicate results by the same laboratory should not be considered suspect unless they differ by more than 0.5 %.

8.2.1 *Reproducibility*—The results submitted by two or more laboratories should not be considered suspect unless they differ by more than 1 %.

Appendix B-3 – Nitrate + Nitrite Nitrogen: Method AP-0058

Although the following procedure lists a post project approval date, the methods described herein accurately describe the procedures used during the study.

Tennessee Valley Authority

Analytical Laboratory of Environmental Applications
Environmental Research Center
Muscle Shoals, AL 35662

Procedure Number : AP-0058

Title: NO_3-N by Flow Injection Analysis

Signature	Title	Date
Prepared by: Sammie Smith Sammie Smith	Analytical Chemist	9/23/97
Concurred: Eugene A. Zarate Eugene A. Zarate	Laboratory Section Leader	9/23/97
Concurred: William J. Rogers William J. Rogers	QA Officer	9/22/97
Concurred:		
Approved: Joseph J. Hoagland Joseph J. Hoagland	Manager	9/23/97

Revision	R0					
Control Date	23-Sep-97					

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1.0 PURPOSE

This procedure provides a method for the determination of nitrate and nitrite in drinking, ground, and surface water, and domestic and industrial wastes.

2.0 SCOPE

2.1 This method covers the determination of nitrate and nitrite in drinking, ground, and surface waters, and domestic and industrial wastes.

2.2 The method is based on reactions that are specific for the nitrate and nitrite (NO_3^- and NO_2^-) ions.

2.3 The applicable range is 0.2 to 20.0 mg N/L.

3.0 SUMMARY

Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm. Nitrite alone can be determined by removing the cadmium column. Nitrate may be determined by difference.

4.0 REFERENCES

4.1 U.S. Environmental Protection Agency, *Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020, Revised March 1983, "Nitrogen, Nitrate-Nitrite, Method 353.2 (Colorimetric, Automated, Cadmium Reduction)."

4.2 *Methods for Determination of Inorganic Substances in Water and Fluvial Sediments*. Book 5. Chapter A1. U.S Department of the Interior, U.S. Geological Survey.

4.3 Lachat Instruments. *QuickChem Automated Ion Analyzer Methods Manual*, QuickChem Method 10-107-04-1-A, "Nitrate/Nitrite, Nitrite in Surface Water, Wastewater."

4.4 Lachat Instruments, *QuickChem 8000 Automated Ion Analyzer Omnion FIA Software Installation and Tutorial Manual.*

5.0 RESPONSIBILITIES

5.1 It is the responsibility of the laboratory manager to ensure that this procedure is followed.

5.2 It is the responsibility of the team leader to review the results of the procedure.

5.3 It is the responsibility of the analysts to follow this procedure, evaluate data, and to report any abnormal results or unusual occurrences to the team leader.

6.0 REQUIREMENTS

6.1 Prerequisites

6.1.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to ensure a representative sample and allow for quality control analysis (at least 100 mL).

6.1.2 Samples may be preserved by addition of a maximum of 2 mL of concentrated H_2SO_4 per liter (preferred - 1 mL of 1N H_2SO_4 per 100 mL) and stored at 4°C. Acid preserved samples have a holding time of 28 days.

6.2 Limitations and Actions

6.2.1 If the analyte concentration is above the analytical range of the calibration curve, the sample must be diluted to bring the analyte concentration within range.

6.2.2 Interferences

6.2.2.1 Residual chlorine can interfere by oxidizing the cadmium column.

6.2.2.2 Low results may be obtained for samples that contain high concentrations of iron, copper of other metals. In this method, EDTA is added to the buffer to reduce this interference.

6.2.2.3 Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference may be eliminated by extracting such samples with an organic solvent prior to analysis.

6.2.2.4 Sample color and turbidity may interfere. Turbidity can be removed by filtration through a 0.45 um pore diameter membrane filter prior to analysis. Sample color may be corrected by running the samples through the manifold without color formation (Sulfanilamide color reagent, reagent 3). The nitrate concentration is determined by subtracting the value obtained without color formation from the value obtained with color formation.

6.3 Apparatus/Equipment

6.3.1 Balance – analytical, capable of accurately weighing to the nearest 0.0001 g.

6.3.2 Glassware – Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3.3 Flow injection analysis equipment (Lachat model 8000) designed to deliver and react samples and reagents in the required order and ratios.

6.3.3.1 Autosampler

6.3.3.2 Multichannel proportioning pump

6.3.3.3 Reaction unit or manifold

6.3.3.4 Colorimeter detector

6.3.3.5 Data system

6.3.4 Special Apparatus

6.3.4.1 Cadmium Granules Column

6.3.4.1.1 Cadmium Preparation: Place **10-20 g of coarse cadmium granules** (0.3 - 1.5 mm diameter, Lachat Part # 50231) in a 250 mL beaker. Wash with **50 mL of acetone**, then **water**, then two **50 mL portions of 1 N hydrochloric acid** (reagent 4). Rinse several times with water. **Cadmium is toxic and carcinogenic. Wear gloves.**

6.3.4.1.2 Copperization: Add a **100 mL** portion of **2% copper sulfate solution** (reagent 5) to the cadmium prepared above. Swirl for about 5 minutes, then decant the liquid and repeat with a fresh **2% copper sulfate solution** (reagent 5). Continue this process until the blue aqueous **copper** color persists. Decant and wash with at least **five portions of ammonium chloride buffer solution** (reagent 2) to remove colloidal copper. The cadmium should be black or dark gray. The copperized granules may be **stored** in a **stoppered bottle under ammonium chloride buffer** (reagent 2).

6.3.4.1.3 Packing the Column

6.3.4.1.3.1 The empty cadmium column is available as Lachat Part # 50230. Wear gloves and do all cadmium transfers over a special tray or beaker dedicated to this purpose. Clamp the empty column upright so that both hands are free. Unscrew one of the colored fittings from an end of the column. Pull out and save the foam plug. The column and threads are glass so be careful not to break or chip them. Fasten this fitting higher than the open end of the column and completely fill the column, attached fittings, and tubing with ammonium chloride buffer (reagent 2).

6.3.4.1.3.2 Scoop up the prepared copperized cadmium granules with a spatula and pour them into the top of the filled column so that they sink down to the bottom of the column. Continue pouring the cadmium in and tapping the column with a screwdriver handle to dislodge any air bubbles and to prevent gaps in the cadmium filling. When the cadmium granules reach to about 5 mm from the open end of the column, push in the foam plug and screw on the top fitting. Rinse the outside of the column with water.

6.3.4.1.3.3 If air remains in the column or is introduced accidentally, connect the column into the manifold at the two state switching valve, pump ammonium chloride buffer (reagent 2) through the column with the pump on maximum, and tap firmly with a screwdriver handle, working up the column until all air is removed.

6.3.4.1.4 Cadmium Granules Column Instillation To Manifold

6.3.4.1.4.1 Before inserting the column, pump all reagents into the manifold.

6.3.4.1.4.2 Turn the pump off and immediately connect both column tubes to the two state switching valve used to place the column in line with the manifold. Do not let air enter the column.

6.3.4.1.4.3 Return the pump to normal speed. The direction of reagent flow through the column is not relevant.

6.3.4.2 Cadmium Wire Column

6.3.4.2.1 Join **two glass tubes, 122 cm x 1.5 mm each**, and bend into a "U" shape about **4 cm** apart. Secure the tubes on a **122 cm x 10 cm board** to prevent breaking. Let the open ends of the tubes **extend over the board about 5 cm** to make connections.

6.3.4.2.2 Cut **two 127 cm lengths of 0.050 inch diameter cadmium alloy wire** (95% cadmium, 5% silver).

6.3.4.2.3 Cadmium Wire Preparation: Wash wire with **acetone** to remove oil and grease, then **water**, then with **1 N hydrochloric acid** (reagent 4) to remove oxides. Rinse several times with **water**. **CAUTION: Collect and store all waste cadmium. Cadmium is toxic and carcinogenic. Wear gloves.**

6.3.4.2.4 Place the two lengths of cadmium wire into the two legs of the column using pliers. Push only about **5 to 6 mm** of wire at a time into the tube to avoid bending and kinking the wire. Push each wire down to the bend in the column as far as it will go. Connect the column to the **two state switching valve** on the manifold using short lengths of Tygon tubing and available fittings. Care should be taken to **minimize any dead volume.**

6.3.4.2.5 Copperization: Pump **2% copper sulfate solution** (reagent 5) through the column until the wire has a metallic appearance. Pump **ammonium chloride solution** (reagent 2) through the column for three to four minutes to remove colloidal copper. **Store** the column filled with **ammonium chloride solution** (reagent 2).

6.3.4.2.6 Cadmium Wire Column Instillation To Manifold

6.3.4.2.6.1 Before inserting the column, pump all reagents into manifold.

6.3.4.2.6.2 Turn the pump off and immediately connect both column tubes to the two state switching valve used to place the column in-line with the manifold.

6.3.4.2.6.3 Set the pump to normal speed.

6.3.4.2.6.4 The direction of reagent flow through the column is not relevant.

6.4 Reagents and Standards

6.4.1 Preparation of Reagents

Use deionized water (10 megohm) for all solutions.

Degassing with helium: To prevent bubble formation, degas all solutions except the standards with helium. Bubble helium through a degassing tube (Lachat Part 50100) through the solution for at least one minute.

Refrigerate all solutions and standards.

6.4.1.1 **Reagent 1. 15 N Sodium Hydroxide**

Add **150 g NaOH pellets** very slowly to **250 mL or g of water** or add **300 g 50% NaOH solution** very slowly to **100 mL or g of water. CAUTION**: The solution will get very hot! Swirl until dissolved. Cool and store in a plastic bottle.

6.4.1.2 **Reagent 2. Ammonium Chloride buffer, pH 8.5**

By Volume: In a **1 L** volumetric flask, dissolve **85.0 g ammonium chloride** (NH_4Cl) and **1.0 g disodium ethylenediamine tetraacetic acid dihydrate** ($Na_2EDTA \cdot 2H_2O$) in about **800 mL water**. Dilute to the mark and shake or stir to mix. Adjust the pH to **8.5** with **15 N sodium hydroxide solution** (reagent 1).

By weight: To a tared **1L** container, add **85.0 g ammonium chloride** (NH_4Cl), **1.0 g disodium ethylenediamine tetraacetic acid dihydrate** ($Na_2EDTA \cdot 2H_2O$) and **938 g water**. Shake or stir until dissolved. Then adjust the pH to **8.5** with **15 N sodium hydroxide solution** (reagent 1).

6.4.1.3 **Reagent 3. Sulfanilamide color reagent**

By Volume: To a **1 L** volumetric flask add about **600 mL water**. Then add **100 mL of 85% phosphoric acid** (H_3PO_4), **40.0 g sulfanilamide**, and **1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride** (NED). Shake to wet, and stir with a stir bar for 30 minutes to dissolve. Dilute to the mark, invert or stir to mix. Store in a dark bottle.

By weight: To a tared, dark **1 L** container add **876 g water, 170 g 85% phosphoric acid** (H_3PO_4), **40.0 g sulfanilamide**, and **1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride** (NED). Shake to wet, and stir with stir bar for 30 minutes until dissolved. Store in a dark bottle.

6.4.1.4 **Reagent 4. 1 N Hydrochloric Acid (HCl)**

By Volume: In a **100 mL** container, add **8 mL concentrated HCl** to **92 mL water**. Stir or shake to mix.

By weight: To a **100 mL** container, add **92 g water** then add **9.6 g concentrated HCl**. Stir or shake to mix.

6.4.1.5 **Reagent 5. 2% Copper Sulfate Solution**

By Volume: In a **1 L** volumetric flask, dissolve **20 g copper sulfate pentahydrate** ($CuSO_4 \cdot 5H_2O$) in about **800 mL water**. Dilute to mark with **water**. Invert to mix thoroughly.

By Weight: To a **1 L** container, add **20 g copper sulfate pentahydrate** ($CuSO_4 \cdot 5H_2O$) to **991 g water**. Stir or shake to dissolve.

6.4.2 Preparation of Standards

Note: Following are standards preparations for running 3 channels simultaneous for PO_4-P, NH_3-N and NO_2-N + NO_3-N. Also included is the preparation of a NO_2-N standard which is used to assess the cadmium reduction column's efficiency.

6.4.2.1 **Standard 1. Stock Orthophosphate Standard - 1000 mg P/L as PO_4^{3-}**

Dry **primary standard grade anhydrous potassium phosphate monobasic** (KH_2PO_4) for one hour at 105°C. In a **1 L** volumetric flask dissolve **4.396 g primary standard grade anhydrous potassium phosphate monobasic** (KH_2PO_4) in about **800 mL water**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.2 **Standard 2. Stock Ammonia Standard - 1000 mg N/L as NH_3**

Dry **ammonium chloride** (NH_4Cl) for two hours at 105°C. In a **1 L** volumetric flask dissolve **3.819 g ammonium chloride** (NH_4Cl) in about **800 mL water**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.3 **Standard 3. Stock Nitrate Standard - 1000 mg N/L as NO_3^-**

In a **1 L** volumetric flask dissolve **7.220 g potassium nitrate** (KNO_3) in about **600 mL water**. Add **2 mL chloroform**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.4 **Standard 4. Stock Nitrite Standard - 1000 mg N/L as NO_2^-**

In a **1 L** volumetric flask dissolve **4.93 g sodium nitrate** ($NaNO_2$) in about **800 mL water**. Add **2 mL chloroform**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.5 **Standard 5. Working Standard - 50 mg/L PO_4-P, NH_3-N and NO_3-N**

In a **1 L** volumetric flask add about **600 mL water**. Pipette **50 mL** from each of the **Stock Orthophosphate Standard** (standard 1), the **Stock Ammonia Standard** (standard 2), and the **Stock Nitrate Standard** (standard 3). Dilute to mark with **water** and mix.

6.4.2.6 **Standard 6. Working Nitrite Standard - 20 mg N/L as NO_2^-**

In a **1 L** volumetric flask add about **700 mL water**. Pipette **20 mL Stock Nitrate Standard** (standard 4). Dilute to mark with **water** and mix.

6.4.2.7 **Standard 7. Working Quality Control Standard - 32.61 mg P/L as PO_4^{3-}, 31.06 mg N/L as NH_4, and 27.11 mg N/L as NO_3^-.**

In a **500 mL** volumetric flask add about **300 mL water**. Pipette **50 mL** of the E M Science **1000 mg/L Phosphate Standard Solution** (326.1 mg P/L), **20 mL** of the E M Science **1000 mg/L Ammonia Standard Solution** (776.5 mg N/L), and **60 mL** of the E M Science **1000 mg/L Nitrate Standard Solution** (225.9 mg N/L). Dilute to mark with **water** and mix.

Note: 1000 mg/L standards by other reputable laboratory vendors may be substituted.

6.4.2.8 **Calibration Standards**

Standards are diluted to **500 mL** with **water.**

	Calibration Standards Concentration mg/L	Prepared From	
		Concentration mg/L	Aliquot mL
1	20.00	50	200
2	10.00	50	100
3	4.00	50	40
4	2.50	50	25
5	1.00	10	50
6	0.10	1	50
7	0.02	0.10	100
8	0.00	Water	0

For standards for samples that have 1 mL of 1 N H_2SO_4 added per 100 mL, add **5 mL** of **1N H_2SO_4** to each standard after building to volume.

Note: If other acid concentrations are used to preserve samples, match for standards.

6.4.2.9 **Cadmium Reduction Column Efficiency Check Standard - 2.00 mg N/L as NO_2^-**

In a **500 mL** volumetric flask add about **300 mL water**. Pipette **50 mL** of the **Working Nitrite Standard** (standard 6). Dilute to mark with **water,** add **5 mL** of **1N H_2SO_4** and mix.

6.4.2.10 **Laboratory Control Standard - 1.63 mg P/L as PO_4^{3-}, 1.55 mg N/L as NH_3, and 1.36 mg N/L as NO_3^-.**

In a **1 L** volumetric flask add about **700 mL water**. Pipette **50 mL** of the **Working Quality Control Standard** (standard 7). Dilute to mark with **water,** add **10 mL** of **1N H_2SO_4** and mix.

6.5 Quality Control Sample Requirements

Begin and end each run by measuring a laboratory control standard, a midpoint calibration standard run as a sample, a cadmium reduction column efficiency check standard, and a reagent blank. When the run is long enough, every twentieth sample should be followed by the above four QC check samples. Recovery should be 90 to 110% of the expected value.

7.0 PROCEDURE

7.1 Procedure Instructions

7.1.1 The instrument is calibrated each day of use and may be calibrated with each sample tray.

7.1.2 Prepare reagents and standards as described in section 6.4.

7.1.3 Set up manifold as shown in section 9.3.

7.1.4 Enter data system parameters as in section 9.1 or 9.2.

7.1.5 Pump deionized water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

7.1.6 Load standard and sample trays.

7.1.7 Place samples and standards in the autosampler. Enter the information required by the data system, such as standard concentration, and sample identification.

7.1.8 Calibrate the instrument by injecting the standards. The data system will then associate the concentration with the instrument responses for each standard.

7.1.9 If samples require color correction, inject the samples with color development, then inject the samples with water replacing the color reagent (reagent 3).

7.1.10 At end of run, turn the two state switching valve to isolate the cadmium reduction column. Remove all transmission lines from reagents and place them in water. Pump for about five minutes.

7.1.11 Remove the transmission lines from the water and pump all lines dry.

7.2 Calculations and Recording Data

7.2.1 Calibration is done by injecting standards. The data system will then automatically prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation provided by the software.

7.2.2 Create a custom report. (Lachat Instruments, QuickChem 8000 Automated Ion Analyzer Omnion FIA Software Installation and Tutorial Manual, page 43, Task 11 - Creating a Custom Report)

7.2.3 Report only those values that fall between the lowest and highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

7.2.4 Samples that require color correction: From the value obtained with color developer added, subtract the value obtained without color developer. When a large number of samples are analyzed, use a spreadsheet to calculate the color correction.

7.2.5 Report results in mg NO_3-N/L.

8.0 SAFETY

8.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Use routine laboratory protective clothing (lab coat, gloves, and eye protection) when handling these

reagents. Thoroughly wash any skin that comes into contact with any of these chemicals. Avoid creating or inhaling dust or fumes from solid chemicals.

9.0 NOTES

9.1 Data System Parameters - Cadmium Granules Reduction Column

Method Filename:	PANHANOA.MET
Method Description:	Ortho P (a) = 4.0 to 0.02 mg P/L
	NH_3-N (a) = 20.0 to 0.1 mg N/L
	NO_2-N/NO_3-N (a) = 20.0 to 0.2 mg N/L

Analyte Data:

Analyte Name:	Nitrate (NO_3)-N
Concentration Units:	mg NO_3-N/L
Chemistry:	Direct
Inject to Peak Start (s):	22.0
Peak Base Width (s):	29.000
% Width Tolerance:	100.000
Threshold:	4100.000
Autodilution Trigger:	Off
QuickChem Method:	10-107-04-1-A

Calibration Data:

Levels: (mg NO_3-N/L)	1: 20.000	2: 10.000	3: 4.000
	5: 1.000	6: 0.100	8: 0.000
Calibration Rep Handling:	Average		
Calibration Fit Type:	1st Order Poly		
Force through Zero:	No		
Weighing Method:	None		

Concentration Scaling: None

Sampler Timing:

Method Cycle Period: 50.0

Min. Probe in Wash Period: 9.0

Probe in Sample Period: 25.0

Valve Timing:

Method Cycle Period: 50.0

Sample Reaches 1st Valve: 18.0

Valve: On

Load Time: 0.0

Load period 20.0

Inject Period: 30.0

Sample Loop: Microloop

9.2 Data System Parameters - Cadmium Wire Reduction Column

Method Filename: PANHANOW.MET

Method Description: Ortho P (a) = 4.0 to 0.02 mg P/L

NH_3-N (a) = 20.0 to 0.1 mg N/L

NO_2-N/NO_3-N (a) = 20.0 to 0.2 mg N/L

Analyte Data:

Analyte Name: Nitrate (NO_3)-N

Concentration Units: mg NO_3-N/L

Chemistry: Direct

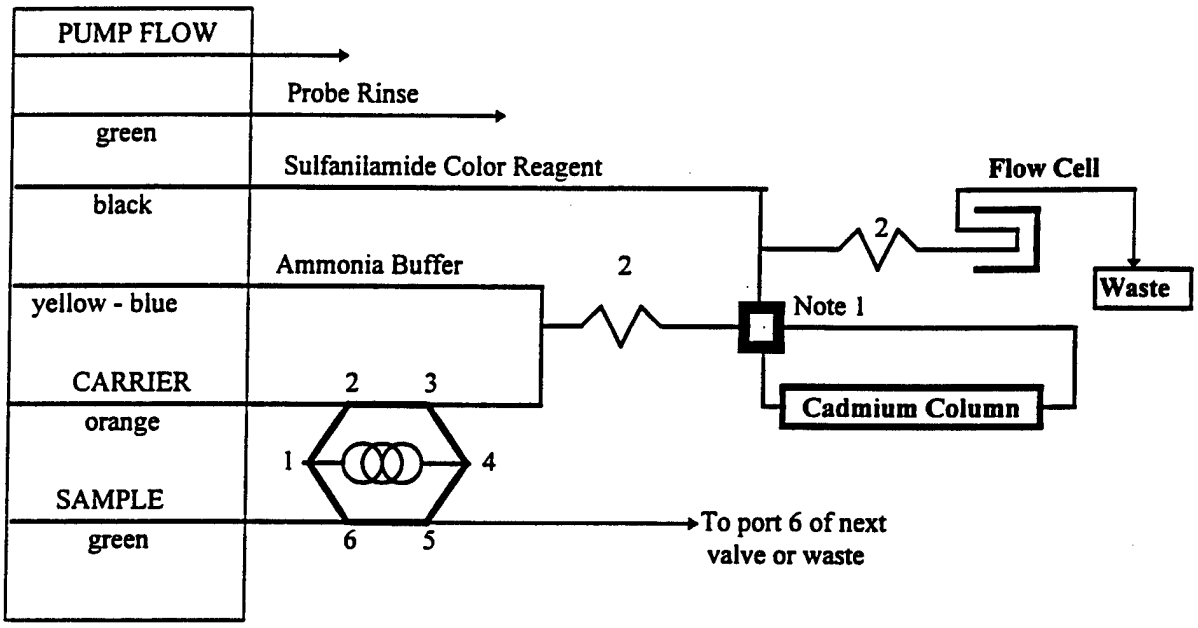
Inject to Peak Start (s): 50.5

Peak Base Width (s): 29.000

% Width Tolerance: 100.000

Threshold:	4100.000		
Autodilution Trigger:	Off		
QuickChem Method:	10-107-04-1-A		
Calibration Data:			
Levels: (mg NO_3-N/L)	1: 20.000	2: 10.000	3: 4.000
	5: 1.000	6: 0.100	8: 0.000
Calibration Rep Handling:	Average		
Calibration Fit Type:	1st Order Poly		
Force through Zero:	No		
Weighing Method:	None		
Concentration Scaling:	None		
Sampler Timing:			
Method Cycle Period:	70.0		
Min. Probe in Wash Period:	9.0		
Probe in Sample Period:	30.0		
Valve Timing:			
Method Cycle Period:	70.0		
Sample Reaches 1st Valve:	18.0		
Valve:	On		
Load Time:	0.0		
Load period	25.0		
Inject Period:	45.0		
Sample Loop:	Microloop		

9.3 Nitrate Manifold Diagram



Sample Loop = Microloop Interference Filter = 520 nm

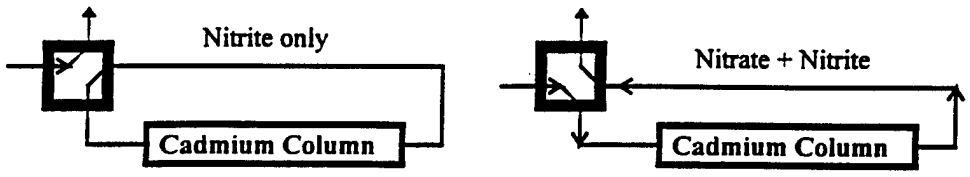
Carrier is DI Water

All manifold tubing is **0.8 mm (0.32 in) i.d.** Lachat Part No. 50028. This is **5.2 uL/cm.**

2 is **70 cm** of tubing on a **4.5 cm** coil support.

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

Note 1: This is a 2 state switching valve used to place the cadmium column in-line with the manifold.



10.0 ATTACHMENTS AND APPENDICES

None

End of Procedure

Appendix B-4 – Ammonium Nitrogen: Method AP-0059

Although the following procedure lists a post project approval date, the methods described herein accurately describe the procedures used during the study.

Tennessee Valley Authority

Analytical Laboratory of Environmental Applications
Environmental Research Center
Muscle Shoals, AL 35662

Procedure Number : AP-0059

Title: NH_4-N by Flow Injection Analysis

Signature	Title	Date
Prepared by: Sammie Smith Sammie Smith	Analytical Chemist	9/24/97
Concurred: Eugene A. Zarate Eugene A. Zarate	Laboratory Section Leader	9/24/97
Concurred: William J. Rogers William J. Rogers	QA Officer	9/24/97
Concurred:		
Approved: Joseph J. Hoagland	Manager	9/24/97

Revision	R0					
Control Date	24-Sep-97					

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1.0 PURPOSE

This procedure provides a method for the determination of ammonia in drinking and surface waters.

2.0 SCOPE

2.1 This method covers the determination of ammonia in drinking and surface waters.

2.2 The method is based on reactions that are specific for the ammonium ion.

2.3 The applicable range is 0.1 to 20.0 mg N/L as NH3.

3.0 SUMMARY

This method is based on the Berthelot reaction. Ammonia reacts with alkaline phenol, then with sodium hypochlorite to form indophenol blue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm, and is directly proportional to the original ammonia concentration in the sample.

4.0 REFERENCES

4.1 U.S. Environmental Protection Agency, *Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020, Revised March 1983, "Nitrogen, Ammonia, Method 350.1 (Colorimetric, Automated Phenate)."

4.2 U.S. Environmental Protection Agency, 40 CFR Part 36 Table 1B, footnote 6, 1994.

4.3 Lachat Instruments, *QuickChem Automated Ion Analyzer Methods Manual*, QuickChem Method 10-107-06-1-A, "Determination Of Ammonia By Flow Injection Analysis, Colorimetry."

4.4 Lachat Instruments, *QuickChem 8000 Automated Ion Analyzer Omnion FIA Software Installation and Tutorial Manual.*

5.0 RESPONSIBILITIES

5.1 It is the responsibility of the laboratory manager to ensure that this procedure is followed.

5.2 It is the responsibility of the team leader to review the results of the procedure.

5.3 It is the responsibility of the Analysts to follow this procedure, evaluate data, and to report any abnormal results or unusual occurrences to the team leader.

6.0 REQUIREMENTS

6.1 Prerequisites

6.1.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to ensure a representative sample and allow for quality control analysis (at least 100 mL).

6.1.2 Samples may be preserved by addition of a maximum of 2 mL of concentrated H_2SO_4 per liter (preferred - 1 mL of 1N H_2SO_4 per 100 mL) and stored at 4°C. Acid preserved samples have a holding time of 28 days.

6.2 Limitations and Actions

6.2.1 If the analyte concentration is above the analytical range of the calibration curve, the sample must be diluted to bring the analyte concentration within range.

6.2.2 Interferences

6.2.2.1 Calcium and magnesium ions may precipitate if present in sufficient concentration. Tartrate or EDTA is added to the sample in-line in order to prevent this problem.

6.2.2.2 Color, turbidity and certain organic species may interfere. Turbidity can be removed by filtration through a 0.45 um pore diameter membrane filter prior to analysis. Sample color may be corrected for by running the samples through the

manifold without color formation (omit Sodium Phenolate, reagent 1). The ammonium concentration is determined by subtracting the value obtained without color formation from the value obtained with color formation.

6.3 Apparatus/Equipment

6.3.1 Balance – analytical, capable of accurately weighing to the nearest 0.0001 g.

6.3.2 Glassware – Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3.3 Flow injection analysis equipment (Lachat model 8000) designed to deliver and react samples and reagents in the required order and ratios.

6.3.3.1 Autosampler

6.3.3.2 Multichannel proportioning pump

6.3.3.3 Reaction unit or manifold

6.3.3.4 Colorimetric detector

6.3.3.5 Data system

6.3.4 Special Apparatus

6.3.4.1 Heating Unit

6.4 Reagents and Standards

6.4.1 Preparation of Reagents -

Use deionized water (10 megohm) for all solutions.

Degassing with helium: To prevent bubble formation, degas all solutions except the standards, Sodium Phenolate (Reagent 1) and Sodium Hypochlorite (Reagent 2) with helium. Bubble helium through a degassing tube (Lachat Part 50100) through the solution for at least one minute.

Refrigerate all solutions and standards.

6.4.1.1 **Reagent 1. Sodium Phenolate**

CAUTION: Wear gloves. Phenol causes severe burns and is rapidly absorbed in the body through the skin.

By Volume: In a **1 L** volumetric flask, dissolve **88 mL** of **88% liquefied phenol** or **83 g crystaline phenol** (C_6H_5OH) in approximately **600 mL water**. While stirring, slowly add **32 g sodium hydroxide** (NaOH). Cool, dilute to the mark, and mix. Do not degas this reagent.

By weight: To a tared **1 L** container, add **888 g water**. Add **94.2 g** of **88 liquefied phenol** or **83 g crystalline phenol** (C_6H_5OH). While stirring, slowly add **32 g sodium hydroxide** (NaOH). Cool and invert to mix. Do not degas this reagent.

6.4.1.2 **Reagent 2. Sodium Hypochlorite**

By Volume: In a **500 mL** volumetric flask, dilute **250 mL Regular Clorox bleach** [5.25% sodium hypochlorite (NaOCl), The Clorox Company, Oakland, CA] to mark with **water**. Invert to mix.

By weight: To a tared **500 mL** container, add **250 g Regular Clorox bleach** [5.25% sodium hypochlorite (NaOCl), The Clorox Company, Oakland, CA] and **250 g water**. Invert to mix.

6.4.1.3 **Reagent 3. Buffer**

By Volume: In a **1 L** volumetric flask, dissolve **50.0 g disodium ethylenediamine tetraacetate dihydrate** ($Na_2EDTA \cdot 2H_2O$) and **5.5 g sodium hydroxide** (NaOH) in about **900 mL water**. Dilute to the mark and invert or stir to mix.

By weight: To a tared **1 L** container, add **50.0 g disodium ethylenediamine tetraacetate dihydrate** ($Na_2EDTA \cdot 2H_2O$) and **5.5 g sodium hydroxide** (NaOH). Add **968 g water**. Invert or stir to mix.

6.4.1.4 **Reagent 4. Sodium Nitroprusside**

By Volume: In a **1 L** volumetric flask, dissolve **3.50 g sodium nitroprusside** (Sodium Nitroferrricyanide [$Na_2Fe(CN)5NO \cdot 2H2O$]) dilute to the mark with **water**. Stir or shake to mix.

By weight: To a tared **1 L** flask, dissolve **3.50 g sodium nitroprusside** (Sodium Nitroferrricyanide [$Na_2Fe(CN)_5NO \cdot 2H_2O$]) and **1000 g water**. Stir or shake to mix.

6.4.2 Preparation of Standards

Note: Following are standards preparations for running 3 channels simultaneously for PO_4-P, NH_3-N and NO_2-N + NO_3-N. Also included is the preparation of a NO_2-N standard which is used to assess the cadmium reduction column's efficiency.

6.4.2.1 **Standard 1. Stock Orthophosphate Standard - 1000 mg P/L as PO_4**

Dry **primary standard grade anhydrous potassium phosphate monobasic** (KH_2PO_4) for one hour at 105°C. In a **1 L** volumetric flask dissolve **4.396 g primary standard grade anhydrous potassium phosphate monobasic** (KH_2PO_4) in about **800 mL water**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.2 **Standard 2. Stock Ammonia Standard - 1000 mg N/L as NH_3**

Dry **ammonium chloride** (NH_4Cl) for two hours at 105°C. In a **1 L** volumetric flask dissolve **3.819 g ammonium chloride** (NH_4Cl) in about **800 mL water**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.3 **Standard 3. Stock Nitrate Standard - 1000 mg N/L as NO_3^-**

In a **1 L** volumetric flask dissolve **7.220 g potassium nitrate** (KNO_3) in about **600 mL water**. Add **2 mL chloroform**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.4 **Standard 4. Stock Nitrite Standard - 1000 mg N/L as NO_2^-**

In a **1 L** volumetric flask dissolve **4.93 g sodium nitrate** ($NaNO_2$) in about **800 mL water**. Add **2 mL chloroform**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.5 **Standard 5. Working Standard - 50 mg/L PO_4-P, NH_3-N and NO_3-N**

In a **1 L** volumetric flask add about **600 mL water**. Pipette **50 mL** from each of the **Stock Orthophosphate Standard** (standard 1), the **Stock Ammonia Standard** (standard 2), and the **Stock Nitrate Standard** (standard 3). Dilute to mark with **water** and mix.

6.4.2.6 **Standard 6. Working Nitrite Standard - 20 mg N/L as NO_2^-**

In a **1 L** volumetric flask add about **700 mL water**. Pipette **20 mL Stock Nitrate Standard** (standard 4). Dilute to mark with **water** and mix.

6.4.2.7 **Standard 7. Working Quality Control Standard - 32.61 mg P/L as PO_4^{3-}, 31.06 mg N/L as NH_4, and 27.11 mg N/L as NO_3^-.**

In a **500 mL** volumetric flask add about **300 mL water**. Pipette **50 mL** of the E M Science **1000 mg/L Phosphate Standard Solution** (326.1 mg P/L), **20 mL** of the E M Science **1000 mg/L Ammonia Standard Solution** (776.5 mg N/L), and **60 mL** of the E M Science **1000 mg/L Nitrate Standard Solution** (225.9 mg N/L). Dilute to mark with **water** and mix.

Note: 1000 mg/L standards by other reputable laboratory vendors may be substituted.

6.4.2.8 **Calibration Standards**

Standards are diluted to **500 mL** with **water.**

	Calibration Standards Concentration mg/L	Prepared From	
		Concentration mg/L	Aliquot mL
1	20.00	50	200
2	10.00	50	100
3	4.00	50	40
4	2.50	50	25
5	1.00	10	50
6	0.10	1	50
7	0.02	0.10	100
8	0.00	Water	0

For standards for samples that have 1 mL of 1 N H_2SO_4 added per 100 mL, add **5 mL** of **1N H_2SO_4** to each standard after building to volume.

Note: If other acid concentrations are used to preserve samples, match for standards.

6.4.2.9 **Cadmium Reduction Column Efficiency Check Standard - 2.00 mg N/L as NO_2^-**

In a **500 mL** volumetric flask add about **300 mL water**. Pipette **50 mL** of the **Working Nitrite Standard** (standard 6). Dilute to mark with **water,** add **5 mL** of **1N H_2SO_4** and mix.

6.4.2.10 **Laboratory Control Standard - 1.63 mg P/L as PO_4, 1.55 mg N/L as NH_3, and 1.36 mg N/L as NO_3^-.**

In a **1 L** volumetric flask add about **700 mL water**. Pipette **50 mL** of the **Working Quality Control Standard** (standard 7). Dilute to mark with **water,** add **10 mL** of **1N H_2SO_4** and mix.

6.5 Quality Control Sample Requirements

Begin and end each run by measuring a laboratory control standard, a midpoint calibration standard run as a sample, and a reagent blank. When the run is long enough, every twentieth sample should be followed by the above three QC check samples. Recovery should be 90 to 110% of the expected value.

7.0 PROCEDURE

7.1 Procedure Instructions

7.11 The instrument is calibrated each day of use and may be calibrated with each sample tray.

7.1.2 Prepare reagents and standards as described in section 6.4.

7.1.3 Set up manifold as shown in section 9.2.

7.1.4 Enter data system parameters as in section 9.1.

7.1.5 Pump deionized water through all reagent lines and check for leaks and smooth flow. Allow 15 minutes for heating unit to warm up to 60°C. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

7.1.6 Load standard and sample trays.

7.1.7 Place samples and standards in the autosampler. Enter the information required by the data system, such as standard concentration, and sample identification.

7.1.8 Calibrate the instrument by injecting the standards. The data system will then associate the concentration with the instrument responses for each standard.

7.1.9 If samples require color correction, inject the samples with color development, then inject the samples with water replacing the color reagent (reagent 1).

7.1.10 At end of run, remove all transmission lines from reagents and place them in water. Pump for about five minutes.

7.1.11 To prevent baseline drifts, peaks that are too wide, or other problems with NH3-N precision, clean the NH_3-N manifold by placing the manifold reagent lines in 1M hydrochloric acid (1 volume concentrated HCl added to 11 volumes of water). Pump for about 5 minutes.

7.1.12 Remove all reagent lines from the hydrochloric acid and place them in water. Pump until the HCl is thoroughly washed out (about 5 minutes).

7.1.13 Remove the transmission lines from the water and pump all lines dry.

7.2 Calculations and Recording Data

7.2.1 Calibration is done by injecting standards. The data system will then automatically prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation provided by the software.

7.2.2 Create a custom report. (Lachat Instruments, *QuickChem 8000 Automated Ion Analyzer Omnion FIA Software Installation and Tutorial Manual*, page 43, "Task 11 - Creating a Custom Report")

7.2.3 Report only those values that fall between the lowest and highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

7.2.4 Samples that require color correction: From the value obtained with color developer added, subtract the value obtained without color developer. When a large number of samples are analyzed, use a spreadsheet to calculate the color correction.

7.2.5 Report results in mg NH_3-N/L.

8.0 SAFETY

8.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Use routine laboratory protective clothing (lab coat, gloves, and eye protection) when handling these reagents. Thoroughly wash any skin that comes into contact with any of these chemicals. Avoid creating or inhaling dust or fumes from solid chemicals.

9.0 NOTES

9.1 Data System Parameters

Method Filename:	PANHANOW.MET
Method Description:	Ortho P (a) = 4.0 to 0.02 mg P/L
	NH_3-N (a) = 20.0 to 0.1 mg N/L
	NO_2-N/NO_3-N (a) = 20.0 to 0.2 mg N/L

Analyte Data:

Analyte Name:	Ammonia (NH_3)-N
Concentration Units:	mg NH_3-N/L
Chemistry:	Direct
Inject to Peak Start (s):	28.0
Peak Base Width (s):	21.000
% Width Tolerance:	100.000
Threshold:	8000.000
Autodilution Trigger:	Off
QuickChem Method:	10-107-06-1-A

Calibration Data:

Levels: (mg NH_3-N/L)	1: 20.000	2: 10.000	3: 4.000
	5: 1.000	6: 0.100	8: 0.000
Calibration Rep Handling:	Average		
Calibration Fit Type:	1st Order Poly		
Force through Zero:	No		
Weighing Method:	None		
Concentration Scaling:	None		

Sampler Timing:

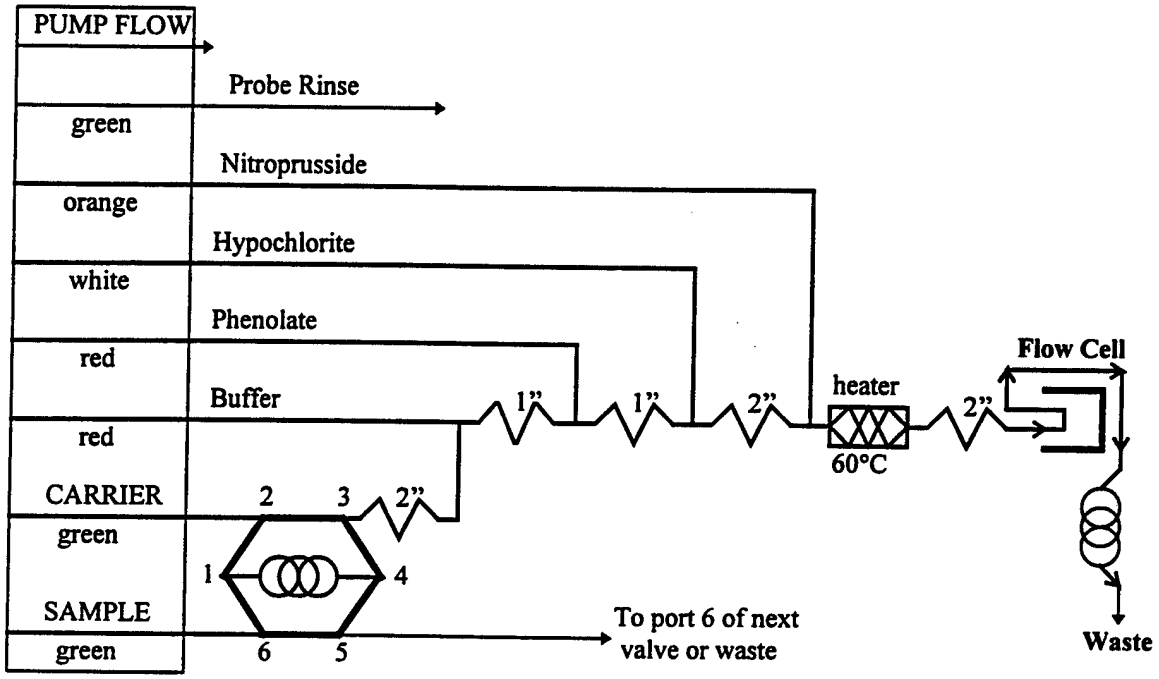
Method Cycle Period:	70.0
Min. Probe in Wash Period:	9.0
Probe in Sample Period:	30.0

Valve Timing:

Method Cycle Period:	70.0
Sample Reaches 1st Valve:	18.0
Valve:	On
Load Time:	0.0
Load period	25.0
Inject Period:	45.0

Sample Loop: 13 cm x 0.5 mm i.d.

9.2 Ammonia Manifold Diagram



Sample Loop = 13 cm x 0.5 mm i.d.

Interference Filter = 630 nm

Carrier is DI Water

All manifold tubing is **0.8 mm (0.32 in) i.d.** Lachat Part No. 50028. This is **5.2 uL/cm**. The sample loop uses **0.5 mm (0.022") i.d.** tubing.

1 is **70 cm** of tubing on a **4.5 cm** coil support.

Apparatus: The 60°C includes 650 cm of tubing wrapped around the heater block at the specified temperature.

10.0 ATTACHMENTS AND APPENDICES

None

End of Procedure

Appendix B-5 – Total Kjeldahl Nitrogen: AP-0064

Although the following procedure lists a post project approval date, the methods described herein accurately describe the procedures used during the study.

Tennessee Valley Authority

Analytical Laboratory of Environmental Applications
Environmental Research Center
Muscle Shoals, AL 35662

Procedure Number : AP-0064

Title: TKN by Flow Injection Analysis (Lachat QuickChem 8000)

Signature	Title	Date
Prepared by: Sammie C. Smith Sammie Smith	Chemist	10/15/97
Concurred: Eugene A. Zarate Eugene A. Zarate	Laboratory Section Leader	10/15/97
Concurred: William J. Rogers William J. Rogers	QA Officer	10/15/97
Concurred:		
Approved: Joseph J. Hoagland	Manager	10/15/97

Revision	R0					
Control Date	17-Oct-97					

Copy No: ___ has been issued to holder on _____

1.0 PURPOSE

This procedure provides a method for the determination of total Kjeldahl nitrogen (TKN) in water and wastewater.

2.0 SCOPE

2.1 This method covers the determination of total Kjeldahl nitrogen in water and wastewater.

2.2 The colorimetric method is based on reactions that are specific for the ammonia ion. The digestion converts organic forms of nitrogen to the ammonium form. Nitrate is not converted to ammonium during digestion.

2.3 The applicable range is 0.1 to 20 mg N/L.

2.4 Samples containing particulates should be filtered or homogenized.

3.0 SUMMARY

3.1 The sample is heated in the presence of sulfuric acid, H_2SO_4, for two and one half hours. The residue is cooled. diluted with water and analyzed for ammonia. This digested sample may also be used for total phosphorus determination.

3.2 Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of the digestion described.

3.3 Organic nitrogen is obtained by subtracting the free-ammonia concentration from the Kjeldahl nitrogen concentration.

3.4 Approximately 0.3 mL of the digested sample is injected onto the chemistry manifold where its pH is controlled by raising it to a known. basic pH by neutralization and with a concentrated buffer. This in-line neutralization converts the ammonium cation to ammonia. and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction which follows.

3.5 The ammonia thus produced is heated with salicylate and hypochlorite to produce blue color which is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of potassium tartrate in the buffer prevents precipitation of calcium and magnesium.

4.0 REFERENCES

4.1 U.S. Environmental Protection Agency, *Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020, Revised March 1983, "Nitrogen, Kjeldahl, Total, Method 351.2 (Colorimetric, Semi-Automatic Block Digestor, AAII) "

4.2 U.S. Environmental Protection Agency, *Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020, Revised March 1983, "Nitrogen, Ammonia, Method 350.1 (Colorimetric, Automated Phenate)."

4.3 ASTM , Water(I), Volume 11.01, Method D3590-89, "Test Methods for Kjeldahl Nitrogen in water", p. 447.

4.4 Code of Federal Regulations 40, Chapter 1, Part 136, Appendix B.

4.5 Lachat Instruments, *QuickChem Automated Ion Analyzer Methods Manual*, QuickChem Method 10-107-06-2-D, "Determination Of Total Kjeldahl Nitrogen By Flow Injection Analysis, Colorimetry (Block Digestor Method)."

4.6 Lachat Instruments, *QuickChem 8000 Automated Ion Analyzer Omnion FIA Software Installation and Tutorial Manual.*

5.0 RESPONSIBILITIES

5.1 It is the responsibility of the laboratory manager to ensure that this procedure is followed.

5.2 It is the responsibility of the team leader to review the results of the procedure.

5.3 It is the responsibility of the Analysts to follow this procedure, evaluate data, and to report any abnormal results or unusual occurrences to the team leader.

6.0 REQUIREMENTS

6.1 Prerequisites

6.1.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to ensure a representative sample and allow for quality control analysis (at least 100 mL).

6.1.2 Samples may be preserved by addition of a maximum of 2 mL of concentrated H_2SO_4 per liter (preferred - 1 mL of 1N H_2SO_4 per 100 mL) and stored at 4°C. Acid preserved samples have a holding time of 28 days.

6.2 Limitations and Actions

6.2.1 If the analyte concentration is above the analytical range of the calibration curve, the sample must be diluted with reagent 7 to bring the analyte concentration within range.

6.2.2 Interferences

6.2.2.1 Samples must not consume more than 10% of the sulfuric acid during digestion (one mL of sulfuric acid should remain after digestion). The buffer will accommodate a range of 4.5-5.0% (v/v) H_2SO_4 in the digested sample with no change in signal intensity.

6.2.2.2 High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

6.2.2.3 Digests must be free of turbidity. Some boiling stones have been shown to crumble upon vigorous vortexing.

6.3. Apparatus/Equipment

6.3.1 Balance – analytical. capable of accurately weighing to the nearest 0.0001 g.

6.3.2 Glassware – Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3.3 Flow injection analysis equipment (Lachat model 8000) designed to deliver and react samples and reagents in the required order and ratios.

6.3.3.1 Autosampler

6.3.3.2 Multichannel proportioning pump

6.3.3.3 Reaction unit or manifold

6.3.3.4 Colorimetric detector

6.3.3.5 Data system

6.3.3.6 10 nm band pass. 80 uL. glass flow cell

6.3.3.7 660 nm interference filter

6.3.3.8 Helium degassing tube

6.3.4 Special Apparatus

6.3.4.1 Heating Unit

6.3.4.2 75 mL digestion tubes with cold fingers

6.3.4.3 Digestion tube rack

6.3.4.4 Cold finger rack assembly

6.3.4.5 Block Digestor

6.3.4.6 5 mL dispenser

6.3.4.7 10 mL dispenser

6.3.4.8 Vortex mixer

6.3.4.9 Countdown timer

6.4 Reagents and Standards

6.4.1 Preparation of Reagents -

Use deionized water (10 megohm) for all solutions.

Degassing with helium: To prevent bubble formation. degas all solutions with helium except the standards. Mercuric Sulfate Solution (Reagent 1) and Digestion Solution (Reagent 2). Bubble helium through a degassing tube (Lachat Part 50100) into the solution for at least one minute.

Refrigerate all solutions and standards.

6.4.1.1 **Reagent 1. Mercuric Sulfate Solution**

By Volume: To a **100 ml** volumetric flask add **40.0 mL water** and **10 mL concentrated sulfuric acid (H_2SO_4)**. Then add **8.0 g red mercuric oxide (HgO)**. Stir until dissolved, dilute to the mark and invert to mix. Warming the solution while stirring may be required to dissolve the mercuric oxide.

6.4.1.2 **Reagent 2 Digestion Solution**

By Volume: To a **1 L** volumetric flask. add **133.0 g potassium sulfate** (K_2SO_4) and **200 mL concentrated sulfuric acid** (H_2SO_4) to approximately **700 mL water**. Add **25.0 mL Reagent 1**. Dilute to the mark with water and invert to mix.

6.4.1.3 **Reagent 3. Buffer**

Note: To reduce the possibility of the potassium tartrate being contaminated, it is recommended that the tartrate buffer is boiled for 10 minutes. To verify that the tartrate buffer is pure enough, compare the reagent baseline to the DI water baseline. The baseline, with all reagents flowing should not be greater than 0.15V different from just the DI water pumping in all lines.

By Volume: In a **1L** container add **900 mL water, 50 g potassium tartrate** (or potassium sodium tartrate, $NaKC_4H_4O_6 \cdot 4H_2O$), **50 g sodium hydroxide** (NaOH), and **26.8 g sodium phosphate dibasic heptahydrate** ($Na_2HPO_4 \cdot 7H_2O$). Mix until dissolved. Boil for 10 minutes. Cool to room temperature and transfer to a **1L** volumetric flask. Dilute to the mark and invert to mix.

6.4.1.4 **Reagent 4. Sodium Hydroxide (0.8 M)**

By Volume: In a **1 L** volumetric flask dissolve **32 g sodium hydroxide** (NaOH) in about **800 mL of water**. Dilute to the mark and stir to mix.

By Weight: In a **1 L** container dissolve **32 g sodium hydroxide** (NaOH) in **985 g of water** and mix.

6.4.1.5 **Reagent 5. Salicylate Nitroprusside**

By Volume: In a **1 L** volumetric flask dissolve **150.0 g sodium salicylate** [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], and **1.00 g sodium nitroprusside** [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$] in about **800 mL water**. Dilute to the mark and mix. Store in a dark bottle.

By Weight: To a tared **1 L** dark container, add **150.0 g sodium salicylate** [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], and **1.00 g sodium nitroprusside** [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$] and **908 g water**. Mix and store in a dark bottle.

6.4.1.6 **Reagent 6. Hypochlorite Solution**

By Volume: In a **250 mL** volumetric flask. dilute **15.0 mL Regular Clorox Bleach** (5.25% sodium hypochlorite. The Clorox Company, Oakland CA) to the mark with water. Invert to mix.

By Weight: To a tared **250 mL** container. add **16 g of Regular Clorox Bleach** (5.25% sodium hypochlorite. The Clorox Company , Oakland CA) and **234 g water**. Shake to mix.

6.4.1.7 **Reagent 7. Diluent**

Note: Diluent is used for the **carrier** and for off line dilutions.

By Volume: In a **1 L** volumetric flask add about **700 mL water.** then add **48 mL concentrated sulfuric acid** (H_2SO_4), (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add **31.7 g potassium sulfate** (K_2SO_4). Dilute to the mark with water and mix.

By Weight: In a tared **1 L** container. add **940 g water** then **88.3 g concentrated sulfuric acid** (H_2SO_4), (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add **31.7 g potassium sulfate** (K_2SO_4) and mix.

6.4.2 Preparation of Standards

Note: Working standards are prepared per instructions below and then processed through the digestion procedure along with the samples.

6.4.2.1 **Standard 1. Stock Standard 1000 mg N/L**

Dry **ammonium chloride** (NH_4Cl) for two hours at 105°C. In a **1 L** volumetric flask dissolve **3.819 g ammonium chloride** (NH_4Cl) in about **800 mL water.** Dilute to mark with **water** and mix.. Refrigerate. This solution is stable for six months.

6.4.2.2 **Standard 2. Working Standard - 50 mg N/L**

In a **1 L** volumetric flask add about **600 mL water**. Pipette **50 mL** of the **1000 mg N/L** stock standard (standard 1), dilute to mark with **water** and mix.

6.4.2.3 **Standard 3. Working Quality Control Standard - 31.06 mg N/L**

In a **500 mL** volumetric flask add about **300 mL water**. Pipette **20 mL** of the E M Science **1000 mg N/L Ammonia Standard Solution** (776.5 mg N/L), dilute to mark with **water** and mix.

Note: 1000 mg/L standards by other reputable laboratory vendors may be substituted.

6.4.2.4 **Calibration Standards**

Standards are diluted to **500 mL** with **water.**

	Calibration Standards Concentration mg/L	Prepared From Concentration mg/L	Aliquot mL
1	20.00	50	200
2	10.00	50	100
3	4.00	50	40
4	2.50	50	25
5	1.00	10	50
6	0.10	1	50
7	0.02	0.10	100
8	0.00	Water	0

6.4.2.5 **Laboratory Control Standard - 1.55 mg N/L**

In a **1 L** volumetric flask add about **700 mL water**. Pipette **50 mL** of the **Working Quality Control Standard** (standard 3). Dilute to mark with **water** and mix.

6.5 Quality Control Sample Requirements

Begin and end each run by measuring a laboratory control standard, a midpoint calibration standard run as a sample, and a reagent blank. When the run is long enough, every twentieth sample should be followed by the above three QC check samples. Recovery should be 90 to 110% of the expected value.

7.0 PROCEDURE

7.1 Procedure Instructions

7.1.1 Digestion Procedure

7.1.1.1 Both standards and samples are carried through this procedure.

7.1.1.2 Using a digestion tube rack to hold the digestion tubes, place **20.0 mL of sample** or standard in the digestion tubes. Use an acid resistant repipet device to add **5 mL of the digestion** solution (Reagent 2). Mix.

7.1.1.3 Add **2-4 Hengar granules** to each tube. Hengar granules are effective for smooth boiling.

7.1.1.4 Verify that boiling stones have been placed in each tube. Place tubes in the preheated block digestor for **one hour** at **160°C**. Water from the samples should have boiled off before increasing the temperature in step 7.1.1.5.

7.1.1.5 After the water has boiled off, place the **cold fingers** on the tubes. Continue to digest for **1.5 additional hours** with the controller set to **380°C**. This time includes the ramp time for the temperature to come up to 380°C. The typical ramp time is 50 - 60 minutes. 380°C must be maintained for 30 minutes.

7.1.1.6 Before removing samples, gather the necessary supplies to dilute the samples with water. Remove the samples from the digestion block and place on a rack stand. Allow tubes to cool for a minimum of **8 minutes**.

7.1.1.7 With the water dispenser calibrated for 10 mL, add **10 mL of water** to each tube.

7.1.1.8 Place the tubes on a block digestor that is heated to **105°C**. Let the tubes stay on the digestor three to five minutes, but no more than five minutes to avoid loss of volume. Remove the tubes to a tube rack stand.

7.1.1.9 Using a vortex mixer and a countdown timer, mix the samples two at a time for one minute. Do not let the unmixed samples remain unheated for more than three minutes. If there are a large number of samples, it will be necessary to return the tubes with unmixed samples back to the 105°C block digestor to keep the samples warm until mixed but for no more than three minutes at a time. Alternate placing the unmixed samples on and off of the heating block as needed until all samples are mixed. Caution must be given in not allowing the samples to get too cool, which will prevent the potassium sulfate and ammonium sulfate crystals from going into solution.

7.1.1.10 Hold the tubes up to a light source and swirl to see if there are any undissolved crystals in the solution (not to be confused with very fine boiling stone residue). If crystals are present, reheat and remix.

7.1.1.11 After all of the samples have been mixed, use the water dispenser to add an addition **10 mL of water** to each tube. The total final volume should be 21 mL. Mix well using the vortex mixer.

7.1.1.12 Allow the samples to cool to room temperature and analyze.

7.1.2 Analysis Procedure

7.1.2.1 The instrument is calibrated each day of use and may be calibrated with each sample tray.

7.1.2.2 Prepare reagents and standards as described in section 6.4

7.1.2.3 Set up manifold as shown in section 9.2

7.1.2.4 Enter data system parameters as in section 9.1

7.1.2.5 Pump deionized water through all reagent lines and check for leaks and smooth flow. Allow 15 minutes for heating unit to warm up to 60°C. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved. Add the buffer line first, pump for about 5 minutes or at least until the air bubbles introduced during the transfer passes through the flow cell. Then place all other transmission lines in the proper reagents.

7.1.2.6 Load standard and sample trays.

7.1.2.7 Place samples and standards in the autosampler. Enter the information required by the data system, such as standard concentration, and sample identification.

7.1.2.8 Calibrate the instrument by injecting the standards. The data system will then associate the concentration with the instrument responses for each standard.

7.1.2.9 After the standards are injected and the system has automatically prepared a calibration curve, the system will inject the samples from the sample tray.

7.1.2.10 If the analyte concentration is above the analytical range of the calibration curve, the sample must be diluted with reagent 7 to bring the analyte concentration within range.

7.1.2.11 At the end of the run, remove all transmission lines from reagents and place them in water. Pump for about five minutes.

7.1.2.12 To prevent baseline drifts, peaks that are too wide, or other problems with precision, clean the manifold by placing the manifold reagent lines in 1 M hydrochloric acid (1 volume of concentrated HCl added to 11 volumes of water). Pump for about five minutes.

7.1.2.13 Remove all reagent lines from the hydrochloric acid and place them in water. Pump until the HCl is thoroughly washed out (about 5 minutes).

7.1.2.14 Remove the transmission lines from the water and pump all lines dry.

7.2 Calculations and Recording Data

7.2.1 Calibration is done by injecting standards. The data system will then automatically prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation provided by the software.

7.2.2 Create a custom report. (Lachat Instruments, *QuickChem 8000 Automated Ion Analyzer Orion FIA Software Installation and Tutorial Manual,* page 43, "Task 11 - Creating a Custom Report")

7.2.3 Report on those values that fall between the lowest and highest calibration standards. Samples exceeding the highest standard must be diluted with reagent 7 and reanalyzed.

7.2.4 Report results in mg N/L.

8.0 SAFETY

8.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Use routine laboratory protective clothing (lab coat, gloves, and eye protection) when handling these reagents. Thoroughly wash any skin that comes into contact with any of these chemicals. Avoid creating of inhaling dust or fumes from solid chemicals.

9.0 NOTES

9.1 Data System Parameters

Method Filename:	TN_D.MET
Method Description:	TKN (d) = 20.0 to 0.1 mg N/L

Analyte Data:

Analyte Name:	Total N
Concentration Units:	mg N/L
Chemistry:	Direct
Inject to Peak Start (s):	42.0
Peak Base Width (s):	39.000
% Width Tolerance:	100.000
Threshold:	8000.000
Autodilution Trigger:	Off
QuickChem Method:	10-107-06-2-D

Calibration Data:

Levels: (mg N/L)	1: 20.000	2: 10.000	3. 4.000
	4: 1.000	5: 0.100	6. 0.000
Calibration Rep Handling:	Average		
Calibration Fit Type:	1st Order Poly		
Force Through Zero:	No		
Weighing Method:	None		
Concentration Scaling:	None		

Sampler Timing:

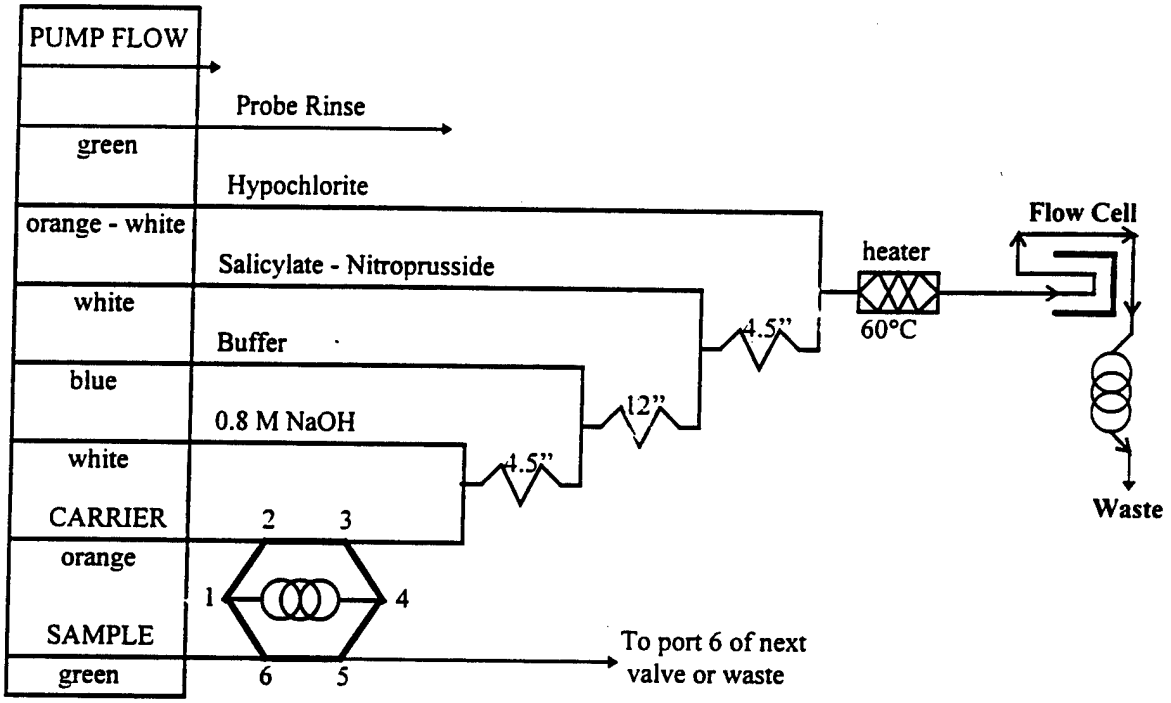
Method Cycle Period (s):	55.0
Min. Probe in Wash Prd. (s):	9.0
Probe in Sample Period (s):	25.0

Valve Timing:

Method Cycle Period (s):	55.0
Sample Reaches 1st Valve (s):	19.0
Valve:	On
Load Time (s):	0.0
Load period (s):	20.0
Inject Period (s):	35.0

Sample Loop: 50 cm

9.2 Total Kjeldahl Nitrogen Manifold Diagram



Sample Loop = 50 cm

Interference Filter = 660 nm

Carrier is Diluent (reagent 7)

All manifold tubing is **0.8 mm (0.32 in) i.d.** Lachat Part No. 50028. This is **5.2 uL/cm**

4.5 is **70 cm** of tubing on a **4.5 cm** coil support.

12 is **255 cm** of tubing on a **12 cm** coil support.

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The 60°C includes 650 cm of tubing wrapped around the heater block at 60°C.

10.0 ATTACHMENTS AND APPENDICES

None

End of Procedure

Appendix B-6 – Total P: Method 24-2.3

ASA 24-2.3 Method (Digestion)
as used for Total P Analysis

A. Reagents

1. Perchloric acid ($HClO_4$), 60% (Warning: Perchloric acid can react violently or explosively with hot organic matter.)
2. Nitric acid (HNO_3), concentrated reagent grade

B. Procedure

1. Mix 2.0 g of finely ground soil (0.5 mm) to a 250-ml volumetric or Erlenmeyer flask. (If the sample is high in organic matter, add 20 ml of HNO_3 and heat to oxidize the sample before adding the perchloric acid.) Add 30 ml of 60% perchloric acid. Digest the mixture at a temperature a few degrees below the boiling point on a hot plate in a hood until the dark color due to organic matter disappears. Then continue heating at the boiling temperature 20 min longer. At this stage, heavy white fumes of perchloric acid appear, and the insoluble material becomes like white sand. If necessary, add 1 or 2 ml of perchloric acid to move down any black particles that stick to the sides of the flask. The total digestion with perchloric acid usually requires about 40 minutes. Cool the mixture. Add distilled water to obtain a volume of 250 ml, and mix the contents. Allow time for the solid material to settle before taking an aliquot for further analysis.

2. Submit the digested sample for analysis by ICP for Total P.

C. References

1. "Method (Digestion)," Section 24-2.3 in *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*, Second Edition, A. L. Page Editor, American Society of Agronomy, Inc. 1982

Appendix B-7 – Inorganic P: Method ASA 24-3.3

ASA 24-3.3
Inorganic P for Organic P Calculation

A. Reagents

1. Sulfuric acid (H_2SO_4), 1*N*

B. Procedure

1. Place a 1-g sample of unignited soil in a 100-ml polypropylene centrifuge tube. Add 50 ml of 1*N* sulfuric acid and place the tube on a shaker for 16 hours.

2. Centrifuge the sample for 15 minutes. If the extract is not clear, filtration may be needed using acid-resistant filter paper.

3. Submit the sample for orthophosphate analysis. This value will be Inorganic P.

4. Calculate:

Organic P = Total P - Inorganic P

C. References

1. "Ignition Method," Section 24-3.3 in *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*, Second Edition, A. L. Page Editor, American Society of Agronomy, Inc. 1982

Appendix B-8 – pH: Method ASA 12-2.6

Soil pH
ASA 12-2.6

Procedure:

1. Calibrate the pH meter according to manufacturer's instructions using two buffers to bracket the expected range of measurements. Buffers should be approximately three pH units apart.

2. Where available, check the calibration with a third buffer.

3. Prepare a slurry of soil and water in the ratio requested by the customer. Note: Some customers may request 0.01 *M* $CaCl_2$ rather than water.

Example: Slurry 10.0 g soil and 10.0 ml water.

4. Stir the slurry vigorously with a glass rod and place the electrode into the slurry. Allow the electrode to come to equilibrium and measure the pH.

5. Record information about the calibration buffers (manufacturer, expiration date, known value), the check buffer and its measurement, and sample measurements.

References:

"pH, Method 150.1 (Electrometric)," *Methods for Chemical Analysis of Water and Wastes* - Revised March 1983, U. S. Environmental Protection Agency, Cincinnati, OH, PB84-128677.

"Glass Electrode - Calomel Electrode pH Meter Method," Section 12-2.6 in *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*, Second Edition, A. L. Page Editor, American Society of Agronomy, Inc. 1982

Appendix B-9– Electrical Conductivity: Method 120 Series

Conductance - Method 120.1 (Specific Conductance, μmhos at 25°C)

1.0 Procedure

Perform conductivity measurements in accordance with Method 120.1 as attached.

2.0 Recordkeeping

Retain all worksheets, notes, and machine printouts as quality assurance records.

3.0 Quality Control Samples

Duplicate samples may be run (one per batch) when sample quantity permits. A quality control sample made from oven-dried (105°C) reagent grade potassium chloride may be used as well.

4.0 References

"2510 Conductivity", *Standard Methods for the Examination of Water and Wastewater*, 18th edition 1992, Edited by Greenberg et. al.

CONDUCTANCE

Method 120.1 (Specific Conductance, umhos at 25°C)

STORET NO. 00095

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline wates, domestic and industrial wastes and acid rain (atmospheric deposition).
2. Summary of Method
   2.1 The specific conductance of a sample is measured by use of a self-contained conductivity meter, Wheatstone bridge-type, or equivalent.
   2.2 Samples are preferable analyzed at 25°C. If not, temprature corrections aremade and results reported at 25°C.
3. Comments
   3.1 Instrument must be standardized with KCl solution before daily use.
   3.2 Conductivity cell must be kept clean.
   3.3 Field measurements with comparable instruments are reliable.
   3.4 Temperature variations and corrections represent the largest source of potential error.
4. Sample Handling and Preservation
   4.1 Analyses can be performed either in the field or laboratory.
   4.2 If analysis is not completed within 24 hours of sample collection, sample should be filtered through a 0.45 micron filter and stored at 4°C. Filter and apparatus must be washed with high quality distilled water and pre-rinsed with sample before use.
5. Apparatus
   5.1 Conductivity bridge, range 1 to 1000 μmho per centimeter.
   5.2 Conductivity cell, cell constant 1.0 or micro dipping type cell with 1.0 constant. YSI #3403 or equivalent.
   5.4 Thermometer
6. Reagents
   6.1 Standard potassium chloride solutions, 0.01 M: Dissolve 0.7456 gm of pre-dried (2 hour at 105°C) KCl in distilled water and dilute to 1 liter at 25°C.
7. Cell Calibration
   7.1 The analyst should use the standard potassium chloride solution (6.1) and the table below to check the accuracy of the cell constant and conductivity bridge.

Approved for NPDES
Issued 1971.
Editorial revision, 1982

Conductivity 0.01 m KCl

°C	Micromhos/cm
21	1305
22	1332
23	1359
24	1386
25	1413
26	1441
27	1468
28	1496

8. Procedure
   - 8.1 Follow the direction of the manufacturer for the operation of the instrument.
   - 8.2 Allow samples to come to room temperature (23 to 27°C), if possible.
   - 8.3 Determine the temperature of samples within 0.5°C. If the temperature of the samples is not 25°C, make temperature correction in accordance with the instruction in Section 9 to convert reading to 25°.
9. Calculation
   - 9.1 These temperature corrections are based on the standard KCl solution.
     - 9.1.1 If the temperature of the sample is below 25°C, add 2% of the reading per degree.
     - 9.1.2 If the temperature is above 25°C, subtract 2% of the reading per degree.
   - 9.2 Report results as Specific Conductance, μmhos/cm at 25°.
10. Precision and Accuracy
    - 10.1 Forty-one analysts in 17 laboratories analyzed six synthetic water samples containing increments of inorganic salts, with the following results:

Increment as Specific Conductance	Precision as Standard Deviation	Accuracy as Bias, %	Accuracy as Bias, μmhos/cm
100	7.55	-2.02	-2.0
106	8.14	-0.76	-0.8
808	66.1	-3.63	-29.3
848	79.6	-4.54	-38.5
1640	106	-5.36	-87.9
1710	119	-5.08	-86.9

(FWPCA Method Study 1, Mineral and Physical Analyses.)

- 10.2 In a single laboratory (EMSL) using surface water samples with an average conductivity of 536 μmhos/cm at 25°C, the standard deviation was ±6.

Bibliography

1. The procedure to be used for this determination is found in:
   Annual Book of ASTM Standards Part 31, "Water," Standard D1125-64, p. 120 (1976).
2. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p. 71, Method 205 (1975).
3. Instruction Manual for YSI Model 31 Conductivity Bridge.
4. Peden, M. E., and Skowron. "Ionic Stability of Precipitation Samples," Atmospheric Environment, Vol. 12, p. 2343-2344, 1978.

Appendix B-10 - Metals: Method 6010B

Although the following procedure lists a post project approval date, the methods described herein accurately describe the procedures used during the study.

Method 6010B - Inductively Coupled Plasma - Atomic Emission Spectroscopy

1.0 Procedure

Perform analysis for metals and certain other elements amenable to ICP analysis in accordance with Method 6010B from SW-846 as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes as quality assurance records.

3.0 Quality Control Samples

For each batch of samples, perform the quality control analyses specified in the method: method blank, reagent blank, calibration check sample.

For each batch, introduce one quality control sample made from a separate stock than that used to calibrate the machine (a laboratory control sample).

Where possible, for each batch analyze one matrix spike sample.

For each batch, analyze a matrix spike duplicate or a sample duplicate.

METHOD 6010B

INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROMETRY

1.0 SCOPE AND APPLICATION

1.1 Inductively coupled plasma-atomic emission spectrometry (ICP-AES) determines trace elements, including metals, in solution. The method is applicable to all of the elements listed in Table 1. All matrices, excluding filtered groundwater samples but including ground water, aqueous samples, TCLP and EP extracts, industrial and organic wastes, soils, sludges, sediments, and other solid wastes, require digestion prior to analysis. Groundwater samples that have been prefiltered and acidified will not need acid digestion. Samples which are not digested must either use an internal standard or be matrix matched with the standards. Refer to Chapter Three for the appropriate digestion procedures.

1.2 Table 1 lists the elements for which this method is applicable. Detection limits, sensitivity, and the optimum and linear concentration ranges of the elements can vary with the wavelength, spectrometer, matrix and operating conditions. Table 1 lists the recommended analytical wavelengths and estimated instrumental detection limits for the elements in clean aqueous matrices. The instrument detection limit data may be used to estimate instrument and method performance for other sample matrices. Elements and matrices other than those listed in Table 1 may be analyzed by this method if performance at the concentration levels of interest (see Section 8.0) is demonstrated.

1.3 Users of the 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 Use of this method is restricted to spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences described in this method.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, samples must be solubilized or digested using appropriate Sample Preparation Methods (e.g. Chapter Three). When analyzing groundwater samples for dissolved constituents, acid digestion is not necessary if the samples are filtered and acid preserved prior to analysis.

2.2 This method describes multielemental determinations by ICP-AES using sequential or simultaneous optical systems and axial or radial viewing of the plasma. The instrument measures characteristic emission spectra by optical spectrometry. Samples are nebulized and the resulting aerosol is transported to the plasma torch. Element-specific emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer, and the intensities of the emission lines are monitored by photosensitive devices. Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. In one mode of analysis the position used should be as free as possible from spectral interference and should reflect the same change in background

intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result. The possibility of additional interferences named in Section 3.0 should also be recognized and appropriate corrections made; tests for their presence are described in Section 8.5. Alternatively, users may choose multivariate calibration methods. In this case, point selections for background correction are superfluous since whole spectral regions are processed.

3.0 INTERFERENCES

3.1 Spectral interferences are caused by background emission from continuous or recombination phenomena, stray light from the line emission of high concentration elements, overlap of a spectral line from another element, or unresolved overlap of molecular band spectra.

3.1.1 Background emission and stray light can usually be compensated for by subtracting the background emission determined by measurements adjacent to the analyte wavelength peak. Spectral scans of samples or single element solutions in the analyte regions may indicate when alternate wavelengths are desirable because of severe spectral interference. These scans will also show whether the most appropriate estimate of the background emission is provided by an interpolation from measurements on both sides of the wavelength peak or by measured emission on only one side. The locations selected for the measurement of background intensity will be determined by the complexity of the spectrum adjacent to the wavelength peak. The locations used for routine measurement must be free of off-line spectral interference (interelement or molecular) or adequately corrected to reflect the same change in background intensity as occurs at the wavelength peak. For multivariate methods using whole spectral regions, background scans should be included in the correction algorithm. Off-line spectral interferences are handled by including spectra on interfering species in the algorithm.

3.1.2 To determine the appropriate location for off-line background correction, the user must scan the area on either side adjacent to the wavelength and record the apparent emission intensity from all other method analytes. This spectral information must be documented and kept on file. The location selected for background correction must be either free of off-line interelement spectral interference or a computer routine must be used for automatic correction on all determinations. If a wavelength other than the recommended wavelength is used, the analyst must determine and document both the overlapping and nearby spectral interference effects from all method analytes and common elements and provide for their automatic correction on all analyses. Tests to determine spectral interference must be done using analyte concentrations that will adequately describe the interference. Normally, 100 mg/L single element solutions are sufficient; however, for analytes such as iron that may be found at high concentration, a more appropriate test would be to use a concentration near the upper analytical range limit.

3.1.3 Spectral overlaps may be avoided by using an alternate wavelength or can be compensated by equations that correct for interelement contributions. Instruments that use equations for interelement correction require the interfering elements be analyzed at the same time as the element of interest. When operative and uncorrected, interferences will produce false positive determinations and be reported as analyte concentrations. More extensive information on interferant effects at various wavelengths and resolutions is available in reference wavelength tables and books. Users may apply interelement

correction equations determined on their instruments with tested concentration ranges to compensate (off line or on line) for the effects of interfering elements. Some potential spectral interferences observed for the recommended wavelengths are given in Table 2. For multivariate methods using whole spectral regions, spectral interferences are handled by including spectra of the interfering elements in the algorithm. The interferences listed are only those that occur between method analytes. Only interferences of a direct overlap nature are listed. These overlaps were observed with a single instrument having a working resolution of 0.035 nm.

3.1.4 When using interelement correction equations, the interference may be expressed as analyte concentration equivalents (i.e. false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that As is to be determined (at 193.696 nm) in a sample containing approximately 10 mg/L of Al. According to Table 2, 100 mg/L of Al would yield a false signal for As equivalent to approximately 1.3 mg/L. Therefore, the presence of 10 mg/L of Al would result in a false signal for As equivalent to approximately 0.13 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary.

3.1.5 Interelement corrections will vary for the same emission line among instruments because of differences in resolution, as determined by the grating, the entrance and exit slit widths, and by the order of dispersion. Interelement corrections will also vary depending upon the choice of background correction points. Selecting a background correction point where an interfering emission line may appear should be avoided when practical. Interelement corrections that constitute a major portion of an emission signal may not yield accurate data. Users should not forget that some samples may contain uncommon elements that could contribute spectral interferences.

3.1.6 The interference effects must be evaluated for each individual instrument whether configured as a sequential or simultaneous instrument. For each instrument, intensities will vary not only with optical resolution but also with operating conditions (such as power, viewing height and argon flow rate). When using the recommended wavelengths, the analyst is required to determine and document for each wavelength the effect from referenced interferences (Table 2) as well as any other suspected interferences that may be specific to the instrument or matrix. The analyst is encouraged to utilize a computer routine for automatic correction on all analyses.

3.1.7 Users of sequential instruments must verify the absence of spectral interference by scanning over a range of 0.5 nm centered on the wavelength of interest for several samples. The range for lead, for example, would be from 220.6 to 220.1 nm. This procedure must be repeated whenever a new matrix is to be analyzed and when a new calibration curve using different instrumental conditions is to be prepared. Samples that show an elevated background emission across the range may be background corrected by applying a correction factor equal to the emission adjacent to the line or at two points on either side of the line and interpolating between them. An alternate wavelength that does not exhibit a background shift or spectral overlap may also be used.

3.1.8 If the correction routine is operating properly, the determined apparent analyte(s) concentration from analysis of each interference solution should fall within a specific concentration range around the calibration blank. The concentration range is calculated by multiplying the concentration of the interfering element by the value of the correction factor being tested and divided by 10. If after the subtraction of the calibration blank the apparent analyte concentration falls outside of this range in either a positive or negative direction, a change in the correction factor of more than 10% should be suspected. The cause of the change should be determined and corrected and the correction factor updated. The interference check solutions should be analyzed more than once to confirm a change has occurred. Adequate rinse time between solutions and before analysis of the calibration blank will assist in the confirmation.

3.1.9 When interelement corrections are applied, their accuracy should be verified, daily, by analyzing spectral interference check solutions. If the correction factors or multivariate correction matrices tested on a daily basis are found to be within the 20% criteria for 5 consecutive days, the required verification frequency of those factors in compliance may be extended to a weekly basis. Also, if the nature of the samples analyzed is such they do not contain concentrations of the interfering elements at ± one reporting limit from zero, daily verification is not required. All interelement spectral correction factors or multivariate correction matrices must be verified and updated every six months or when an instrumentation change, such as in the torch, nebulizer, injector, or plasma conditions occurs. Standard solution should be inspected to ensure that there is no contamination that may be perceived as a spectral interference.

3.1.10 When interelement corrections are <u>not</u> used, verification of absence of interferences is required.

3.1.10.1 One method is to use a computer software routine for comparing the determinative data to limits files for notifying the analyst when an interfering element is detected in the sample at a concentration that will produce either an apparent false positive concentration, (i.e., greater than) the analyte instrument detection limit, or false negative analyte concentration, (i.e., less than the lower control limit of the calibration blank defined for a 99% confidence interval).

3.1.10.2 Another method is to analyze an Interference Check Solution(s) which contains similar concentrations of the major components of the samples (>10 mg/L) on a continuing basis to verify the absence of effects at the wavelengths selected. These data must be kept on file with the sample analysis data. If the check solution confirms an operative interference that is $\geq$ 20% of the analyte concentration, the analyte must be determined using (1) analytical and background correction wavelengths (or spectral regions) free of the interference, (2) by an alternative wavelength, or (3) by another documented test procedure.

3.2 Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. If physical interferences are present, they must be reduced by diluting the sample or by using a peristaltic pump, by using an internal standard or by using a high solids nebulizer. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, affecting aerosol flow rate

and causing instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, using a high solids nebulizer or diluting the sample. Also, it has been reported that better control of the argon flow rate, especially to the nebulizer, improves instrument performance: this may be accomplished with the use of mass flow controllers. The test described in Section 8.5.1 will help determine if a physical interference is present.

3.3 Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique, but if observed, can be minimized by careful selection of operating conditions (incident power, observation position, and so forth), by buffering of the sample, by matrix matching, and by standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

3.4 Memory interferences result when analytes in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the uptake tubing to the nebulizer and from the build up of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples. The possibility of memory interferences should be recognized within an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for a particular element must be estimated prior to analysis. This may be achieved by aspirating a standard containing elements at a concentration ten times the usual amount or at the top of the linear dynamic range. The aspiration time for this sample should be the same as a normal sample analysis period, followed by analysis of the rinse blank at designated intervals. The length of time required to reduce analyte signals to within a factor of two of the method detection limit should be noted. Until the required rinse time is established, this method suggests a rinse period of at least 60 seconds between samples and standards. If a memory interference is suspected, the sample must be reanalyzed after a rinse period of sufficient length. Alternate rinse times may be established by the analyst based upon their DQOs.

3.5 Users are advised that high salt concentrations can cause analyte signal suppressions and confuse interference tests. If the instrument does not display negative values, fortify the interference check solution with the elements of interest at 0.5 to 1 mg/L and measure the added standard concentration accordingly. Concentrations should be within 20% of the true spiked concentration or dilution of the samples will be necessary. In the absence of measurable analyte, overcorrection could go undetected if a negative value is reported as zero.

3.6 The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferant concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

4.0 APPARATUS AND MATERIALS

4.1 Inductively coupled argon plasma emission spectrometer:

4.1.1 Computer-controlled emission spectrometer with background correction.

4.1.2 Radio-frequency generator compliant with FCC regulations.

4.1.3 Optional mass flow controller for argon nebulizer gas supply.

4.1.4 Optional peristaltic pump.

4.1.5 Optional Autosampler.

4.1.6 Argon gas supply - high purity.

4.2 Volumetric flasks of suitable precision and accuracy.

4.3 Volumetric pipets of suitable precision and accuracy.

5.0 REAGENTS

5.1 Reagent or trace metals 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. If the purity of a reagent is in question analyze for contamination. If the concentration of the contamination is less than the MDL then the reagent is acceptable.

5.1.1 Hydrochloric acid (conc), HCl.

5.1.2 Hydrochloric acid (1:1), HCl. Add 500 mL concentrated HCl to 400 mL water and dilute to 1 liter in an appropriately sized beaker.

5.1.3 Nitric acid (conc), HNO_3.

5.1.4 Nitric acid (1:1), HNO_3. Add 500 mL concentrated HNO_3 to 400 mL water and dilute to 1 liter in an appropriately sized beaker.

5.2 Reagent Water. All references to water in the method refer to reagent water unless otherwise specified. Reagent water will be interference free. Refer to Chapter One for a definition of reagent water.

5.3 Standard stock solutions may be purchased or prepared from ultra- high purity grade chemicals or metals (99.99% pure or greater). All salts must be dried for 1 hour at 105°C, unless otherwise specified.

Note: This section does not apply when analyzing samples that have been prepared by Method 3040.

CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

Typical stock solution preparation procedures follow. Concentrations are calculated based upon the weight of pure metal added, or with the use of the element fraction and the weight of the metal salt added.

For metals:

$$\text{Concentration (ppm)} = \frac{\text{weight (mg)}}{\text{volume (L)}}$$

For metal salts:

$$\text{Concentration (ppm)} = \frac{\text{weight (mg) x mole fraction}}{\text{volume (L)}}$$

5.3.1 Aluminum solution, stock, 1 mL = 1000 µg Al: Dissolve 1.000 g of aluminum metal, weighed accurately to at least four significant figures, in an acid mixture of 4.0 mL of (1:1) HCl and 1.0 mL of concentrated $HN0_3$ in a beaker. Warm beaker slowly to effect solution. When dissolution is complete, transfer solution quantitatively to a 1-liter flask, add an additional 10.0 mL of (1:1) HCl and dilute to volume with reagent water.

NOTE: Weight of analyte is expressed to four significant figures for consistency with the weights below because rounding to two decimal places can contribute up to 4 % error for some of the compounds.

5.3.2 Antimony solution, stock, 1 mL = 1000 µg Sb: Dissolve 2.6673 g $K(SbO)C_4H_4O_6$ (element fraction Sb = 0.3749), weighed accurately to at least four significant figures, in water, add 10 mL (1:1) HCl, and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.3 Arsenic solution, stock, 1 mL = 1000 µg As: Dissolve 1.3203 g of As_2O_3 (element fraction As = 0.7574), weighed accurately to at least four significant figures, in 100 mL of water containing 0.4 g NaOH. Acidify the solution with 2 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.4 Barium solution, stock, 1 mL = 1000 µg Ba: Dissolve 1.5163 g $BaCl_2$ (element fraction Ba = 0.6595), dried at 250°C for 2 hours, weighed accurately to at least four significant figures, in 10 mL water with 1 mL (1:1) HCl. Add 10.0 mL (1:1) HCl and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.5 Beryllium solution, stock, 1 mL = 1000 µg Be: Do not dry. Dissolve 19.6463 g $BeSO_4{\cdot}4H_2O$ (element fraction Be = 0.0509), weighed accurately to at least four significant figures, in water, add 10.0 mL concentrated HNO_3, and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.6 Boron solution, stock, 1 mL = 1000 µg B: Do not dry. Dissolve 5.716 g anhydrous H_3BO_3 (B fraction = 0.1749), weighed accurately to at least four significant figures, in reagent water and dilute in a 1-L volumetric flask with reagent water. Transfer immediately after mixing in a clean polytetrafluoroethylene (PTFE) bottle to minimize any leaching of boron from the glass volumetric container. Use of a non-glass volumetric flask is recommended to avoid boron contamination from glassware.

5.3.7 Cadmium solution, stock, 1 mL = 1000 µg Cd: Dissolve 1.1423 g CdO (element fraction Cd = 0.8754), weighed accurately to at least four significant figures, in a

minimum amount of (1:1) HNO_3. Heat to increase rate of dissolution. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.8 Calcium solution, stock, 1 mL = 1000 µg Ca: Suspend 2.4969 g $CaCO_3$ (element Ca fraction = 0.4005), dried at 180°C for 1 hour before weighing, weighed accurately to at least four significant figures, in water and dissolve cautiously with a minimum amount of (1:1) HNO_3. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.9 Chromium solution, stock, 1 mL = 1000 µg Cr: Dissolve 1.9231 g CrO_3 (element fraction Cr = 0.5200), weighed accurately to at least four significant figures, in water. When solution is complete, acidify with 10 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.10 Cobalt solution, stock, 1 mL = 1000 µg Co: Dissolve 1.00 g of cobalt metal, weighed accurately to at least four significant figures, in a minimum amount of (1:1) HNO_3. Add 10.0 mL (1:1) HCl and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.11 Copper solution, stock, 1 mL = 1000 µg Cu: Dissolve 1.2564 g CuO (element fraction Cu = 0.7989), weighed accurately to at least four significant figures), in a minimum amount of (1:1) HNO_3. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.12 Iron solution, stock, 1 mL = 1000 µg Fe: Dissolve 1.4298 g Fe_2O_3 (element fraction Fe = 0.6994), weighed accurately to at least four significant figures, in a warm mixture of 20 mL (1:1) HCl and 2 mL of concentrated HNO_3. Cool, add an additional 5.0 mL of concentrated HNO_3, and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.13 Lead solution, stock, 1 mL = 1000 µg Pb: Dissolve 1.5985 g $Pb(NO_3)_2$ (element fraction Pb = 0.6256), weighed accurately to at least four significant figures, in a minimum amount of (1:1) HNO_3. Add 10 mL (1:1) HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.14 Lithium solution, stock, 1 mL = 1000 µg Li: Dissolve 5.3248 g lithium carbonate (element fraction Li = 0.1878), weighed accurately to at least four significant figures, in a minimum amount of (1:1) HCl and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.15 Magnesium solution, stock, 1 mL = 1000 µg Mg: Dissolve 1.6584 g MgO (element fraction Mg = 0.6030), weighed accurately to at least four significant figures, in a minimum amount of (1:1) HNO_3. Add 10.0 mL (1:1) concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.16 Manganese solution, stock, 1 mL = 1000 µg Mn: Dissolve 1.00 g of manganese metal, weighed accurately to at least four significant figures, in acid mixture (10 mL concentrated HCl and 1 mL concentrated HNO_3) and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.17 Mercury solution, stock, 1 mL = 1000 µg Hg: Do not dry, highly toxic element. Dissolve 1.354 g $HgCl_2$ (Hg fraction = 0.7388) in reagent water. Add 50.0 mL concentrated HNO_3 and dilute to volume in 1-L volumetric flask with reagent water.

5.3.18 Molybdenum solution, stock, 1 mL = 1000 µg Mo: Dissolve 1.7325 g $(NH_4)_6Mo_7O_{24}.4H_2O$ (element fraction Mo = 0.5772), weighed accurately to at least four significant figures, in water and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.19 Nickel solution, stock, 1 mL = 1000 µg Ni: Dissolve 1.00 g of nickel metal, weighed accurately to at least four significant figures, in 10.0 mL hot concentrated HNO_3, cool, and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.20 Phosphate solution, stock, 1 mL = 1000 µg P: Dissolve 4.3937 g anhydrous KH_2PO_4 (element fraction P = 0.2276), weighed accurately to at least four significant figures, in water. Dilute to volume in a 1,000 mL volumetric flask with water.

5.3.21 Potassium solution, stock, 1 mL = 1000 µg K: Dissolve 1.9069 g KCl (element fraction K = 0.5244) dried at 110°C, weighed accurately to at least four significant figures, in water, and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.22 Selenium solution, stock, 1 mL = 1000 µg Se: Do not dry. Dissolve 1.6332 g H_2SeO_3 (element fraction Se = 0.6123), weighed accurately to at least four significant figures, in water and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.23 Silica solution, stock, 1 mL = 1000 µg SiO_2: Do not dry. Dissolve 2.964 g NH_4SiF_6, weighed accurately to at least four significant figures, in 200 mL (1:20) HCl with heating at 85°C to effect dissolution. Let solution cool and dilute to volume in a 1-L volumetric flask with reagent water.

5.3.24 Silver solution, stock, 1 mL = 1000 µg Ag: Dissolve 1.5748 g $AgNO_3$ (element fraction Ag = 0.6350), weighed accurately to at least four significant figures, in water and 10 mL concentrated HNO_3. Dilute to volume in a 1,000 mL volumetric flask with water.

5.3.25 Sodium solution, stock, 1 mL = 1000 µg Na: Dissolve 2.5419 g NaCl (element fraction Na = 0.3934), weighed accurately to at least four significant figures, in water. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.26 Strontium solution, stock, 1 mL = 1000 µg Sr: Dissolve 2.4154 g of strontium nitrate ($Sr(NO_3)_2$) (element fraction Sr = 0.4140), weighed accurately to at least four significant figures, in a 1-liter flask containing 10 mL of concentrated HCl and 700 mL of water. Dilute to volume in a 1,000 mL volumetric flask with water.

5.3.27 Thallium solution, stock, 1 mL = 1000 µg Tl: Dissolve 1.3034 g $TlNO_3$ (element fraction Tl = 0.7672), weighed accurately to at least four significant figures, in water. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.28 Tin solution, stock, 1 mL = 1000 µg Sn: Dissolve 1.000 g Sn shot, weighed accurately to at least 4 significant figures, in 200 mL (1:1) HCl with heating to effect dissolution. Let solution cool and dilute with (1:1) HCl in a 1-L volumetric flask.

5.3.29 Vanadium solution, stock, 1 mL = 1000 µg V: Dissolve 2.2957 g NH_4VO_3 (element fraction V = 0.4356), weighed accurately to at least four significant figures, in a minimum amount of concentrated HNO_3. Heat to increase rate of dissolution. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.30 Zinc solution, stock, 1 mL = 1000 µg Zn: Dissolve 1.2447 g ZnO (element fraction Zn = 0.8034), weighed accurately to at least four significant figures, in a minimum amount of dilute HNO_3. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.4 Mixed calibration standard solutions - Prepare mixed calibration standard solutions by combining appropriate volumes of the stock solutions in volumetric flasks (see Table 3). Add the appropriate types and volumes of acids so that the standards are matrix matched with the sample digestates. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interference or the presence of impurities. Care should be taken when preparing the mixed standards to ensure that the elements are compatible and stable together. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Some typical calibration standard combinations are listed in Table 3.

NOTE: If the addition of silver to the recommended acid combination results in an initial precipitation, add 15 mL of water and warm the flask until the solution clears. Cool and dilute to 100 mL with water. For this acid combination, the silver concentration should be limited to 2 mg/L. Silver under these conditions is stable in a tap-water matrix for 30 days. Higher concentrations of silver require additional HCl.

5.5 Two types of blanks are required for the analysis for samples prepared by any method other than 3040. The calibration blank is used in establishing the analytical curve, and the method blank is used to identify possible contamination resulting from varying amounts of the acids used in the sample processing.

5.5.1 The calibration blank is prepared by acidifying reagent water to the same concentrations of the acids found in the standards and samples. Prepare a sufficient quantity to flush the system between standards and samples. The calibration blank will also be used for all initial and continuing calibration blank determinations (see Sections 7.3 and 7.4).

5.5.2 The method blank must contain all of the reagents in the same volumes as used in the processing of the samples. The method blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

5.6 The Initial Calibration Verification (ICV) is prepared by the analyst by combining compatible elements from a standard source different than that of the calibration standard and at concentrations within the linear working range of the instrument (see Section 8.6.1 for use).

5.7 The Continuing Calibration Verification (CCV)) should be prepared in the same acid matrix using the same standards used for calibration at a concentration near the mid-point of the calibration curve (see Section 8.6.1 for use).

5.8 The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest, particularly those with known interferences at 0.5 to 1 mg/L. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material in Chapter Three, Inorganic Analytes, Sections 3.1 through 3.3.

7.0 PROCEDURE

7.1 Preliminary treatment of most matrices is necessary because of the complexity and variability of sample matrices. Groundwater samples which have been prefiltered and acidified will not need acid digestion. Samples which are not digested must either use an internal standard or be matrix matched with the standards. Solubilization and digestion procedures are presented in Sample Preparation Methods (Chapter Three, Inorganic Analytes).

7.2 Set up the instrument with proper operating parameters established as detailed below. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 minutes of operation prior to calibration). Operating conditions - The analyst should follow the instructions provided by the instrument manufacturer.

7.2.1 Before using this procedure to analyze samples, there must be data available documenting initial demonstration of performance. The required data document the selection criteria of background correction points; analytical dynamic ranges, the applicable equations, and the upper limits of those ranges; the method and instrument detection limits; and the determination and verification of interelement correction equations or other routines for correcting spectral interferences. This data must be generated using the same instrument, operating conditions and calibration routine to be used for sample analysis. These documented data must be kept on file and be available for review by the data user or auditor.

7.2.2 Specific wavelengths are listed in Table 1. Other wavelengths may be substituted if they can provide the needed sensitivity and are corrected for spectral interference. Because of differences among various makes and models of spectrometers, specific instrument operating conditions cannot be provided. The instrument and operating conditions utilized for determination must be capable of providing data of acceptable quality to the program and data user. The analyst should follow the instructions provided by the instrument manufacturer unless other conditions provide similar or better performance for

a task. Operating conditions for aqueous solutions usually vary from 1100 to 1200 watts forward power, 14 to 18 mm viewing height, 15 to 19 liters/min argon coolant flow, 0.6 to 1.5 L/min argon nebulizer flow, 1 to 1.8 mL/min sample pumping rate with a 1 minute preflush time and measurement time near 1 second per wavelength peak for sequential instruments and 10 seconds per sample for simultaneous instruments. For an axial plasma, the conditions will usually vary from 1100-1500 watts forward power, 15-19 liters/min argon coolant flow, 0.6-1.5 L/min argon nebulizer flow, 1-1.8 mL/min sample pumping rate with a 1 minute preflush time and measurement time near 1 second per wavelength peak for sequential instruments and 10 seconds per sample for simultaneous instruments. Reproduction of the Cu/Mn intensity ratio at 324.754 nm and 257.610 nm respectively, by adjusting the argon aerosol flow has been recommended as a way to achieve repeatable interference correction factors.

7.2.3 The plasma operating conditions need to be optimized prior to use of the instrument. This routine is not required on a daily basis, but only when first setting up a new instrument or following a change in operating conditions. The following procedure is recommended or follow manufacturer's recommendations. The purpose of plasma optimization is to provide a maximum signal to background ratio for some of the least sensitive elements in the analytical array. The use of a mass flow controller to regulate the nebulizer gas flow or source optimization software greatly facilitates the procedure.

7.2.3.1 Ignite the radial plasma and select an appropriate incident RF power. Allow the instrument to become thermally stable before beginning, about 30 to 60 minutes of operation. While aspirating a 1000 ug/L solution of yttrium, follow the instrument manufacturer's instructions and adjust the aerosol carrier gas flow rate through the nebulizer so a definitive blue emission region of the plasma extends approximately from 5 to 20 mm above the top of the load coil. Record the nebulizer gas flow rate or pressure setting for future reference. The yttrium solution can also be used for coarse optical alignment of the torch by observing the overlay of the blue light over the entrance slit to the optical system.

7.2.3.2 After establishing the nebulizer gas flow rate, determine the solution uptake rate of the nebulizer in mL/min by aspirating a known volume of calibration blank for a period of at least three minutes. Divide the volume aspirated by the time in minutes and record the uptake rate; set the peristaltic pump to deliver the rate in a steady even flow.

7.2.3.3 Profile the instrument to align it optically as it will be used during analysis. The following procedure can be used for both horizontal and vertical optimization in the radial mode, but is written for vertical. Aspirate a solution containing 10 ug/L of several selected elements. These elements can be As, Se, Tl or Pb as the least sensitive of the elements and most needing to be optimize or others representing analytical judgement (V, Cr, Cu, Li and Mn are also used with success). Collect intensity data at the wavelength peak for each analyte at 1 mm intervals from 14 to 18 mm above the load coil. (This region of the plasma is referred to as the analytical zone.) Repeat the process using the calibration blank. Determine the net signal to blank intensity ratio for each analyte for each viewing height setting. Choose the height for viewing the plasma that provides the best net intensity ratios for the elements analyzed or the highest intensity ratio for the least

sensitive element. For optimization in the axial mode, follow the instrument manufacturer's instructions.

7.2.3.4 The instrument operating condition finally selected as being optimum should provide the lowest reliable instrument detection limits and method detection limits.

7.2.3.5 If either the instrument operating conditions, such as incident power or nebulizer gas flow rate are changed, or a new torch injector tube with a different orifice internal diameter is installed, the plasma and viewing height should be re-optimized.

7.2.3.6 After completing the initial optimization of operating conditions, but before analyzing samples, the laboratory must establish and initially verify an interelement spectral interference correction routine to be used during sample analysis. A general description concerning spectral interference and the analytical requirements for background correction in particular are discussed in the section on interferences. Criteria for determining an interelement spectral interference is an apparent positive or negative concentration for the analyte that falls within ± one reporting limit from zero. The upper control limit is the analyte instrument detection limit. Once established the entire routine must be periodically verified every six months. Only a portion of the correction routine must be verified more frequently or on a daily basis. Initial and periodic verification of the routine should be kept on file. Special cases where continual verification is required are described elsewhere.

7.2.3.7 Before daily calibration and after the instrument warmup period, the nebulizer gas flow rate must be reset to the determined optimized flow. If a mass flow controller is being used, it should be set to the recorded optimized flow rate, In order to maintain valid spectral interelement correction routines the nebulizer gas flow rate should be the same (< 2% change) from day to day.

7.2.4 For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements.

7.2.5 Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on each particular instrument. All measurements must be within the instrument linear range where the correction equations are valid.

7.2.5.1 Method detection limits must be established for all wavelengths utilized for each type of matrix commonly analyzed. The matrix used for the MDL calculation must contain analytes of known concentrations within 3-5 times the anticipated detection limit. Refer to Chapter One for additional guidance on the performance of MDL studies.

7.2.5.2 Determination of limits using reagent water represent a best case situation and do not represent possible matrix effects of real world samples.

7.2.5.3 If additional confirmation is desired, reanalyze the seven replicate aliquots on two more non consecutive days and again calculate the method detection limit values for each day. An average of the three values for each analyte may provide for a more appropriate estimate. Successful analysis of samples with added analytes or using method of standard additions can give confidence in the method detection limit values determined in reagent water.

7.2.5.4 The upper limit of the linear dynamic range must be established for each wavelength utilized by determining the signal responses from a minimum for three, preferably five, different concentration standards across the range. One of these should be near the upper limit of the range. The ranges which may be used for the analysis of samples should be judged by the analyst from the resulting data. The data, calculations and rationale for the choice of range made should be documented and kept on file. The upper range limit should be an observed signal no more than 10% below the level extrapolated from lower standards. Determined analyte concentrations that are above the upper range limit must be diluted and reanalyzed. The analyst should also be aware that if an interelement correction from an analyte above the linear range exists, a second analyte where the interelement correction has been applied may be inaccurately reported. New dynamic ranges should be determined whenever there is a significant change in instrument response. For those analytes that periodically approach the upper limit, the range should be checked every six months. For those analytes that are known interferences, and are present at above the linear range, the analyst should ensure that the interelement correction has not been inaccurately applied.

NOTE: Many of the alkali and alkaline earth metals have non-linear response curves due to ionization and self absorption effects. These curves may be used if the instrument allows; however the effective range must be checked and the second order curve fit should have a correlation coefficient of 0.995 or better. Third order fits are not acceptable. These non-linear response curves should be revalidated and recalculated every six months. These curves are much more sensitive to changes in operating conditions than the linear lines and should be checked whenever there have been moderate equipment changes.

7.2.6 The analyst must (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.

7.3 Profile and calibrate the instrument according to the instrument manufacturer's recommended procedures, using the typical mixed calibration standard solutions described in Section 5.4. Flush the system with the calibration blank (Section 5.5.1) between each standard or as the manufacturer recommends. (Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.) The calibration curve must consist of a minimum of a blank and a standard.

7.4 For all analytes and determinations, the laboratory must analyze an ICV (Section 5.6), a calibration blank (Section 5.5.1), and a continuing calibration verification (CCV) (Section 5.7) immediately following daily calibration. A calibration blank and either a calibration verification (CCV) or an ICV must be analyzed after every tenth sample and at the end of the sample run. Analysis of

the check standard and calibration verification must verify that the instrument is within ± 10% of calibration with relative standard deviation < 5% from replicate (minimum of two) integrations. 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 ICV, CCV or check standard must be reanalyzed. The analysis data of the calibration blank, check standard, and ICV or CCV must be kept on file with the sample analysis data.

7.5 Rinse the system with the calibration blank solution (Section 5.5.1) before the analysis of each sample. The rinse time will be one minute. Each laboratory may establish a reduction in this rinse time through a suitable demonstration.

7.6 Calculations: If dilutions were performed, the appropriate factors must be applied to sample values. All results should be reported with up to three significant figures.

7.7 The MSA should be used if an interference is suspected or a new matrix is encountered. When the method of standard additions is used, standards are added at one or more levels to portions of a prepared sample. This technique compensates for enhancement or depression of an analyte signal by a matrix. It will not correct for additive interferences, such as contamination, interelement interferences, or baseline shifts. This technique is valid in the linear range when the interference effect is constant over the range, the added analyte responds the same as the endogenous analyte, and the signal is corrected for additive interferences. The simplest version of this technique is the single addition method. This procedure calls for two identical aliquots of the sample solution to be taken. To the first aliquot, a small volume of standard is added; while to the second aliquot, a volume of acid blank is added equal to the standard addition. The sample concentration is calculated by: multiplying the intensity value for the unfortified aliquot by the volume (Liters) and concentration (mg/L or mg/kg) of the standard addition to make the numerator; the difference in intensities for the fortified sample and unfortified sample is multiplied by the volume (Liters) of the sample aliquot for the denominator. The quotient is the sample concentration.

For more than one fortified portion of the prepared sample, linear regression analysis can be applied using a computer or calculator program to obtain the concentration of the sample solution.

NOTE: Refer to Method 7000 for a more detailed discussion of the MSA.

7.8 An alternative to using the method of standard additions is the internal standard technique. Add one or more elements not in the samples and verified not to cause an interelement spectral interference to the samples, standards and blanks; yttrium or scandium are often used. The concentration should be sufficient for optimum precision but not so high as to alter the salt concentration of the matrix. The element intensity is used by the instrument as an internal standard to ratio the analyte intensity signals for both calibration and quantitation. This technique is very useful in overcoming matrix interferences especially in high solids matrices.

8.0 QUALITY CONTROL

8.1 All quality control data should be maintained and available for easy reference or inspection. All quality control measures described in Chapter One should be followed.

8.2 Dilute and reanalyze samples that exceed the linear calibration range or use an alternate, less sensitive line for which quality control data is already established.

8.3 Employ a minimum of one method blank per sample batch to determine if contamination or any memory effects are occurring. A method blank is a volume of reagent water carried through the same preparation process as a sample (refer to Chapter One).

8.4 Analyze matrix spiked duplicate samples at a frequency of one per matrix batch. A matrix duplicate sample is a sample brought through the entire sample preparation and analytical process in duplicate.

8.4.1.1 The relative percent difference between spiked matrix duplicate determinations is to 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 (replicate).

(A control limit of ± 20% RPD or within the documented historical acceptance limits for each matrix shall be used for sample values greater than ten times the instrument detection limit.)

8.4.1.2 The spiked sample or spiked duplicate sample recovery is to be within ± 25% of the actual value or within the documented historical acceptance limits for each matrix.

8.5 It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests, as outlined in Sections 8.5.1 and 8.5.2, will ensure that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

8.5.1 Dilution Test: If the analyte concentration is sufficiently high (minimally, a factor of 10 above the instrumental detection limit after dilution), an analysis of a 1:5 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

8.5.2 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% of the known value. The spike addition should produce a minimum level of 10 times and a maximum of 100 times the instrumental detection limit. If the spike is not recovered within the specified limits, a matrix effect should be suspected.

CAUTION: If spectral overlap is suspected, use of computerized compensation, an alternate wavelength, or comparison with an alternate method is recommended.

8.6 Check the instrument standardization by analyzing appropriate QC samples as follows.

8.6.1 Verify calibration with the Continuing Calibration Verification (CCV) Standard immediately following daily calibration, after every ten samples, and at the end of an analytical run. Check calibration with an ICV following the initial calibration (Section 5.6). At the laboratory's discretion, an ICV may be used in lieu of the continuing calibration verifications. If used in this manner, the ICV should be at a concentration near the mid-point of the calibration curve. Use a calibration blank (Section 5.5.1) immediately following daily calibration, after every 10 samples and at the end of the analytical run.

8.6.1.1 The results of the ICV and CCVs are to agree within 10% of the expected value; if not, terminate the analysis, correct the problem, and recalibrate the instrument.

8.6.1.2 The results of the check standard are to agree within 10% of the expected value; if not, terminate the analysis, correct the problem, and recalibrate the instrument.

8.6.1.3 The results of the calibration blank are to agree within three times the IDL. If not, repeat the analysis two more times and average the results. If the average is not within three standard deviations of the background mean, terminate the analysis, correct the problem, recalibrate, and reanalyze the previous 10 samples. If the blank is less than 1/10 the concentration of the action level of interest, and no sample is within ten percent of the action limit, analyses need not be rerun and recalibration need not be performed before continuation of the run.

8.6.2 Verify the interelement and background correction factors at the beginning of each analytical run. Do this by analyzing the interference check sample (Section 5.8). Results should be within ± 20% of the true value.

9.0 METHOD PERFORMANCE

9.1 In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

9.2 Performance data for aqueous solutions and solid samples from a multilaboratory study (9) are provided in Tables 5 and 6.

10.0 REFERENCES

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5. Jones, C.L. et al. An Interlaboratory Study of Inductively Coupled Plasma Atomic Emission Spectroscopy Method 6010 and Digestion Method 3050. EPA-600/4-87-032, U.S. Environmental Protection Agency, Las Vegas, Nevada, 1987.

TABLE 1
RECOMMENDED WAVELENGTHS AND ESTIMATED INSTRUMENTAL DETECTION LIMITS

Detection Element	Wavelength[a] (nm)	Estimated IDL[b] (µg/L)
Aluminum	308.215	30
Antimony	206.833	21
Arsenic	193.696	35
Barium	455.403	0.87
Beryllium	313.042	0.18
Boron	249.678x2	3.8
Cadmium	226.502	2.3
Calcium	317.933	6.7
Chromium	267.716	4.7
Cobalt	228.616	4.7
Copper	324.754	3.6
Iron	259.940	4.1
Lead	220.353	28
Lithium	670.784	2.8
Magnesium	279.079	20
Manganese	257.610	0.93
Mercury	194.227x2	17
Molybdenum	202.030	5.3
Nickel	231.604x2	10
Phosphorus	213.618	51
Potassium	766.491	See note c
Selenium	196.026	50
Silica (SiO_2)	251.611	17
Silver	328.068	4.7
Sodium	588.995	19
Strontium	407.771	0.28
Thallium	190.864	27
Tin	189.980x2	17
Titanium	334.941	5.0
Vanadium	292.402	5.0
Zinc	213.856x2	1.2

[a]The wavelengths listed (where x2 indicates second order) are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted (e.g., in the case of an interference) if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see Section 3.1). In time, other elements may be added as more information becomes available and as required.

[b]The estimated instrumental detection limits shown are provided as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

[c]Highly dependent on operating conditions and plasma position.

TABLE 2
POTENTIAL INTERFERENCES
ANALYTE CONCENTRATION EQUIVALENTS ARISING FROM
INTERFERENCE AT THE 100-mg/L LEVEL[c]

Analyte	Wavelength (nm)	Interferant[a,b] Al	Ca	Cr	Cu	Fe	Mg	Mn	Ni	Ti	V
Aluminum	308.215	--	--	--	--	--	--	0.21	--	--	1.4
Antimony	206.833	0.47	--	2.9	--	0.08	--	--	--	0.25	0.45
Arsenic	193.696	1.3	--	0.44	--	--	--	--	--	--	1.1
Barium	455.403	--	--	--	--	--	--	--	--	--	--
Beryllium	313.042	--	--	--	--	--	--	--	--	0.04	0.05
Cadmium	226.502	--	--	--	--	0.03	--	--	0.02	--	--
Calcium	317.933	--	--	0.08	--	0.01	0.01	0.04	--	0.03	0.03
Chromium	267.716	--	--	--	--	0.003	--	0.04	--	--	0.04
Cobalt	228.616	--	--	0.03	--	0.005	--	--	0.03	0.15	--
Copper	324.754	--	--	--	--	0.003	--	--	--	0.05	0.02
Iron	259.940	--	--	--	--	--	--	0.12	--	--	--
Lead	220.353	0.17	--	--	--	--	--	--	--	--	--
Magnesium	279.079	--	0.02	0.11	--	0.13	--	0.25	--	0.07	0.12
Manganese	257.610	0.005	--	0.01	--	0.002	0.002	--	--	--	--
Molybdenum	202.030	0.05	--	--	--	0.03	--	--	--	--	--
Nickel	231.604	--	--	--	--	--	--	--	--	--	--
Selenium	196.026	0.23	--	--	--	0.09	--	--	--	--	--
Sodium	588.995	--	--	--	--	--	--	--	--	0.08	--
Thallium	190.864	0.30	--	--	--	--	--	--	--	--	--
Vanadium	292.402	--	--	0.05	--	0.005	--	--	--	0.02	--
Zinc	213.856	--	--	--	0.14	--	--	--	0.29	--	--

[a] Dashes indicate that no interference was observed even when interferents were introduced at the following levels:

Al -	1000 mg/L	Mg -	1000 mg/L
Ca -	1000 mg/L	Mn -	200 mg/L
Cr -	200 mg/L	Tl -	200 mg/L
Cu -	200 mg/L	V -	200 mg/L
Fe -	1000 mg/L		

[b] The figures recorded as analyte concentrations are not the actual observed concentrations; to obtain those figures, add the listed concentration to the interferant figure.

[c] Interferences will be affected by background choice and other interferences may be present.

TABLE 3
MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo
IV	Al, Ca, Cr, K, Na, Ni,Li, and Sr
V	Ag (see "NOTE" to Section 5.4), Mg, Sb, and Tl
VI	P

TABLE 4. ICP PRECISION AND ACCURACY DATA[a]

Element	Sample No. 1				Sample No. 2				Sample No. 3			
	True Conc. (ug/L)	Mean Conc. (ug/L)	RSD[b] (%)	Accuracy[d] (%)	True Conc. (ug/L)	Mean Conc. (ug/L)	RSD[b]	Accuracy[d] (%)	True Conc. (ug/L)	Mean Conc. (ug/L)	RSD[b] (%)	Accuracy[d] (%)
Be	750	733	6.2	98	20	20	9.8	100	180	176	5.2	98
Mn	350	345	2.7	99	15	15	6.7	100	100	99	3.3	99
V	750	749	1.8	100	70	69	2.9	99	170	169	1.1	99
As	200	208	7.5	104	22	19	23	86	60	63	17	105
Cr	150	149	3.8	99	10	10	18	100	50	50	3.3	100
Cu	250	235	5.1	94	11	11	40	100	70	67	7.9	96
Fe	600	594	3.0	99	20	19	15	95	180	178	6.0	99
Al	700	696	5.6	99	60	62	33	103	160	161	13	101
Cd	50	48	12	96	2.5	2.9	16	116	14	13	16	93
Co	700	512	10	73	20	20	4.1	100	120	108	21	90
Ni	250	245	5.8	98	30	28	11	93	60	55	14	92
Pb	250	236	16	94	24	30	32	125	80	80	14	100
Zn	200	201	5.6	100	16	19	45	119	80	82	9.4	102
Se[c]	40	32	21.9	80	6	8.5	42	142	10	8.5	8.3	85

[a] Not all elements were analyzed by all laboratories.
[b] RSD = relative standard deviation.
[c] Results for Se are from two laboratories.
[d] Accuracy is expressed as the mean concentration divided by the true concentration times 100.

TABLE 5

ICP-AES PRECISION AND ACCURACY FOR AQUEOUS SOLUTIONS[a]

Element	Mean Conc. (mg/L)	N[b]	RSD[b] (%)	Accuracy[c] (%)
Al	14.8	8	6.3	100
Sb	15.1	8	7.7	102
As	14.7	7	6.4	99
Ba	3.66	7	3.1	99
Be	3.78	8	5.8	102
Cd	3.61	8	7.0	97
Ca	15.0	8	7.4	101
Cr	3.75	8	8.2	101
Co	3.52	8	5.9	95
Cu	3.58	8	5.6	97
Fe	14.8	8	5.9	100
Pb	14.4	7	5.9	97
Mg	14.1	8	6.5	96
Mn	3.70	8	4.3	100
Mo	3.70	8	6.9	100
Ni	3.70	7	5.7	100
K	14.1	8	6.6	95
Se	15.3	8	7.5	104
Ag	3.69	6	9.1	100
Na	14.0	8	4.2	95
Tl	15.1	7	8.5	102
V	3.51	8	6.6	95
Zn	3.57	8	8.3	96

[a]these performance values are independent of sample preparation because the labs analyzed portions of the same solutions

[b]N = Number of measurements for mean and relative standard deviation (RSD).

[c]Accuracy is expressed as a percentage of the nominal value for each analyte in acidified, multi-element solutions.

TABLE 6

ICP-AES PRECISION AND BIAS FOR SOLID WASTE DIGESTS[a]

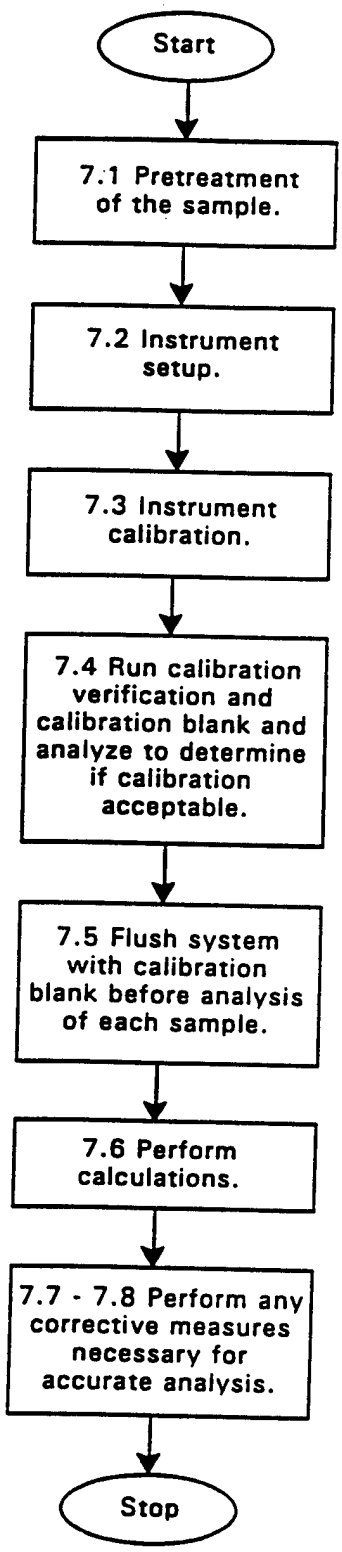
Element	Spiked Coal Fly Ash (NIST-SRM 1633a) Mean Conc. (mg/L)	N[b]	RSD[b] (%)	Bias[c] (%AAS)	Spiked Electroplating Sludge Mean Conc. (mg/L)	N[b]	RSD[b] (%)	Bias[c] (%AAS)
Al	330	8	16	104	127	8	13	110
Sb	3.4	6	73	96	5.3	7	24	120
As	21	8	83	270	5.2	7	8.6	87
Ba	133	8	8.7	101	1.6	8	20	58
Be	4.0	8	57	460	0.9	7	9.9	110
Cd	0.97	6	5.7	101	2.9	7	9.9	90
Ca	87	6	5.6	208	954	7	7.0	97
Cr	2.1	7	36	106	154	7	7.8	93
Co	1.2	6	21	94	1.0	7	11	85
Cu	1.9	6	9.7	118	156	8	7.8	97
Fe	602	8	8.8	102	603	7	5.6	98
Pb	4.6	7	22	94	25	7	5.6	98
Mg	15	8	15	110	35	8	20	84
Mn	1.8	7	14	104	5.9	7	9.6	95
Mo	891	8	19	105	1.4	7	36	110
Ni	1.6	6	8.1	91	9.5	7	9.6	90
K	46	8	4.2	98	51	8	5.8	82
Se	6.4	5	16	73	8.7	7	13	101
Ag	1.4	3	17	140	0.75	7	19	270
Na	20	8	49	130	1380	8	9.8	95
Tl	6.7	4	22	260	5.0	7	20	180
V	1010	5	7.5	100	1.2	6	11	80
Zn	2.2	6	7.6	93	266	7	2.5	101

[a]These performance values are independent of sample preparation because the labs analyzed portions of the same digests.

[b]N = Number of measurements for mean and relative standard deviation (RSD).

[c]Bias for the ICP-AES data is expressed as a percentage of atomic absorption spectroscopy (AA) data for the same digests.

INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROMETRY



Appendix B-11 - Metals (Hg): Method 7470A/71A

Method 7470A/7471A Mercury (Manual Cold-Vapor Technique)

1.0 Procedure

For liquid samples for mercury, perform analysis in accordance with Method 7470A as attached. For solid samples for mercury, perform analysis in accordance with Method 7471A as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes as quality assurance records.

3.0 Quality Control Samples

For each batch of samples, perform the quality control analyses specified in the method: method blank, reagent blank, calibration check sample.

For each batch, introduce one quality control sample made from a separate stock than that used to calibrate the machine (a laboratory control sample).

Where possible, for each batch analyze one matrix spike sample.

For each batch, analyze a matrix spike duplicate or a sample duplicate.

MERCURY IN LIQUID WASTE (MANUAL COLD-VAPOR TECHNIQUE)

1.0 SCOPE AND APPLICATION

1.1 Method 7470 is a cold-vapor atomic absorption procedure approved for determining the concentration of mercury in mobility-procedure extracts, aqueous wastes, and ground waters. (Method 7470 can also be used for analyzing certain solid and sludge-type wastes; however, Method 7471 is usually the method of choice for these waste types.) All samples must be subjected to an appropriate dissolution step prior to analysis.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, the liquid samples must be prepared according to the procedure discussed in this method.

2.2 Method 7470, a cold-vapor atomic absorption technique, is based on the absorption of radiation at 253.7-nm 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 detection limit for this method is 0.0002 mg/L.

3.0 INTERFERENCES

3.1 Potassium permanganate is added to eliminate possible interference from sulfide. Concentrations as high as 20 mg/L of sulfide as sodium sulfide do not interfere with the recovery of added inorganic mercury from reagent water.

3.2 Copper has also been reported to interfere; however, copper concentrations as high as 10 mg/L had no effect on recovery of mercury from spiked samples.

3.3 Seawaters, brines, and industrial effluents 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 253.7 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. Both inorganic and organic mercury spikes have been quantitatively recovered from seawater by using this technique.

3.4 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.

4.0 APPARATUS AND MATERIALS

4.1 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.

4.2 Mercury hollow cathode lamp or electrodeless discharge lamp.

4.3 Recorder: Any multirange variable-speed recorder that is compatible with the UV detection system is suitable.

4.4 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-in.-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.

4.5 Air pump: Any peristaltic pump capable of delivering 1 liter air/min may be used. A Masterflex pump with electronic speed control has been found to be satisfactory.

4.6 Flowmeter: Capable of measuring an air flow of 1 liter/min.

4.7 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.

4.8 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.

4.9 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. 0.25% Iodine in a 3% KI solution.

A specially treated charcoal that will adsorb mercury vapor is also available from Barnebey and Cheney, East 8th Avenue and North Cassidy Street, Columbus, Ohio 43219, Cat. #580-13 or #580-22.

4.10 Hot plate or equivalent - Adjustable and capable of maintaining a temperature of 90-95°C.

4.11 Graduated cylinder or equivalent.

5.0 REAGENTS

5.1 Reagent Water: Reagent water will be interference free. All references to water in this method will refer to reagent water unless otherwise specified.

5.2 Sulfuric acid (H_2SO_4), concentrated: Reagent grade.

5.3 Sulfuric acid, 0.5 N: Dilute 14.0 mL of concentrated sulfuric acid to 1.0 liter.

5.4 Nitric acid (HNO_3), concentrated: Reagent grade of low mercury content. If a high reagent blank is obtained, it may be necessary to distill the nitric acid.

5.5 Stannous sulfate: Add 25 g stannous sulfate to 250 mL of 0.5 N H_2SO_4. This mixture is a suspension and should be stirred continuously during use. (Stannous chloride may be used in place of stannous sulfate.)

5.6 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.)

5.7 Potassium permanganate, mercury-free, 5% solution (w/v): Dissolve 5 g of potassium permanganate in 100 mL of reagent water.

5.8 Potassium persulfate, 5% solution (w/v): Dissolve 5 g of potassium persulfate in 100 mL of reagent water.

5.9 Stock mercury solution: Dissolve 0.1354 g of mercuric chloride in 75 mL of reagent water. Add 10 mL of concentrated HNO_3 and adjust the volume to 100.0 mL (1 mL = 1 mg Hg). Stock solutions may also be purchased.

5.10 Mercury working standard: Make successive dilutions of the stock mercury solution to obtain a working standard containing 0.1 ug per mL. This working standard and the dilutions of the stock mercury solution 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 addition of the aliquot.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be prewashed with detergents, acids, and reagent water. Plastic and glass containers are both suitable.

6.3 Aqueous samples must be acidified to a pH <2 with HNO_3. The suggested maximum holding times for mercury is 28 days.

6.4 Nonaqueous samples shall be refrigerated, when possible, and analyzed as soon as possible.

7.0 PROCEDURE

7.1 Sample preparation: Transfer 100 mL, or an aliquot diluted to 100 mL, containing <1.0 g of mercury, to a 300-mL BOD bottle or equivalent. Add 5 mL of H_2SO_4 and 2.5 mL of concentrated HNO_3, mixing after each addition. Add 15 mL of potassium permanganate solution to each sample bottle. Sewage samples may require additional permanganate. Ensure that equal amounts of permanganate are added to standards and blanks. Shake and add additional portions of potassium permanganate solution, if necessary, until the purple color persists for at least 15 min. Add 8 mL of potassium persulfate to each bottle and heat for 2 hr in a water bath maintained at 95°C. Cool and add 6 mL of sodium chloride-hydroxylamine sulfate to reduce the excess permanganate. After a delay of at least 30 sec, add 5 mL of stannous sulfate, immediately attach the bottle to the aeration apparatus, and continue as described in Paragraph 7.3.

7.2 Standard preparation: Transfer 0-, 0.5-, 1.0-, 2.0-, 5.0-, and 10.0-mL aliquots of the mercury working standard, containing 0-1.0 ug of mercury, to a series of 300-mL BOD bottles. Add enough reagent water to each bottle to make a total volume of 100 mL. Mix thoroughly and add 5 mL of concentrated H_2SO_4 and 2.5 mL of concentrated HNO_3 to each bottle. Add 15 mL of $KMnO_4$ solution to each bottle and allow to stand at least 15 min. Add 8 mL of potassium persulfate to each bottle and heat for 2 hr in a water bath maintained at 95°C. Cool and add 6 mL of sodium chloride-hydroxylamine sulfate solution to reduce the excess permanganate. When the solution has been decolorized, wait 30 sec, add 5 mL of the stannous sulfate solution, immediately attach the bottle to the aeration apparatus, and continue as described in Paragraph 7.3.

7.3 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 liter/min, is allowed to run continuously. The absorbance will increase and reach a maximum within 30 sec. As soon as the recorder pen levels off (approximately 1 min), open the bypass valve and continue the aeration until the absorbance returns to its minimum value. Close the bypass valve, remove the stopper and frit from the BOD bottle, and continue the aeration. Because of instrument variation refer to the manufacturers recommended operating conditions when using this method.

7.4 Construct a calibration curve by plotting the absorbances of standards versus micrograms of mercury. Determine the peak height of the unknown from the chart and read the mercury value from the standard curve. Duplicates, spiked samples, and check standards should be routinely analyzed.

7.5 Calculate metal concentrations (1) by the method of standard additions, or (2) from a calibration curve. All dilution or concentration factors must be taken into account. Concentrations reported for multiphased or wet samples must be appropriately qualified (e.g., 5 ug/g dry weight).

8.0 QUALITY CONTROL

8.1 Refer to section 8.0 of Method 7000.

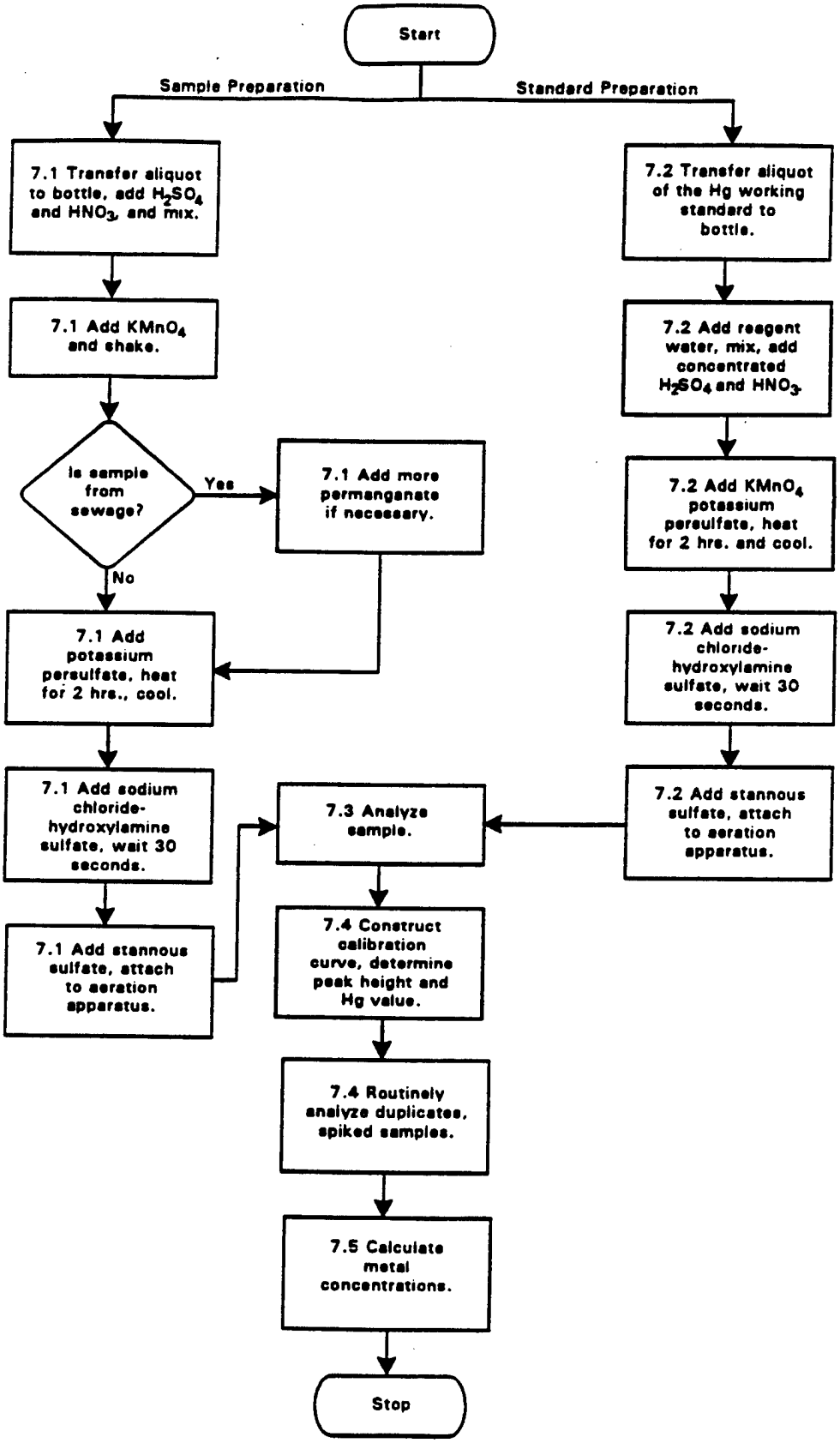
9.0 METHOD PERFORMANCE

9.1 Precision and accuracy data are available in Method 245.1 of Methods for Chemical Analysis of Water and Wastes.

10.0 REFERENCES

1. Methods for Chemical Analysis of Water and Wastes, EPA-600/4-82-055, December 1982, Method 245.1.

METHOD 7470A
MERCURY IN LIQUID WASTE (MANUAL COLD-VAPOR TECHNIQUE)



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 Method 7471, a cold-vapor atomic absorption method, 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 INTERFERENCES

3.1 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.

3.2 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.

3.3 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 253 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.

3.4 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.

4.0 APPARATUS AND MATERIALS

4.1 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.

4.2 Mercury hollow cathode lamp or electrodeless discharge lamp.

4.3 Recorder: Any multirange variable-speed recorder that is compatible with the UV detection system is suitable.

4.4 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-in.-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.

4.5 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.

4.6 Flowmeter: Capable of measuring an air flow of 1 L/min.

4.7 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.

4.8 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.

4.9 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. 0.25% iodine 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.

4.10 Hot plate or equivalent - Adjustable and capable of maintaining a temperature of 90-95°C.

4.11 Graduated cylinder or equivalent.

5.0 REAGENTS

5.1 Reagent Water: Reagent water will be interference free. All references to water in this method refer to reagent water unless otherwise specified.

5.2 Aqua regia: Prepare immediately before use by carefully adding three volumes of concentrated HCl to one volume of concentrated HNO_3.

5.3 Sulfuric acid, 0.5 N: Dilute 14.0 mL of concentrated sulfuric acid to 1 liter.

5.4 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.

5.5 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.

5.6 Potassium permanganate, mercury-free, 5% solution (w/v): Dissolve 5 g of potassium permanganate in 100 mL of reagent water.

5.7 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).

5.8 Mercury working standard: Make successive dilutions of the stock mercury solution to obtain a working standard containing 0.1 ug/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.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be prewashed with detergents, acids, and reagent water. Plastic and glass containers are both suitable.

6.3 Non-aqueous samples shall be refrigerated, when possible, and analyzed as soon as possible."

7.0 PROCEDURE

7.1 Sample preparation: Weigh triplicate 0.2-g portions of untreated sample and place in the bottom of a BOD bottle. Add 5 mL of reagent water and 5 mL of aqua regia. Heat 2 min in a water bath at 95°C. Cool; then add 50 mL reagent water and 15 mL potassium permanganate solution to each sample bottle. Mix thoroughly and place in the water bath for 30 min at 95°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 step 7.4.

7.2 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 0.2 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°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 step 7.4. Refer to the caution statement in section 7.1 for the proper protocol in reducing the excess permanganate solution and adding stannous sulfate.

7.3 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 in a water bath at 95°C. Allow the sample to cool; add 50 mL reagent water and 15 mL of $KMnO_4$ solution to each bottle and return to the water bath 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 Step 7.4.

7.4 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 sec. As soon as the recorder pen levels off (approximately 1 min), 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.

7.5 Construct a calibration curve by plotting the absorbances of standards versus micrograms of mercury. Determine the peak height of the unknown from the chart and read the mercury value from the standard curve. Duplicates, spiked samples, and check standards should be routinely analyzed.

7.6 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 ug/g dry weight).

8.0 QUALITY CONTROL

8.1 Refer to section 8.0 of Method 7000.

9.0 METHOD PERFORMANCE

9.1 Precision and accuracy data are available in Method 245.5 of Methods for Chemical Analysis of Water and Wastes.

9.2 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.

10.0 REFERENCES

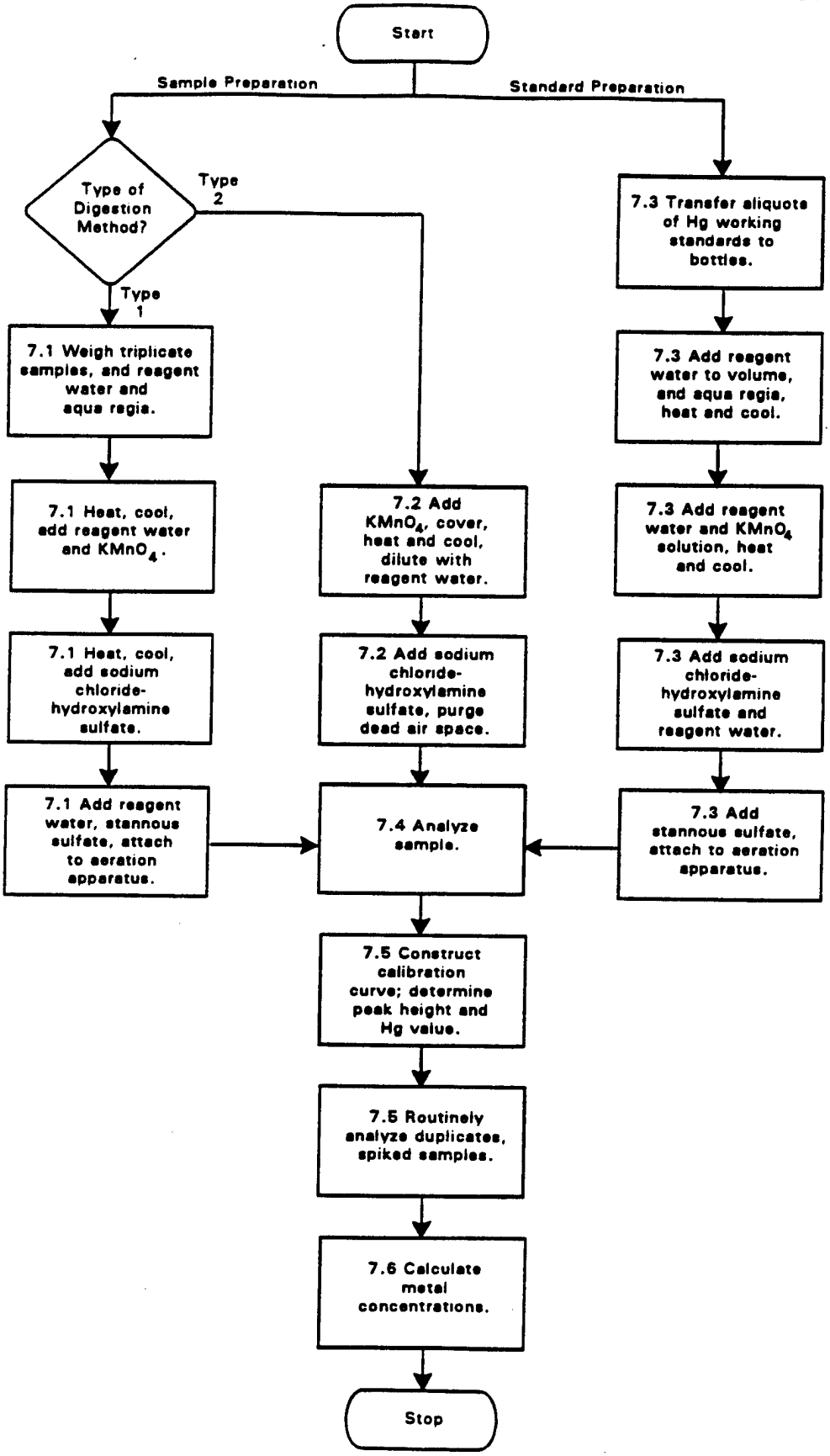
1. Methods for Chemical Analysis of Water and Wastes, EPA-600/4-82-055, December 1982, Method 245.5.

2. Gaskill, A., Compilation and Evaluation of RCRA Method Performance Data, Work Assignment No. 2, EPA Contract No. 68-01-7075, September 1986.

TABLE 1. METHOD PERFORMANCE DATA

Sample Matrix	Preparation Method	Laboratory Replicates
Emission control dust	Not known	12, 12 ug/g
Wastewater treatment sludge	Not known	0.4, 0.28 ug/g

METHOD 7471A
MERCURY IN SOLID OR SEMISOLID WASTE (MANUAL COLD-VAPOR TECHNIQUE)



Revision 1
September 1994

Appendix B-12 - Metals (Se): Method 7740

Method 7740 - Selenium (Atomic Absorption, Furnace Technique)

1.0 Procedure

Perform analysis for selenium in accordance with Method 7740 as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes as quality assurance records.

3.0 Quality Control Samples

For each batch of samples, perform the quality control analyses specified in the method: method blank, reagent blank, calibration check sample.

For each batch, introduce one quality control sample made from a separate stock than that used to calibrate the machine (a laboratory control sample).

Where possible, for each batch analyze one matrix spike sample.

For each batch, analyze a matrix spike duplicate or a sample duplicate.

SELENIUM (ATOMIC ABSORPTION, FURNACE TECHNIQUE)

1.0 SCOPE AND APPLICATION

1.1 Method 7740 is an atomic absorption procedure approved for determining the concentration of selenium in wastes, mobility-procedure extracts, soils, and ground water. All samples must be subjected to an appropriate dissolution step prior to analysis.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis by Method 7740, samples must be prepared in order to convert organic forms of selenium to inorganic forms, to minimize organic interferences, and to convert samples to suitable solutions for analysis. The sample-preparation procedure varies, depending on the sample matrix. Aqueous samples are subjected to the acid-digestion procedure described in this method. Sludge samples are prepared using the procedure described in Method 3050.

2.2 Following the appropriate dissolution of the sample, a representative aliquot is placed manually or by means of an automatic sampler into a graphite tube furnace. The sample aliquot is then slowly evaporated to dryness, charred (ashed), and atomized. The absorption of lamp radiation during atomization will be proportional to the selenium concentration.

2.3 The typical detection limit for this method is 2 ug/L.

3.0 INTERFERENCES

3.1 Elemental selenium and many of its compounds are volatile; therefore, samples may be subject to losses of selenium during sample preparation. Spike samples and relevant standard reference materials should be processed to determine if the chosen dissolution method is appropriate.

3.2 Likewise, caution must be employed during the selection of temperature and times for the dry and char (ash) cycles. A nickel nitrate solution must be added to all digestates prior to analysis to minimize volatilization losses during drying and ashing.

3.3 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 is required to avoid erroneously high results. High iron levels can give overcorrection with deuterium background. Zeeman background correction can be useful in this situation.

3.4 If the analyte is not completely volatilized and removed from the furnace during atomization, memory effects will occur. If this situation is detected, the tube should be cleaned by operating the furnace at full power at regular intervals in the analytical scheme.

3.5 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.

4.0 APPARATUS AND MATERIALS

4.1 250-mL Griffin beaker.

4.2 10-mL volumetric flasks.

4.3 Atomic absorption spectrophotometer: Single- or dual-channel, single- or double-beam instrument with a grating monochromator, photomultiplier detector, adjustable slits, a wavelength range of 190-800 nm, and provisions for simultaneous background correction and interfacing with a strip-chart recorder.

4.4 Selenium hollow cathode lamp, or electrodeless discharge lamp (EDL): EDLs provide better sensitivity for the analysis of Se.

4.5 Graphite furnace: Any graphite furnace device with the appropriate temperature and timing controls.

4.6 Strip-chart recorder: A recorder is strongly recommended for furnace work so that there will be a permanent record and so that any problems with the analysis, such as drift, incomplete atomization, losses during charring, changes in sensitivity, etc., can easily be recognized.

4.7 Pipets: Microliter with disposable tips. Sizes can range from 5 to 1,000 uL, as required.

5.0 REAGENTS

5.1 ASTM Type II water (ASTM D1193): Water should be monitored for impurities.

5.2 Concentrated nitric acid (HNO_3): Acid should be analyzed to determine levels of impurities. If a method blank made with the acid is <MDL, the acid can be used.

5.3. Hydrogen peroxide (30%): Oxidant should be analyzed to determine levels of impurities. If a method blank made with the oxidant is <MDL, the oxidant can be used.

5.4 Selenium standard stock solution (1,000 mg/L): Either procure a certified aqueous standard from a supplier and verify by comparison with a second standard, or dissolve 0.3453 g of selenious acid (actual assay 94.6% H_2SeO_3, analytical reagent grade) or equivalent in Type II water and dilute to 200 mL.

5.5 Nickel nitrate solution (5%): Dissolve 24.780 g of ACS reagent grade $Ni(NO_3)_2 \cdot 6H_2O$ or equivalent in Type II water and dilute to 100 mL.

5.6 Nickel nitrate solution (1%): Dilute 20 mL of the 5% nickel nitrate to 100 mL with Type II water.

5.7 Selenium working standards: Prepare dilutions of the stock solution to be used as calibration standards at the time of the analysis. Withdraw appropriate aliquots of the stock solution, add 1 mL of concentrated HNO_3, 2 mL of 30% H_2O_2, and 2 mL of the 5% nickel nitrate solution. Dilute to 100 mL with Type II water.

5.8 Air: Cleaned and dried through a suitable filter to remove oil, water, and other foreign substances. The source may be a compressor or a cylinder of industrial-grade compressed air.

5.9 Hydrogen: Suitable for instrumental analysis.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be prewashed with detergents, acids, and Type II water. Plastic and glass containers are both suitable.

6.3 Special containers (e.g., containers used for volatile organic analysis) may have to be used if very volatile selenium compounds are to be analyzed.

6.4 Aqueous samples must be acidified to a pH of <2 with nitric acid.

6.5 Nonaqueous samples shall be refrigerated, when possible, and analyzed as soon as possible.

7.0 PROCEDURE

7.1 Sample preparation: Aqueous samples should be prepared in the manner described in Steps 7.1.1 to 7.1.3. Sludge-type samples should be prepared according to Method 3050. The applicability of a sample-preparation technique to a new matrix type must be demonstrated by analyzing spiked samples and/or relevant standard reference materials.

7.1.1 Transfer 100 mL of well-mixed sample to a 250-mL Griffin beaker; add 2 mL of 30% H_2O_2 and sufficient concentrated HNO_3 to result in an acid concentration of 1% (v/v). Heat for 1 hr at 95°C or until the volume is slightly less than 50 mL.

7.1.2 Cool and bring back to 50 mL with Type II water.

7.1.3 Pipet 5 mL of this digested solution into a 10-mL volumetric flask, add 1 mL of the 1% nickel nitrate solution, and dilute to 10 mL with Type II water. The sample is now ready for injection into the furnace.

7.2 The 196.0-nm wavelength line and a background correction system must be employed. Follow the manufacturer's suggestions for all other spectrophotometer parameters.

7.3 Furnace parameters suggested by the manufacturer should be employed as guidelines. Because temperature-sensing mechanisms and temperature controllers can vary between instruments or with time, the validity of the furnace parameters must be periodically confirmed by systematically altering the furnace parameters while analyzing a standard. In this manner, losses of analyte due to overly high temperature settings or losses in sensitivity due to less than optimum settings can be minimized. Similar verification of furnace parameters may be required for complex sample matrices.

7.4 Inject a measured uL-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.

7.5 Analyze all EP extracts, all samples analyzed as part of a delisting petition, and all samples that suffer from matrix interferences by the method of standard additions.

7.6 Run a check standard after approximately every 10 sample injections. 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.

7.7 Duplicates, spiked samples, and check standards should be analyzed every 20 samples.

7.8 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.

8.0 QUALITY CONTROL

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

8.2 Calibration curves must be composed of a minimum of a blank and three standards. A calibration curve should be made for every hour of continuous sample analysis.

8.3 Dilute samples if they are more concentrated than the highest standard or if they fall on the plateau of a calibration curve.

8.4 Employ a minimum of one blank per sample batch to determine if contamination or any memory effects are occurring.

8.5 Verify calibration with an independently prepared check standard every 15 samples.

8.6 Run one spike duplicate sample for every 10 samples. A duplicate sample is a sample brought through the entire sample preparation and analytical process.

8.7 The method of standard additions (see Method 7000, Section 8.7) shall be used for the analysis of all EP extracts, on all analyses submitted as part of a delisting petition, and whenever a new sample matrix is being analyzed.

9.0 METHOD PERFORMANCE

9.1 Precision and accuracy data are available in Method 270.2 of Methods for Chemical Analysis of Water and Wastes.

9.2 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.

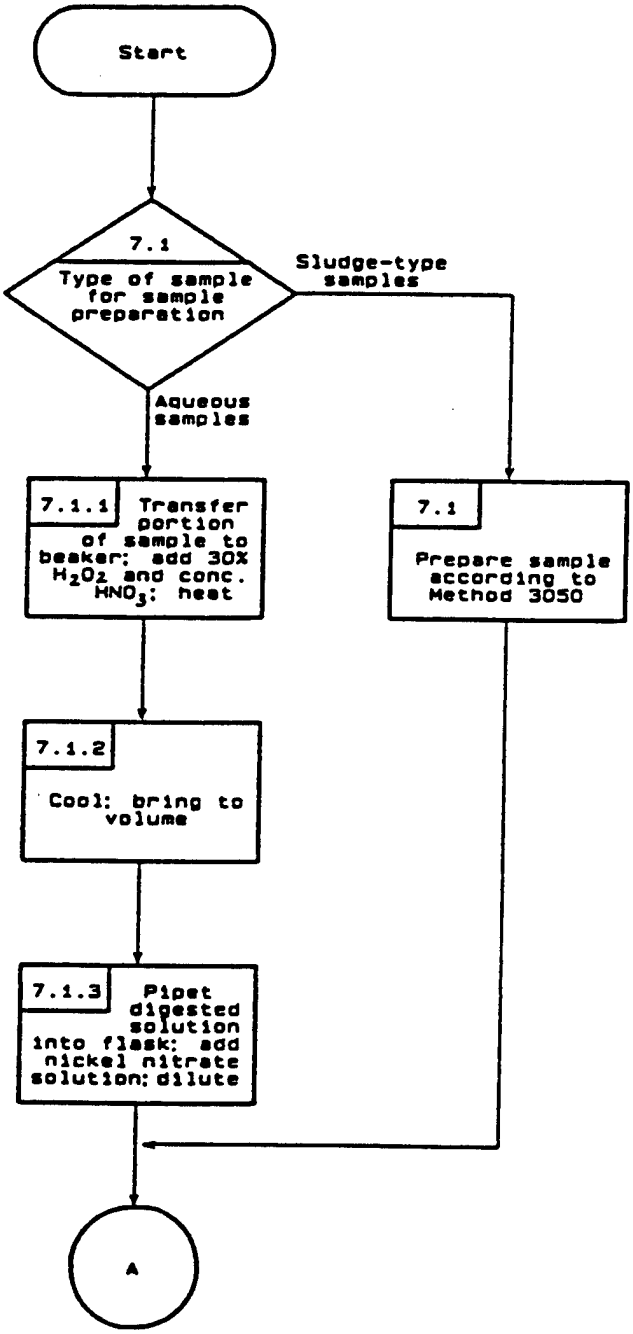
10.0 REFERENCES

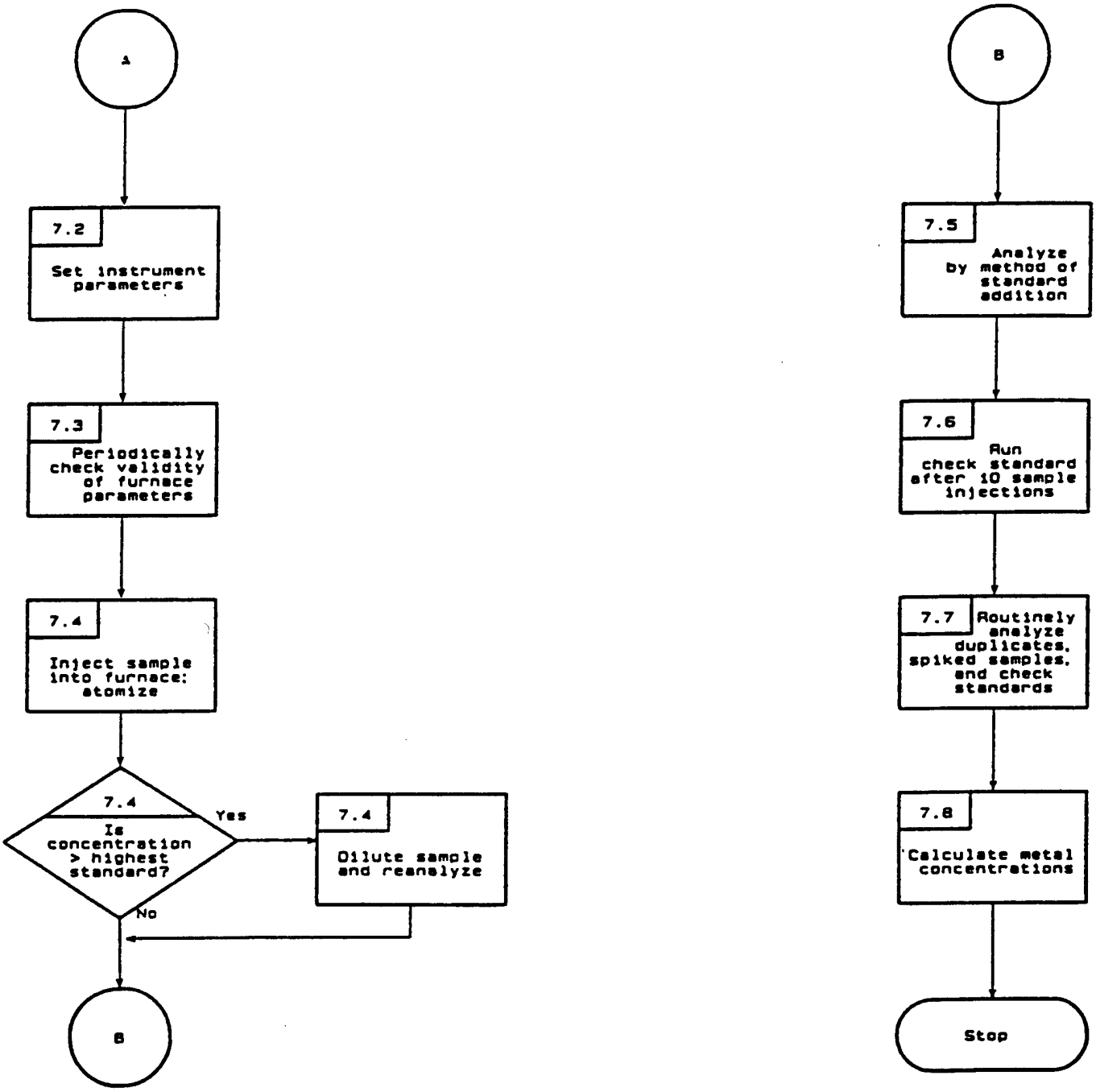
1. Methods for Chemical Analysis of Water and Wastes, EPA-600/4-82-055, December 1982, Method 270.2.

2. Gaskill, A., Compilation and Evaluation of RCRA Method Performance Data, Work Assignment No. 2, EPA Contract No. 68-01-7075, September 1986.

TABLE 1. METHOD PERFORMANCE DATA

Sample Matrix	Preparation Method	Laboratory Replicates
Emission control dust	3050	14, 11 ug/g


Start
7.1
Type of sample for sample preparation
Sludge-type samples
Aqueous samples
7.1.1 Transfer portion of sample to beaker; add 30% H_2O_2 and conc. HNO_3; heat
7.1 Prepare sample according to Method 3050
7.1.2 Cool; bring to volume
7.1.3 Pipet digested solution into flask; add nickel nitrate solution; dilute
A


A
7.2
Set instrument parameters
7.3
Periodically check validity of furnace parameters
7.4
Inject sample into furnace: atomize
7.4
Is concentration > highest standard?
Yes
No
7.4
Dilute sample and reanalyze
B
B
7.5
Analyze by method of standard addition
7.6
Run check standard after 10 sample injections
7.7
Routinely analyze duplicates, spiked samples, and check standards
7.8
Calculate metal concentrations
Stop

Appendix B-13 - Metals (As): Method 7060A

Method 7060A - Arsenic (Atomic Absorption, Furnace Technique)

1.0 Procedure

Perform analysis for arsenic in accordance with Method 7060 as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes as quality assurance records.

3.0 Quality Control Samples

For each batch of samples, perform the quality control analyses specified in the method: method blank, reagent blank, calibration check sample.

For each batch, introduce one quality control sample made from a separate stock than that used to calibrate the machine (a laboratory control sample).

Where possible, for each batch analyze one matrix spike sample.

For each batch, analyze a matrix spike duplicate or a sample duplicate.

ARSENIC (ATOMIC ABSORPTION, FURNACE TECHNIQUE)

1.0 SCOPE AND APPLICATION

1.1 Method 7060 is an atomic absorption procedure approved for determining the concentration of arsenic in wastes, mobility procedure extracts, soils, and ground water. All samples must be subjected to an appropriate dissolution step prior to analysis.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis by Method 7060, samples must be prepared in order to convert organic forms of arsenic to inorganic forms, to minimize organic interferences, and to convert the sample to a suitable solution for analysis. The sample preparation procedure varies depending on the sample matrix. Aqueous samples are subjected to the acid digestion procedure described in this method. Sludge samples are prepared using the procedure described in Method 3050.

2.2 Following the appropriate dissolution of the sample, a representative aliquot of the digestate is spiked with a nickel nitrate solution and is placed manually or by means of an automatic sampler into a graphite tube furnace. The sample aliquot is then slowly evaporated to dryness, charred (ashed), and atomized. The absorption of hollow cathode or EDL radiation during atomization will be proportional to the arsenic concentration. Other modifiers may be used in place of nickel nitrate if the analyst documents the chemical and concentration used.

2.3 The typical detection limit for water samples using this method is 1 ug/L. This detection limit may not be achievable when analyzing waste samples.

3.0 INTERFERENCES

3.1 Elemental arsenic and many of its compounds are volatile; therefore, samples may be subject to losses of arsenic during sample preparation. Spike samples and relevant standard reference materials should be processed to determine if the chosen dissolution method is appropriate.

3.2 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 must be added to all digestates prior to analysis to minimize volatilization losses during drying and ashing.

3.3 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 interferent 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.

3.4 If the analyte is not completely volatilized and removed from the furnace during atomization, memory effects will occur. If this situation is detected by means of blank burns, the tube should be cleaned by operating the furnace at full power at regular intervals in the analytical scheme.

4.0 APPARATUS AND MATERIALS

4.1 Griffin beaker or equivalent: 250 mL.

4.2 Class A Volumetric flasks: 10-mL.

4.3 Atomic absorption spectrophotometer: Single or dual channel, single- or double-beam instrument having a grating monochromator, photo-multiplier detector, adjustable slits, a wavelength range of 190 to 800 nm, and provisions for simultaneous background correction and interfacing with a suitable recording device.

4.4 Arsenic hollow cathode lamp, or electrodeless discharge lamp (EDL): EDLs provide better sensitivity for arsenic analysis.

4.5 Graphite furnace: Any graphite furnace device with the appropriate temperature and timing controls.

4.6 Data systems recorder: A recorder is strongly recommended for furnace work so that there will be a permanent record and so that any problems with the analysis such as drift, incomplete atomization, losses during charring, changes in sensitivity, etc., can easily be recognized.

4.7 Pipets: Microliter with disposable tips. Sizes can range from 5 to 1,000 uL, as required.

5.0 REAGENTS

5.1 Reagent water: Water should be monitored for impurities. All references to water will refer to reagent water.

5.2 Concentrated nitric acid: Acid should be analyzed to determine levels of impurities. If a method blank using the acid is <MDL, the acid can be used.

5.3. Hydrogen peroxide (30%): Oxidant should be analyzed to determine levels of impurities. If a method blank using the H_2O_2 is <MDL, the reagent can be used.

5.4 Arsenic standard stock solution (1,000 mg/L): Either procure a certified aqueous standard from a supplier and verify by comparison with a second standard, or dissolve 1.320 g of arsenic trioxide (As_2O_3, analytical reagent grade) or equivalent in 100 mL of reagent water containing 4 g NaOH. Acidify the solution with 20 mL concentrated HNO_3 and dilute to 1 liter (1 mL = 1 mg As).

5.5 Nickel nitrate solution (5%): Dissolve 24.780 g of ACS reagent grade $Ni(NO_3)_2 \cdot 6H_2O$ or equivalent in reagent water and dilute to 100 mL.

5.6 Nickel nitrate solution (1%): Dilute 20 mL of the 5% nickel nitrate to 100 mL with reagent water.

5.7 Arsenic working standards: Prepare dilutions of the stock solution to be used as calibration standards at the time of the analysis. Withdraw appropriate aliquots of the stock solution, add concentrated HNO_3, 30% H_2O_2, and 5% nickel nitrate solution or other appropriate matrix modifier. Amounts added should be representative of the concentrations found in the samples. Dilute to 100 mL with reagent water.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be prewashed with detergents, acids, and reagent water. Plastic and glass containers are both suitable.

6.3 Special containers (e.g., containers used for volatile organic analysis) may have to be used if very volatile arsenic compounds are to be analyzed.

6.4 Aqueous samples must be acidified to a pH of <2 with nitric acid and refrigerated prior to analysis.

6.5 Although waste samples do not need to be refrigerated sample handling and storage must comply with the minimum requirements established in Chapter One.

7.0 PROCEDURE

7.1 Sample preparation: Aqueous samples should be prepared in the manner described in Paragraphs 7.1.1-7.1.3. Sludge-type samples should be prepared according to Method 3050A. The applicability of a sample-preparation technique to a new matrix type must be demonstrated by analyzing spiked samples and/or relevant standard reference materials.

7.1.1 Transfer a known volume of well-mixed sample to a 250-mL Griffin beaker or equivalent; add 2 mL of 30% H_2O_2 and sufficient concentrated HNO_3 to result in an acid concentration of 1% (v/v). Heat, until digestion is complete, at 95°C or until the volume is slightly less than 50 mL.

7.1.2 Cool, transfer to a volumetric flask, and bring back to 50 mL with reagent water.

7.1.3 Pipet 5 mL of this digested solution into a 10-mL volumetric flask, add 1 mL of the 1% nickel nitrate solution or other appropriate matrix modifier, and dilute to 10 mL with reagent water. The sample is now ready for injection into the furnace.

7.2 The 193.7-nm wavelength line and a background correction system are required. Follow the manufacturer's suggestions for all other spectrophotometer parameters.

7.3 Furnace parameters suggested by the manufacturer should be employed as guidelines. Because temperature-sensing mechanisms and temperature controllers can vary between instruments or with time, the validity of the furnace parameters must be periodically confirmed by systematically altering the furnace parameters while analyzing a standard. In this manner, losses of analyte due to overly high temperature settings or losses in sensitivity due to less than optimum settings can be minimized. Similar verification of furnace parameters may be required for complex sample matrices.

7.4 Inject a measured microliter 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.

8.0 QUALITY CONTROL

8.1 Refer to section 8.0 of Method 7000.

9.0 METHOD PERFORMANCE

9.1 Precision and accuracy data are available in Method 206.2 of Methods for Chemical Analysis of Water and Wastes.

9.2 The optimal concentration range for aqueous samples using this method is 5-100 ug/L. Concentration ranges for non-aqueous samples will vary with matrix type.

9.3 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.

10.0 REFERENCES

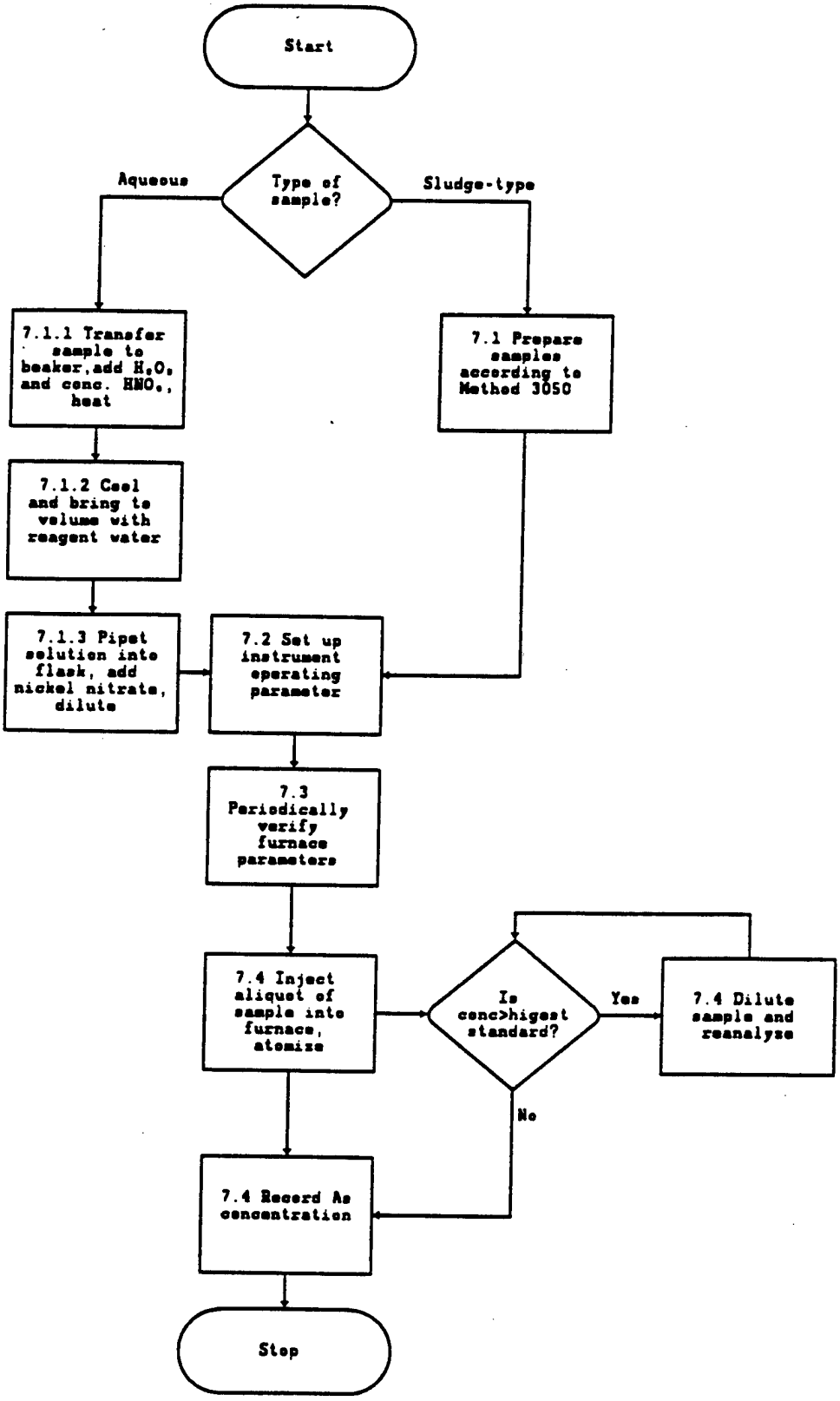
1. Methods for Chemical Analysis of Water and Wastes, EPA-600/4-82-055, December 1982, Method 206.2.

2. Gaskill, A., Compilation and Evaluation of RCRA Method Performance Data, Work Assignment No. 2, EPA Contract No. 68-01-7075, September 1986.

TABLE 1. METHOD PERFORMANCE DATA

Sample Matrix	Preparation Method	Laboratory Replicates
Contaminated soil	3050	2.0, 1.8 ug/g
Oily soil	3050	3.3, 3.8 ug/g
NBS SRM 1646 Estuarine sediment	3050	8.1, 8.33 ug/g[a]
Emission control dust	3050	430, 350 ug/g

[a]Bias of -30 and -28% from expected, respectively.


Start
Type of sample?
Aqueous
Sludge-type
7.1.1 Transfer sample to beaker, add H_2O_2 and conc. HNO_3, heat
7.1.2 Cool and bring to volume with reagent water
7.1.3 Pipet solution into flask, add nickel nitrate, dilute
7.1 Prepare samples according to Method 3050
7.2 Set up instrument operating parameter
7.3 Periodically verify furnace parameters
7.4 Inject aliquot of sample into furnace, atomize
Is conc>higest standard?
Yes
7.4 Dilute sample and reanalyze
No
7.4 Record As concentration
Stop

Appendix B-14- Metals Digestion: Method 3050 B

Although the following procedure lists a post project approval date, the methods described herein accurately describe the procedures used during the study.

Method 3050B - Acid Digestion of Sediments, Sludges, and Soils

1.0 Procedure

Prepare solid samples for further analysis by AA or ICP in accordance with Method 3050B as attached.

2.0 Recordkeeping

Retain all worksheets, percent recovery calculations, weights, volumes, preparation information, spiking solution concentrations, and notes as quality assurance records.

3.0 Quality Control Samples

For each batch of samples, perform the quality control analyses specified in the method: method blank, reagent blank, calibration check sample.

For each batch, introduce one quality control sample made from a separate stock than that used to calibrate the machine (a laboratory control sample).

Where possible, for each batch analyze one matrix spike sample.

For each batch, analyze a matrix spike duplicate or a sample duplicate.

METHOD 3050B

ACID DIGESTION OF SEDIMENTS, SLUDGES, AND SOILS

1.0 SCOPE AND APPLICATION

1.1 This method has been written to provide two separate digestion procedures, one for the preparation of sediments, sludges, and soil samples for analysis by flame atomic absorption spectrometry (FLAA) or inductively coupled plasma atomic emission spectrometry (ICP-AES) and one for the preparation of sediments, sludges, and soil samples for analysis of samples by Graphite Furnace AA (GFAA) or inductively coupled plasma mass spectrometry (ICP-MS). The extracts from these two procedures are not interchangeable and should only be used with the analytical determinations outlined in this section. Samples prepared by this method may be analyzed by ICP-AES or GFAA for all the listed metals as long as the detecion limits are adequate for the required end-use of the data. Alternative determinative techniques may be used if they are scientifically valid and the QC criteria of the method, including those dealing with interferences, can be achieved. Other elements and matrices may be analyzed by this method if performance is demonstrated for the analytes of interest, in the matrices of interest, at the concentration levels of interest (See Section 8.0). The recommended determinative techniques for each element are listed below:

FLAA/ICP-AES		GFAA/ICP-MS
Aluminum	Magnesium	Arsenic
Antimony	Manganese	Beryllium
Barium	Molybdenum	Cadmium
Beryllium	Nickel	Chromium
Cadmium	Potassium	Cobalt
Calcium	Silver	Iron
Chromium	Sodium	Lead
Cobalt	Thallium	Molybdenum
Copper	Vanadium	Selenium
Iron	Zinc	Thallium
Lead		
Vanadium		

1.2 This method is not a total digestion technique for most samples. It is a very strong acid digestion that will dissolve almost all elements that could become "environmentally available." By design, elements bound in silicate structures are not normally dissolved by this procedure as they are not usually mobile in the environment. If absolute total digestion is required use Method 3052.

2.0 SUMMARY OF METHOD

2.1 For the digestion of samples, a representative 1-2 gram (wet weight) or 1 gram (dry weight) sample is digested with repeated additions of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2).

2.2 For GFAA or ICP-MS analysis, the resultant digestate is reduced in volume while heating and then diluted to a final volume of 100 mL.

2.3 For ICP-AES or FLAA analyses, hydrochloric acid (HCl) is added to the initial digestate and the sample is refluxed. In an optional step to increase the solubility of some metals (see Section 7.3.1: NOTE), this digestate is filtered and the filter paper and residues are rinsed, first

with hot HCl and then hot reagent water. Filter paper and residue are returned to the digestion flask, refluxed with additional HCl and then filtered again. The digestate is then diluted to a final volume of 100 mL.

2.4 If required, a separate sample aliquot shall be dried for a total percent solids determination.

3.0 INTERFERENCES

3.1 Sludge samples can contain diverse matrix types, each of which may present its own analytical challenge. Spiked samples and any relevant standard reference material should be processed in accordance with the quality control requirements given in Sec. 8.0 to aid in determining whether Method 3050B is applicable to a given waste.

4.0 APPARATUS AND MATERIALS

4.1 Digestion Vessels - 250-mL.

4.2 Vapor recovery device (e.g., ribbed watch glasses, appropriate refluxing device, appropriate solvent handling system).

4.3 Drying ovens - able to maintain 30°C ± 4°C.

4.4 Temperature measurement device capable of measuring to at least 125°C with suitable precision and accuracy (e.g., thermometer, IR sensor, thermocouple, thermister, etc.)

4.5 Filter paper - Whatman No. 41 or equivalent.

4.6 Centrifuge and centrifuge tubes.

4.7 Analytical balance - capable of accurate weighings to 0.01 g.

4.8 Heating source - Adjustable and able to maintain a temperature of 90-95°C. (e.g., hot plate, block digestor, microwave, etc.)

4.9 Funnel or equivalent.

4.10 Graduated cylinder or equivalent volume measuring device.

4.11 Volumetric Flasks - 100-mL.

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. If the purity of a reagent is questionable, analyze the reagent to determine the level of impurities. The reagent blank must be less than the MDL in order to be used.

5.2 Reagent Water. Reagent water will be interference free. All references to water in the method refer to reagent water unless otherwise specified. Refer to Chapter One for a definition of reagent water.

5.3 Nitric acid (concentrated), HNO_3. Acid should be analyzed to determine level of impurities. If method blank is < MDL, the acid can be used.

5.4 Hydrochloric acid (concentrated), HCl. Acid should be analyzed to determine level of impurities. If method blank is < MDL, the acid can be used.

5.5 Hydrogen peroxide (30%), H_2O_2. Oxidant should be analyzed to determine level of impurities. If method blank is < MDL, the peroxide can be used.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be demonstrated to be free of contamination at or below the reporting limit. Plastic and glass containers are both suitable. See Chapter Three, Section 3.1.3, for further information.

6.3 Nonaqueous samples should be refrigerated upon receipt and analyzed as soon as possible.

6.4 It can be difficult to obtain a representative sample with wet or damp materials. Wet samples may be dried, crushed, and ground to reduce subsample variability as long as drying does not affect the extraction of the analytes of interest in the sample.

7.0 PROCEDURE

7.1 Mix the sample thoroughly to achieve homogeneity and sieve, if appropriate and necessary, using a USS #10 sieve. All equipment used for homogenization should be cleaned according to the guidance in Sec. 6.0 to minimize the potential of cross-contamination. For each digestion procedure, weigh to the nearest 0.01 g and transfer a 1-2 g sample (wet weight) or 1 g sample (dry weight) to a digestion vessel. For samples with high liquid content, a larger sample size may be used as long as digestion is completed.

> NOTE: All steps requiring the use of acids should be conducted under a fume hood by properly trained personnel using appropriate laboratory safety equipment. The use of an acid vapor scrubber system for waste minimization is encouraged.

7.2 For the digestion of samples for analysis by GFAA or ICP-MS, add 10 mL of 1:1 HNO_3, mix the slurry, and cover with a watch glass or vapor recovery device. Heat the sample to 95°C ± 5°C and reflux for 10 to 15 minutes without boiling. Allow the sample to cool, add 5 mL of concentrated HNO_3, replace the cover, and reflux for 30 minutes. If brown fumes are generated, indicating oxidation of the sample by HNO_3, repeat this step (addition of 5 mL of conc. HNO_3) over and over until no brown fumes are given off by the sample indicating the complete reaction with HNO_3. Using a ribbed watch glass or vapor recovery system, either allow the solution to evaporate to approximately 5 mL without boiling or heat at 95°C ± 5°C without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all times.

NOTE: Alternatively, for direct energy coupling devices, such as a microwave, digest samples for analysis by GFAA or ICP-MS by adding 10 mL of 1:1 HNO_3, mixing the slurry and then covering with a vapor recovery device. Heat the sample to 95°C ± 5°C and reflux for 5 minutes at 95°C ± 5°C without boiling. Allow the sample to cool for 5 minutes, add 5 mL of concentrated HNO_3, heat the sample to 95°C ± 5°C and reflux for 5 minutes at 95°C ± 5°C. If brown fumes are generated, indicating oxidation of the sample by HNO_3, repeat this step (addition of 5 mL concentrated HNO_3) until no brown fumes are given off by the sample indicating the complete reaction with HNO_3. Using a vapor recovery system, heat the sample to 95°C ± 5°C and reflux for 10 minutes at 95°C ± 5°C without boiling.

7.2.1 After the step in Section 7.2 has been completed and the sample has cooled, add 2 mL of water and 3 mL of 30% H_2O_2. Cover the vessel with a watch glass or vapor recovery device and return the covered vessel to the heat source for warming and to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. Heat until effervescence subsides and cool the vessel.

NOTE: Alternatively, for direct energy coupled devices: After the Sec. 7.2 "NOTE" step has been completed and the sample has cooled for 5 minutes, add slowly 10 mL of 30% H_2O_2. Care must be taken to ensure that losses do not occur due to excessive vigorous effervesence. Go to Section 7.2.3.

7.2.2 Continue to add 30% H_2O_2 in 1-mL aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged.

NOTE: Do not add more than a total of 10 mL 30% H_2O_2.

7.2.3 Cover the sample with a ribbed watch glass or vapor recovery device and continue heating the acid-peroxide digestate until the volume has been reduced to approximately 5 mL or heat at 95°C ± 5°C without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all times.

NOTE: Alternatively, for direct energy coupled devices: Heat the acid-peroxide digestate to 95°C ± 5°C in 6 minutes and remain at 95°C ± 5°C without boiling for 10 minutes.

7.2.4 After cooling, dilute to 100 mL with water. Particulates in the digestate should then be removed by filtration, by centrifugation, or by allowing the sample to settle. The sample is now ready for analysis by GFAA or ICP-MS.

7.2.4.1 Filtration - Filter through Whatman No. 41 filter paper (or equivalent).

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

7.2.4.3 The diluted digestate solution contains approximately 5% (v/v) HNO_3. For analysis, withdraw aliquots of appropriate volume and add any required reagent or matrix modifier.

7.3 For the analysis of samples for FLAA or ICP-AES, add 10 mL conc. HCl to the sample digest from 7.2.3 and cover with a watch glass or vapor recovery device. Place the sample on/in the heating source and reflux at 95°C ± 5°C for 15 minutes.

NOTE: Alternatively, for direct energy coupling devices, such as a microwave, digest samples for analysis by FLAA and ICP-AES by adding 5 mL HCl and 10 mL H_2O to the sample digest from 7.2.3 and heat the sample to 95°C ± 5°C, Reflux at 95°C ± 5°C without boiling for 5 minutes.

7.4 Filter the digestate through Whatman No. 41 filter paper (or equivalent) and collect filtrate in a 100-mL volumetric flask. Make to volume and analyze by FLAA or ICP-AES.

NOTE: Section 7.5 may be used to improve the solubilities and recoveries of antimony, barium, lead, and silver when necessary. These steps are optional and are not required on a routine basis.

7.5 Add 2.5 mL conc. HNO_3 and 10 mL conc. HCl to a 1-2 g sample (wet weight) or 1 g sample (dry weight) and cover with a watchglass or vapor recovery device. Place the sample on/in the heating source and reflux for 15 minutes.

7.5.1 Filter the digestate through Whatman No. 41 filter paper (or equivalent) and collect filtrate in a 100-mL volumetric flask. Wash the filter paper, while still in the funnel, with no more than 5 mL of hot (~95°C) HCl, then with 20 mL of hot (~95°C) reagent water. Collect washings in the same 100-mL volumetric flask.

7.5.2 Remove the filter and residue from the funnel, and place them back in the vessel. Add 5 mL of conc. HCl, place the vessel back on the heating source, and heat at 95°C ± 5°C until the filter paper dissolves. Remove the vessel from the heating source and wash the cover and sides with reagent water. Filter the residue and collect the filtrate in the same 100-mL volumetric flask. Allow filtrate to cool, then dilute to volume.

NOTE: High concentrations of metal salts with temperature-sensitive solubilities can result in the formation of precipitates upon cooling of primary and/or secondary filtrates. If precipitation occurs in the flask upon cooling, do not dilute to volume.

7.5.3 If a precipitate forms on the bottom of a flask, add up to 10 mL of concentrated HCl to dissolve the precipitate. After precipitate is dissolved, dilute to volume with reagent water. Analyze by FLAA or ICP-AES.

7.6 Calculations

7.6.1 The concentrations determined are to be reported on the basis of the actual weight of the sample. If a dry weight analysis is desired, then the percent solids of the sample must also be provided.

7.6.2 If percent solids is desired, a separate determination of percent solids must be performed on a homogeneous aliquot of the sample.

8.0 QUALITY CONTROL

8.1 All quality control measures described in Chapter One should be followed.

8.2 For each batch of samples processed, a method blank should 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 method blanks.

8.3 Spiked duplicate samples should be processed on a routine basis and whenever a new sample matrix is being analyzed. Spiked duplicate samples will be used to determine precision and bias. The criteria of the determinative method will dictate frequency, but 5% (one per batch) is recommended or whenever a new sample matrix is being analyzed. Refer to Chapter One for the proper protocol when analyzing spiked replicates.

8.4 Limitations for the FLAA and ICP-AES optional digestion procedure. Analysts should be aware that the upper linear range for silver, barium, lead, and antimony may be exceeded with some samples. If there is a reasonable possibility that this range may be exceeded, or if a sample's analytical result exceeds this upper limit, a smaller sample size should be taken through the entire procedure and re-analyzed to determine if the linear range has been exceeded. The approximate linear upper ranges for a 2 gram sample size:

Ag	2,000 mg/kg
As	1,000,000 mg/kg
Ba	2,500 mg/kg
Be	1,000,000 mg/kg
Cd	1,000,000 mg/kg
Co	1,000,000 mg/kg
Cr	1,000,000 mg/kg
Cu	1,000,000 mg/kg
Mo	1,000,000 mg/kg
Ni	1,000,000 mg/kg
Pb	200,000 mg/kg
Sb	200,000 mg/kg
Se	1,000,000 mg/kg
Tl	1,000,000 mg/kg
V	1,000,000 mg/kg
Zn	1,000,000 mg/kg

NOTE: These ranges will vary with sample matrix, molecular form, and size.

9.0 METHOD PERFORMANCE

9.1 In a single laboratory, the recoveries of the three matrices presented in Table 2 were obtained using the digestion procedure outlined for samples prior to analysis by FLAA and ICP-AES. The spiked samples were analyzed in duplicate. Tables 3-5 represents results of analysis of NIST Standard Reference Materials that were obtained using both atmospheric pressure microwave digestion techniques and hot-plate digestion procedures.

10.0 REFERENCES

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

3. Edgell, K.; USEPA Method Study 37 - SW-846 Method 3050 Acid Digestion of Sediments, Sludges, and Soils. EPA Contract No. 68-03-3254, November 1988.

4. Kimbrough, David E., and Wakakuwa, Janice R. Acid Digestion for Sediments, Sludges, Soils, and Solid Wastes. A Proposed Alternative to EPA SW 846 Method 3050, Environmental Science and Technology, Vol. 23, Page 898, July 1989.

5. Kimbrough, David E., and Wakakuwa, Janice R. Report of an Interlaboratory Study Comparing EPA SW 846 Method 3050 and an Alternative Method from the California Department of Health Services, Fifth Annual Waste Testing and Quality Assurance Symposium, Volume I, July 1989. Reprinted in Solid Waste Testing and Quality Assurance: Third Volume, ASTM STP 1075, Page 231, C.E. Tatsch, Ed., American Society for Testing and Materials, Philadelphia, 1991.

6. Kimbrough, David E., and Wakakuwa, Janice R. A Study of the Linear Ranges of Several Acid Digestion Procedures, Environmental Science and Technology, Vol. 26, Page 173, January 1992. Presented Sixth Annual Waste Testing and Quality Assurance Symposium, July 1990.

7. Kimbrough, David E., and Wakakuwa, Janice R. A Study of the Linear Ranges of Several Acid Digestion Procedures, Sixth Annual Waste Testing and Quality Assurance Symposium, Reprinted in Solid Waste Testing and Quality Assurance: Fourth Volume, ASTM STP 1076, Ed., American Society for Testing and Materials, Philadelphia, 1992.

8. NIST published leachable concentrations. Found in addendum to certificate of analysis for SRMs 2709, 2710, 2711 - August 23, 1993.

9. Kingston, H.M. Haswell, S.J. ed., Microwave Enhanced Chemistry, Professional Reference Book Series, American Chemical Society, Washington, D.C., Chapter 3, 1997.

TABLE 1

STANDARD RECOVERY (%) COMPARISON FOR METHODS 3050A AND 3050B[a]

Analyte	METHOD 3050A[a]	METHOD 3050B w/option[a]
Ag	9.5	98
As	86	102
Ba	97	103
Be	96	102
Cd	101	99
Co	99	105
Cr	98	94
Cu	87	94
Mo	97	96
Ni	98	92
Pb	97	95
Sb	87	88
Se	94	91
Tl	96	96
V	93	103
Zn	99	95

[a] All values are percent recovery. Samples: 4 mL of 100 mg/mL multistandard; n = 3.

TABLE 2

PERCENT RECOVERY COMPARISON FOR METHODS 3050A AND 3050B

Analyte	Percent Recovery[a,c]							
	Sample 4435		Sample 4766		Sample HJ		Average	
	3050A	3050B	3050A	3050B	3050A	3050B	3050A	3050B
Ag	9.8	103	15	89	56	93	27	95
As	70	102	80	95	83	102	77	100
Ba	85	94	78	95	b	b	81	94
Be	94	102	108	98	99	94	99	97
Cd	92	88	91	95	95	97	93	94
Co	90	94	87	95	89	93	89	94
Cr	90	95	89	94	72	101	83	97
Cu	81	88	85	87	70	106	77	94
Mo	79	92	83	98	87	103	83	98
Ni	88	93	93	100	87	101	92	98
Pb	82	92	80	91	77	91	81	91
Sb	28	84	23	77	46	76	32	79
Se	84	89	81	96	99	96	85	94
Tl	88	87	69	95	66	67	74	83
V	84	97	86	96	90	88	87	93
Zn	96	106	78	75	b	b	87	99

a - Samples: 4 mL of 100 mg/mL multi-standard in 2 g of sample. Each value is percent recovery and is the average of duplicate spikes.

b - Unable to accurately quantitate due to high background values.

c - Method 3050B using optional section.

Table 3
Results of Analysis of Nist Standard Reference Material 2704
"River Sediment" Using Method 3050B (µg/g ± SD)

Element	Atm. Pressure Microwave Assisted Method with Power Control	Atm. Pressure Microwave Assisted Method with Temperature Control (gas-bulb)	Atm. Pressure Microwave Assisted Method with Temperature Control (IR-sensor)	Hot-Plate	NIST Certified Values for Total Digestion (µg/g ±95% CI)
Cu	101 ± 7	89 ± 1	98 ± 1.4	100 ± 2	98.6 ± 5.0
Pb	160 ± 2	145 ± 6	145 ± 7	146 ± 1	161 ± 17
Zn	427 ± 2	411 ± 3	405 ± 14	427 ± 5	438 ± 12
Cd	NA	3.5 ± 0.66	3.7 ± 0.9	NA	3.45 ± 0.22
Cr	82 ± 3	79 ± 2	85 ± 4	89 ± 1	135 ± 5
Ni	42 ± 1	36 ± 1	38 ± 4	44 ± 2	44.1 ± 3.0

NA - Not Available

Table 4
Results of Analysis of NIST Standard Reference Material 2710
"Montana Soil (Highly Elevated Trace Element Concentrations)" Using Method 3050B
(µg/g ± SD)

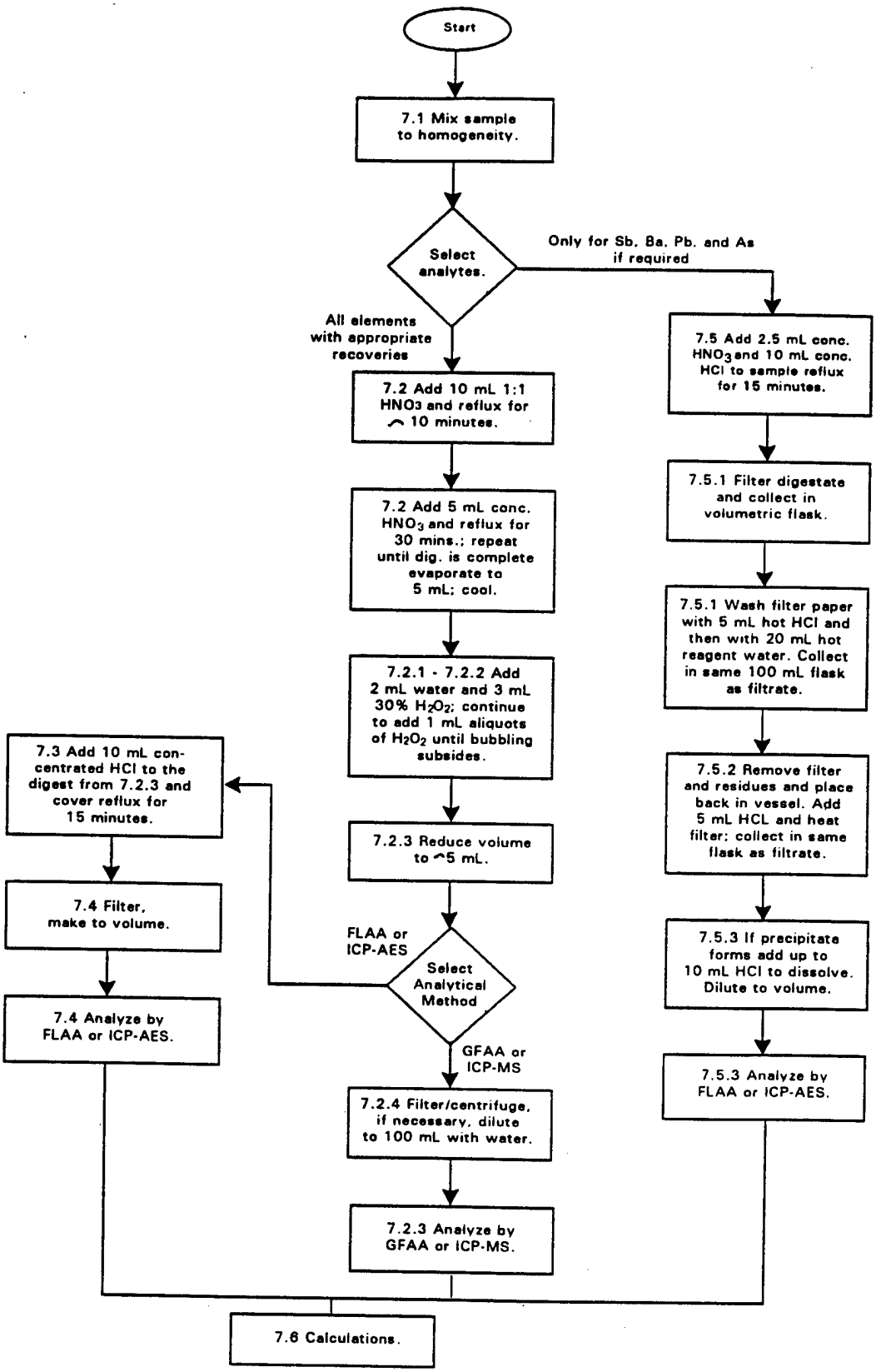
Element	Atm. Pressure Microwave Assisted Method with Power Control	Atm. Pressure Microwave Assisted Method with Temperature Control (gas-bulb)	Atm. Pressure Microwave Assisted Method with Temperature Control (IR-sensor)	Hot-Plate	NIST Leachable Concentrations Using Method 3050	NIST Certified Values for Total Digestion (µg/g ±95% CI)
Cu	2640 ± 60	2790 ± 41	2480 ± 33	2910 ± 59	2700	2950 ± 130
Pb	5640 ± 117	5430 ± 72	5170 ± 34	5720 ± 280	5100	5532 ± 80
Zn	6410 ± 74	5810 ± 34	6130 ± 27	6230 ± 115	5900	6952 ± 91
Cd	NA	20.3 ± 1.4	20.2 ± 0.4	NA	20	21.8 ± 0.2
Cr	20 ± 1.6	19 ± 2	18 ± 2.4	23 ± 0.5	19	39*
Ni	7.8 ± 0.29	10 ± 1	9.1 ± 1.1	7 ± 0.44	10.1	14.3 ± 1.0

NA - Not Available * Non-certified values, for information only.

Table 5
Results of Analysis of NIST Standard Reference Material 2711
"Montana Soil (Moderately Elevated Trace Element Concentrations)" Using Method 3050B
(µg/g ± SD)

Element	Atm. Pressure Microwave Assisted Method with Power Control	Atm. Pressure Microwave Assisted Method with Temperature Control (gas-bulb)	Atm. Pressure Microwave Assisted Method with Temperature Control (IR-sensor)	Hot-Plate	NIST Leachable Concentrations Using Method 3050	NIST Certified Values for Total Digestion (µg/g ±95% CI)
Cu	107 ± 4.6	98 ± 5	98 ± 3.8	111 ± 6.4	100	114 ± 2
Pb	1240 ± 68	1130 ± 20	1120 ± 29	1240 ± 38	1100	1162 ± 31
Zn	330 ± 17	312 ± 2	307 ± 12	340 ± 13	310	350.4 ± 4.8
Cd	NA	39.6 ± 3.9	40.9 ± 1.9	NA	40	41.7 ± 0.25
Cr	22 ± 0.35	21 ± 1	15 ± 1.1	23 ± 0.9	20	47*
Ni	15 ± 0.2	17 ± 2	15 ± 1.6	16 ± 0.4	16	20.6 ± 1.1

NA - Not Available
* Non-certified values, for information only.


Start
7.1 Mix sample to homogeneity.
Select analytes.
Only for Sb, Ba, Pb, and As if required
All elements with appropriate recoveries
7.5 Add 2.5 mL conc. HNO_3 and 10 mL conc. HCl to sample reflux for 15 minutes.
7.2 Add 10 mL 1:1 HNO_3 and reflux for ~ 10 minutes.
7.5.1 Filter digestate and collect in volumetric flask.
7.2 Add 5 mL conc. HNO_3 and reflux for 30 mins.; repeat until dig. is complete evaporate to 5 mL; cool.
7.5.1 Wash filter paper with 5 mL hot HCl and then with 20 mL hot reagent water. Collect in same 100 mL flask as filtrate.
7.2.1 - 7.2.2 Add 2 mL water and 3 mL 30% H_2O_2; continue to add 1 mL aliquots of H_2O_2 until bubbling subsides.
7.3 Add 10 mL concentrated HCl to the digest from 7.2.3 and cover reflux for 15 minutes.
7.5.2 Remove filter and residues and place back in vessel. Add 5 mL HCL and heat filter; collect in same flask as filtrate.
7.2.3 Reduce volume to ~5 mL.
7.4 Filter, make to volume.
7.5.3 If precipitate forms add up to 10 mL HCl to dissolve. Dilute to volume.
FLAA or ICP-AES
Select Analytical Method
7.4 Analyze by FLAA or ICP-AES.
GFAA or ICP-MS
7.5.3 Analyze by FLAA or ICP-AES.
7.2.4 Filter/centrifuge, if necessary, dilute to 100 mL with water.
7.2.3 Analyze by GFAA or ICP-MS.
7.6 Calculations.

Appendix B-15 – Total Organic Carbon: Method 415 Series

Total Organic Carbon - Method 415.1 with Dohrmann DC-190

1.0 Procedure

Perform Total Organic Carbon (or Non-purgeable Organic Carbon[1]) in accordance with "Organic Cargon, Total", Method 415.1 (Combustion or Oxidation) and in accordance with chapters 6 and 10 of the operating manual for the Dorhmann DC-190 high temperature organic carbon analyzer as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples and notes as quality assurance records.

3.0 Quality Control Samples

For each batch of samples, perform a method blank and a calibration check sample. For each batch introduce one quality control sample made from a separate stock than that used to calibrate the instrument. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.

[1] Note: Non-Purgeable Organic Carbon is run when no effort has been made to sample or retain volatiles. This is the analysis of interest for most soil research.

ORGANIC CARBON, TOTAL

Method 415.1 (Combustion or Oxidation)

STORET NO. Total 00680
Dissolved 00681

1. Scope and Application
   1.1 This method includes the measurement of organic carbon in drinking, surface and saline waters, domestic and industrial wastes. Exclusions are noted under Definitions and Interferences.
   1.2 The method is most applicable to measurement of organic carbon above 1 mg/1.
2. Summary of Method
   2.1 Organic carbon in a sample is converted to carbon dioxide (CO_2) by catalytic combustion or wet chemical oxidation. The CO_2 formed can be measured directly by an infrared detector or converted to methane (CH_4) and measured by a flame ionization detector. The amount of CO_2 or CH_4 is directly proportional to the concentration of carbonaceous material in the sample.
3. Definitions
   3.1 The carbonaceous analyzer measures all of the carbon in a sample. Because of various properties of carbon-containing compounds in liquid samples, preliminary treatment of the sample prior to analysis dictates the definition of the carbon as it is measured. Forms of carbon that are measured by the method are:
      A) soluble, nonvolatile organic carbon; for instance, natural sugars.
      B) soluble, volatile organic carbon; for instance, mercaptans.
      C) insoluble, partially volatile carbon; for instance, oils.
      D) insoluble, particulate carbonaceous materials, for instance; cellulose fibers.
      E) soluble or insoluble carbonaceous materials adsorbed or entrapped on insoluble inorganic suspended matter; for instance, oily matter adsorbed on silt particles.
   3.2 The final usefulness of the carbon measurement is in assessing the potential oxygen-demanding load of organic material on a receiving stream. This statement applies whether the carbon measurement is made on a sewage plant effluent, industrial waste, or on water taken directly from the stream. In this light, carbonate and bicarbonate carbon are not a part of the oxygen demand in the stream and therefore should be discounted in the final calculation or removed prior to analysis. The manner of preliminary treatment of the sample and instrument settings defines the types of carbon which are measured. Instrument manufacturer's instructions should be followed.

Approved for NPDES
Issued 1971
Editorial revision 1974

4. Sample Handling and Preservation

4.1 Sampling and storage of samples in glass bottles is preferable. Sampling and storage in plastic bottles such as conventional polyethylene and cubitainers is permissible if it is established that the containers do not contribute contaminating organics to the samples. **NOTE 1:** A brief study performed in the EPA Laboratory indicated that distilled water stored in new, one quart cubitainers did not show any increase in organic carbon after two weeks exposure.

4.2 Because of the possibility of oxidation or bacterial decomposition of some components of aqueous samples, the lapse of time between collection of samples and start of analysis should be kept to a minimum. Also, samples should be kept cool (4°C) and protected from sunlight and atmospheric oxygen.

4.3 In instances where analysis cannot be performed within two hours (2 hours) from time of sampling, the sample is acidified ($pH \leq 2$) with HCl or H_2SO_4.

5. Interferences

5.1 Carbonate and bicarbonate carbon represent an interference under the terms of this test and must be removed or accounted for in the final calculation.

5.2 This procedure is applicable only to homogeneous samples which can be injected into the apparatus reproducibly by means of a microliter type syringe or pipette. The openings of the syringe or pipette limit the maximum size of particles which may be included in the sample.

6. Apparatus

6.1 Apparatus for blending or homogenizing samples: Generally, a Waring-type blender is satisfactory.

6.2 Apparatus for total and dissolved organic carbon:

6.2.1 A number of companies manufacture systems for measuring carbonaceous material in liquid samples. Considerations should be made as to the types of samples to be analyzed, the expected concentration range, and forms of carbon to be measured.

6.2.2 No specific analyzer is recommended as superior.

7. Reagents

7.1 Distilled water used in preparation of standards and for dilution of samples should be ultra pure to reduce the carbon concentration of the blank. Carbon dioxide-free, double distilled water is recommended. Ion exchanged waters are not recommended because of the possibilities of contamination with organic materials from the resins.

7.2 Potassium hydrogen phthalate, stock solution, 1000 mg carbon/liter: Dissolve 0.2128 g of potassium hydrogen phthalate (Primary Standard Grade) in distilled water and dilute to 100.0 ml.

NOTE 2: Sodium oxalate and acetic acid are not recommended as stock solutions.

7.3 Potassium hydrogen phthalate, standard solutions: Prepare standard solutions from the stock solution by dilution with distilled water.

7.4 Carbonate-bicarbonate, stock solution, 1000 mg carbon/liter: Weigh 0.3500 g of sodium bicarbonate and 0.4418 g of sodium carbonate and transfer both to the same 100 ml volumetric flask. Dissolve with distilled water.

7.5 Carbonate-bicarbonate, standard solution: Prepare a series of standards similar to step 7.3.

NOTE 3: This standard is not required by some instruments.

7.6 Blank solution: Use the same distilled water (or similar quality water) used for the preparation of the standard solutions.

8. Procedure

8.1 Follow instrument manufacturer's instructions for calibration, procedure, and calculations.

8.2 For calibration of the instrument, it is recommended that a series of standards encompassing the expected concentration range of the samples be used.

9. Precision and Accuracy

9.1 Twenty-eight analysts in twenty-one laboratories analyzed distilled water solutions containing exact increments of oxidizable organic compounds, with the following results:

Increment as TOC mg/liter	Precision as Standard Deviation TOC, mg/liter	Accuracy as Bias, %	Accuracy as Bias, mg/liter
4.9	3.93	+15.27	+0.75
107	8.32	+ 1.01	+1.08

(FWPCA Method Study 3, Demand Analyses)

Bibliography

1. Annual Book of ASTM Standards, Part 31, "Water", Standard D 2574–79, p 469 (1976).
2. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 532, Method 505, (1975).

SECTION 6
OPERATION

INTRODUCTION

This section contains instructions for routine operation along with detailed descriptions on how to operate and calibrate the different modes.

6.1 ROUTINE OPERATION

SUMMARY
* Daily Start-Up * Daily Operation * Daily and Long-term Shutdown

DAILY START-UP

Check utility supply.

Enough carrier gas for a day's operation.

Acid reservoir at least 1/3 full.

Replenish IC chamber.

Confirm the IC chamber is half full (gas off).

Fill the IC chamber by using the "Acid to IC chamber" function (press **MAIN 2 5**). Each use of this function will result in 20 pulses and is equivalent to 2 ml of acid.

Turn on gas.

Press **CARRIER** .

For Boat Users :

Connect the 1/8 inch PTFE line from the boat module furnace to the DC-190 dehumidifier (see Figure 4.8).

Check system status.

(Press **MAIN 1** to view the status menu.)

Flow rate = 180 - 220 cc/min.

Dryer temperature = 0 - 10 ^{o}C

Furnace Temperature = Furnace set point (Furnace light is green.) For most applications, the temperature should be 680^{o}C.

Confirm or change set-up number on display (see Section 6.8).

Check set-up.

(See Section 6.2 for help in choosing set-up.)

Modes last used are lit up. Make any changes for the day and print the set-up parameters. System is ready for analysis.

Press START when ready.

It is good practice to run a check standard at the beginning of the day before analyzing unknowns, especially if any conditions have been changed. Update calibration if needed. See Section 6.3 for notes on operating and calibrating.

DAILY SHUTDOWN

Check the RUN status.

The unit should not be in a RUN mode.

For Boat Users :

Disconnect the 1/8 inch PTFE line which runs from the boat furnace to the dehumidifier.

Shut off the gas.

Press CARRIER .

NOTE: The furnace and the NDIR should be left on unless the unit is going to be relocated or will not be used for a long time. Frequently turning the furnace on/off reduces the life of the heater element. The NDIR requires at least 2 hours for stabilization after power up.

6.2 SELECTING THE ANALYSIS PARAMETERS

Most analysis have three parameters:

1) Analysis mode.
2) Inlet mode.
3) Volume.

NOTE: The ASM and RSM operating modes have other parameters which must be selected. See Sections 6.4 and 6.5 for guidelines in selecting these parameters.

SELECT THE ANALYSIS MODE

Use Table 6.1 to match your application to an analysis mode. The default mode is **NPOC**. To set another mode, press the corresponding button.

Table 6.1
ANALYSIS MODE SELECTION

ANALYSIS MODE	APPLICATION	METHOD
NPOC	Any water sample.	IC purged from sample at sparging station. Inject into TC port. NPOC ---TC/Furnace---> CO_2
TOC	Any water sample. Method of choice when sample has no volatiles.	TOC = TC - IC Two (2) injections per analysis. DC-190 calculates the difference. See See TC and IC descriptions.
IC	Any sample where dissolved CO_2 or carbonate concentration is of interest.	Sample injected into IC port. IC ---IC/Chamber---> CO_2
TC	Any water sample.	Sample injected into TC port. TC ---TC/Furnace---> CO_2
POC	Water sampler where volatile organics or other purgeables are of interest.	Sample is sparged at POC sparge station. LiOH scrubber removes IC from sparged gas. POC Gas --> IC Scrubber --> POC---TC/Furnace---> CO_2
Boat Option, TC	Solids, sludges, slurries and waters with particulates greater than 0.5 mm.	Sample introduced onto platinum boat. Boat pushed into 183 furnace. Sample ---800°C---> CO_2

SELECT THE INLET MODE

The default inlet mode is **SYRINGE** . To select a different mode, refer to the following Table, then press the button corresponding to the new inlet mode.

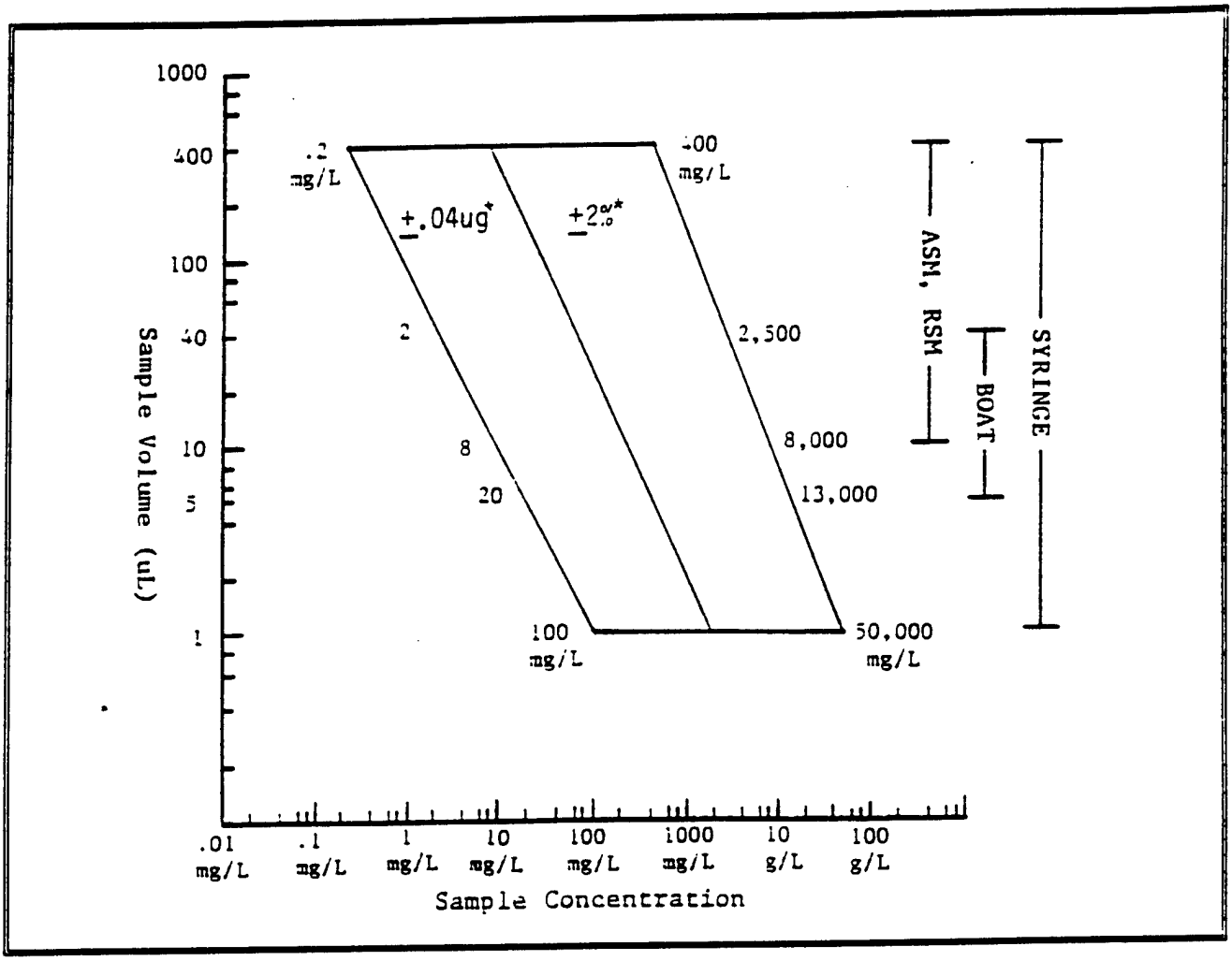
Table 6.2
INLET MODE SELECTION

ANALYSIS MODE	INLET MODE	DEFAULT		POSSIBLE VOLUME (ul)
		Volume (ul)	Range (mgC/L)	
NPOC TOC IC TC	Syringe	50	1 - 2000	1 - 400 20 - 200 *
	ASM	50	1 - 2000	10 - 400
TOC IC TC	RSM	50	1 - 2000	10 - 400
TC NPOC	Boat	40	2 - 4000	5 - 40
POC	N/A	10 mL	.01 - 20	2 - 10 mL

* This is the range for the manual micropipettor which is used with the **SYRINGE** mode.

SELECT VOLUME

The default volume and corresponding concentration range for each inlet mode are shown in the previous table. If the default concentration range is unsuitable, a better sample volume may be selected using Figure 6.1. Enter the new sample volume on the inlet mode menu.



* Expected precision. See Section 1.4.

FIGURE 6.1 Concentration Range vs. Sample Volume

EXAMPLE: Expected sample concentration range = 5 to 5,000 ppm.
From Figure 6.1, 20 ul gives 4 to 6,000 ppm.
(Note the logarithmic scales.)
20 ul is compatible with all inlet modes, except POC.

6.3 MANUAL OPERATION

Use these instructions for syringe or micropipettor operation in NPOC, TOC, IC, TC or POC modes. The following table shows the general operation sequence for syringe injections. Specific notes for each analysis mode follow the table.

GENERAL OPERATION SEQUENCE - ALL MODES

* If it is desired to save the current operating parameters before making any changes, select a new set-up number (see Section 6.7).
* Choose set-up.
* Have the syringe filled and ready. (Have the septum installed as shown in Figure 6.2.)
* Press **START** .
* Inject the sample. (Review the injection technique for the mode selected.)
* At the conclusion of the analysis, the screen will display the final ppm value along with:

  Continue Y/N ?

  (This question must be answered before the system will perform any other action.)
* Press **YES** to make more injections.
* Press **NO** to end the run.
* Press **STOP** to end the run after the current analysis. To terminate the run, immediately press **STOP** five times.

ABOUT SYRINGES

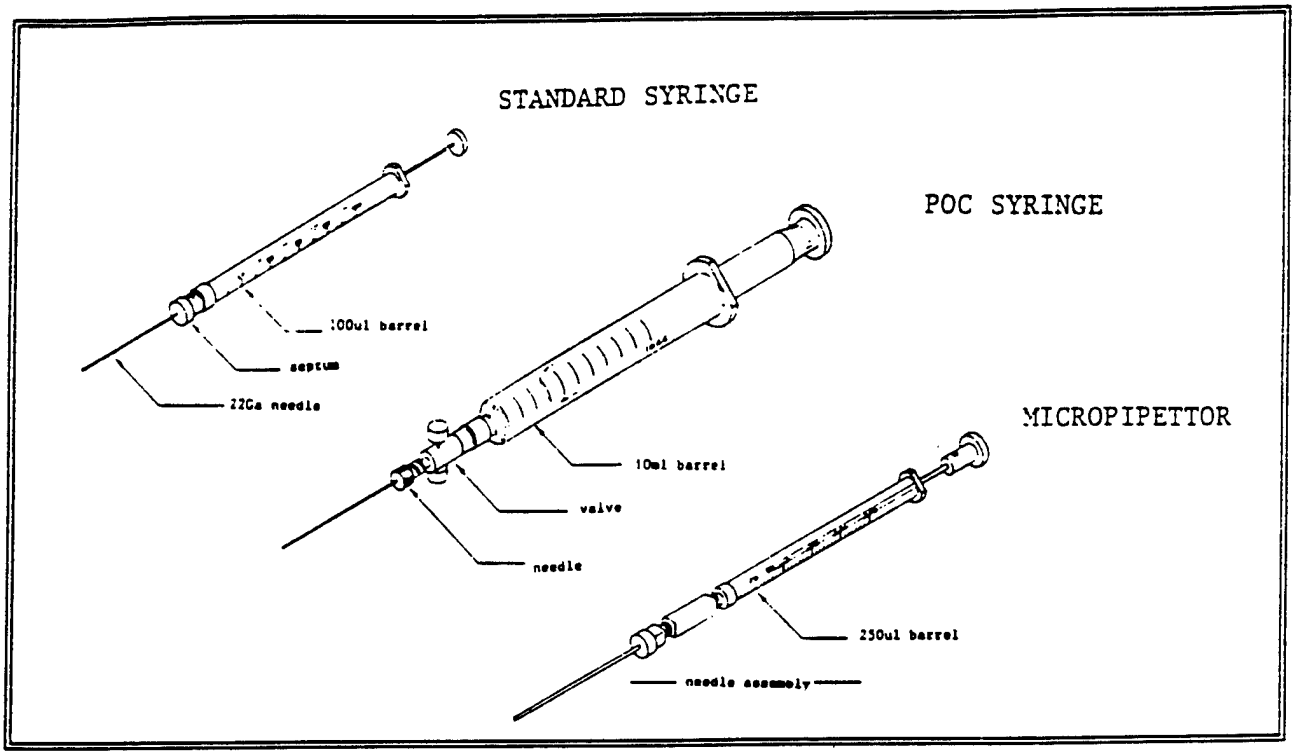


FIGURE 6.2. SYRINGE ASSEMBLIES

Assemble the syringes and micropipettor as shown in Figure 6.2. Always have a grey septum attached to the syringe or pipettor.

It is important for reliable sample introduction to use blunt-point needles such as those supplied with the DC-190. Side-port needles should not be used except on the POC syringe.

The 100 uL syringe (P/N 060-871) provided with the DC-190 has a 22S gauge (0.006 inch I.D.) needle. The 22 gauge (0.016 inch I.D.) replacement needles (P/N 060-872) are provided in the DC-190 operating kit for sample types requiring a larger I.D. needle.

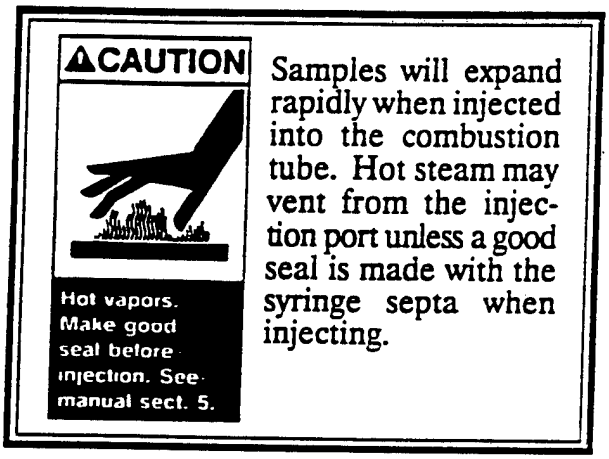
Also available are a micropipettor barrel (250 uL syringe barrel, P/N 060-875) and a micropipettor needle (P/N 888-297). The micropipettor is used for samples containing particulates up to 0.5 mm diameter or samples which are incompatible with (react with or corrode) a stainless steel needle. The micropipettor probe should be used with a 250 uL syringe barrel only.

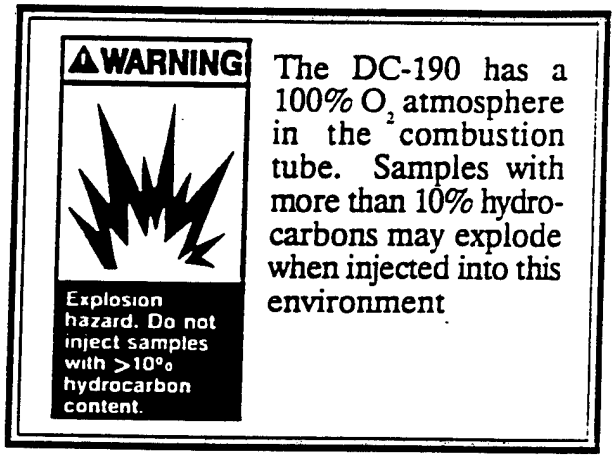
Injection Technique

As soon as the INJECT light comes on, press **OPEN/CLOSE**

Immediately insert the syringe into the injection port that has the illuminated LED.

Make seal during injection by pressing the grey septum against the port.





Inject at 50 ul/sec rate.

Withdraw the syringe and immediately press **OPEN/CLOSE** to close the port.

For 1 - 10 ul volumes, wait 5 seconds in between injecting and withdrawing syringe.

Micropipettor Users:

When using a micropipettor, wipe off the outside of the probe after drawing up the sample.

For volumes below 50 uL, the injection rate is crucial to obtaining reproducible results. Make the injection rapidly without jarring the syringe. (**HINT:** After withdrawing the syringe, look at the tip. If it is wet on the outside, inject faster; if it is partially empty, inject slower.)

Wait 10 seconds after injecting before withdrawing the pipettor for all volumes.

Sample Pretreatment

None, unless the samples are inhomogeneous or contain large particulates (> 0.5 mm diameter).

TOC

(This is a combination of the TC and IC modes.)

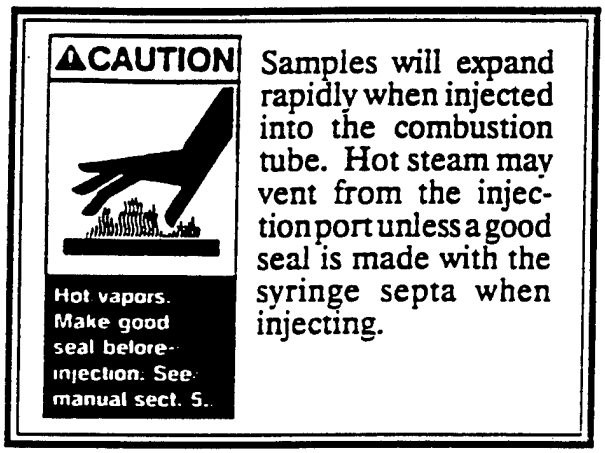
Injection Technique

Use the same technique as for the TC and IC modes.

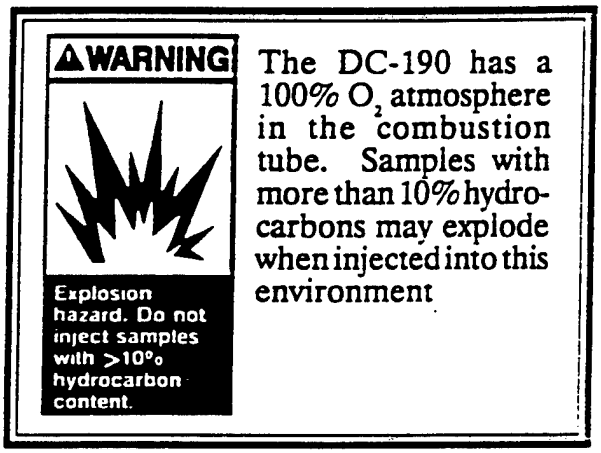
Make two injections per analysis.

The first injection goes in the TC port.

Have the syringe filled and ready for the second injection which is made to the IC port. Look for the prompt from the display.



Samples will expand rapidly when injected into the combustion tube. Hot steam may vent from the injection port unless a good seal is made with the syringe septa when injecting.



The DC-190 has a 100% O_2 atmosphere in the combustion tube. Samples with more than 10% hydrocarbons may explode when injected into this environment.

NOTE: When high pH samples are expected, treat combustion tube with 2 injections of 100 ul of pH1 HCl or HNO_3 solution.

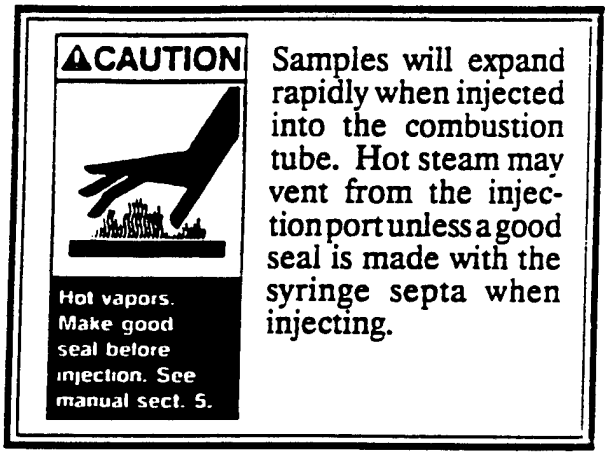
NPOC

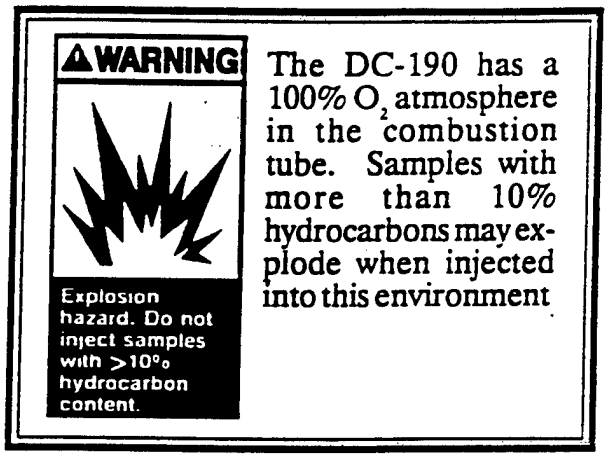
(This is the default analysis mode.)

Injection Technique

Use the same technique as for the TC and IC modes.

Inject into the TC port only.





Sample Pretreatment

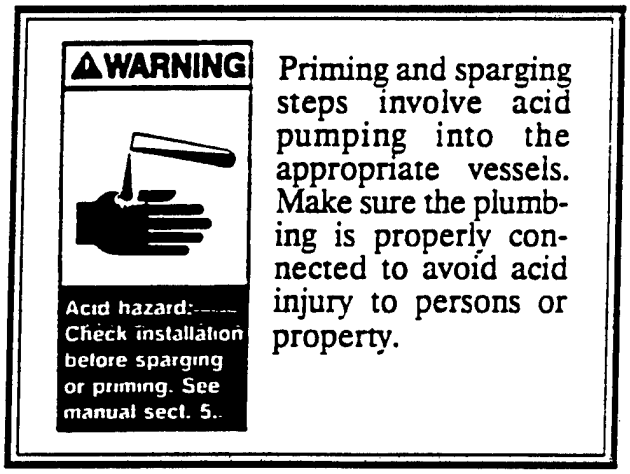
The sample must be sparged prior to injection to remove the IC.

To sparge the sample:

- Pour about 10 mL of sample into a 20 mL vial (P/N 889-726).
- Screw the vial into Sparger A or Sparger B.
- Press A or B, and then 1 to start sparging.
- The sample will be automatically acidified. Each unit of "Add acid" is equivalent to 100 ul.
- Sparging will stop automatically at the end of sparge time.
- Remove the vial and cap it until the analysis is run.

Two samples can be sparged simultaneously.

Samples containing large particulates (> 0.5 mm) must be pretreated as directed in Section 10.2.



Injection Technique

As soon as the INJECT light comes on, inject the sample into the POC sparger through the injection port.

When the analysis is over, withdraw the remaining sample from the sparger with the syringe.

Sample Pretreatment

None.

How to Fill the Syringe

Remove the plunger from the syringe and close the syringe valve and needle. Open the sample or standard container, which has been allowed to come to ambient temperature, and carefully pour the sample into the syringe barrel to just short of overflowing. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 10 ml.

This process of taking an aliquot destroys the validity of the liquid sample for future analysis. If there is only one sample container, the analyst should fill a second syringe at this time in case the first analysis is unsuccessful.

6.4 AUTOSAMPLER OPERATION

INTRODUCTION

The DC-190 Autosampler (ASM) option is designed for unattended operation for many hours. The sample tray holds 32 8 mL vials. Automatic acid addition and sparging are provided by the sparge tower to remove inorganic carbon for NPOC analysis. The sample probe may be rinsed with either water and/or sample between analyses. The ASM can handle samples with particulates up to 0.5 mm and the sample may be stirred with gas before the sample is drawn to insure uniform sampling. Cross-contamination is minimized by the use of non-wetting materials for all sample contacting parts. Sample vials may be marked as blanks or standards for automatic calibration of the system during the ASM run.

The ASM offers an autoranging capability which will adjust the sample volume to maintain the peak integral within the range of the detector. Since the dynamic range of the DC-190 system is very wide (10,000 to 1), activation of the autoranging will normally be a very rare event. When this feature is active, the DC-190 will check the first replicate of a vial in the ASM mode to verify that the peak integral is within range. If the peak integral is below range, the result will be printed, but ignored in future statistical calculations. The injection will then be repeated, but with a volume 5 times larger than the original injection. If the peak integral is over range, a similar procedure is followed with a volume one fifth the original volume. The volume adjustment will be repeated until the peak integral is within range. If an adjustment would result in a volume outside the 10 to 400 uL range, the volume will be set to either 10 or 400 uL as appropriate and no further adjustment will be made. The original injection volume will be restored at the beginning of the next sample vial. The accuracy of the autoranged data may suffer somewhat because the ASM was not calibrated with the new volume. The inaccuracy without autoranging is potentially much worse, however, than with autoranging. If desired, the results of autoranged data may be rechecked later.

Below is a table of expected and observed volumes for the ASM. These values are approximate and will vary from instrument to instrument. This volume variation only affects autoranged data. This will not apply to normal calibrated ASM data because the same volume is used for analysis.

VOLUME (uL)	
Expected	Observed
10	10.3
20	19.5
40	35.4
80	70
100	92
200	194
400	400

OPERATION

* Refer to DAILY START-UP in Section 6.1 to prepare the analyzer for operation.

* If it is desired to save the current operating parameters before making any changes, select a new Set-up number (see Section 6.8).

* Refer to Section 6.2 and select the analysis mode and volume desired. See Table 6.3 for guidelines to set the other operating parameters.

* Place the vials in the sample tray beginning with tray position 1. Refer to Table 6.4 and mark the vials as blanks, standards, or samples as appropriate. Mark the first empty tray position after the samples as indicated in Table 6.4 to terminate the run.

* Clean and fill the rinse bottle with DI water if water rinses were called for on the Rinse\stir menu.

* Check that the acid bottle is at least 1/3 full of acid solution if set up for NPOC analysis.

* Check that the printer is ready and has sufficient paper.

* Press **START**.

* There are two ways to end the run before completion. Press **STOP** to end the run after the current analysis. To terminate the run immediately, press **STOP** five times. After an immediate bail out, the ASM may have to be returned to its resting position. The sparge arm may be raised by selecting "Raise sparge arm" (**1**) on the "Sparge arm menu" (**MAIN 2 5 3 3**). The sample arm may be returned to the rinse bottle position by selecting "Move arm to rinse" (**4**) on the "Sample arm" menu (**MAIN 2 5 3 2**). Always check the "Furnace/IC ports" menu (**MAIN 2 5 5**) to be sure the inlet ports are shut (even if the indicator lights next to the ports are not lit).

TABLE 6.3
ASM OPERATION PARAMETER GUIDELINES

# of repeats	Select a number that is statistically comfortable. The allowed range is 1 - 5 repeats, with 3 being the default.
Sparge time (min)	The default time (3 minutes) should be satisfactory for almost all samples as long as the pH is in the proper range (see "Acid volume" below). This option is applicable to the NPOC mode only.
Acid volume	The pH must be adjusted to a value less than 4. It may be necessary to check a few samples after acid addition and make adjustments by trial and error until the acid addition matches the particular samples being analyzed. The default is 1 (each unit of acid volume is equivalent to 100 ul). This option is applicable to the NPOC mode only.

The following selections are on the "Rinse and /or stir" menu:

# of rinses w/water	This option specifies the number of times the ASM sample probe and loop will be rinsed with water between each vial.
# of rinses w/sample	Similar to the above option except that the ASM will rinse with sample before the first injection from each vial.
Sample stir time (sec)	Specifies the time that the sample will be stirred before the sample is drawn into the sample loop. The allowed range is 0 - 30 seconds (default = 0). In most applications, 15 seconds will provide effective stirring. Stirring is accomplished by bubbling gas out of the sample probe to suspend particulates and obtain a more uniform sample.
Auto-range	When set to "Yes", the DC-190 will automatically adjust the injection volume. "No" is the default setting. See the INTRODUCTION to this section for details on this feature.
CG off after	The default "No" means the carrier gas (CG) will not be turned off at the end of an ASM run. A "Yes" will cause the carrier gas to be turned off 10 minutes after the end of an ASM run. During this period, the red light in the **START/STOP** button will blink as if the run is still in progress.

TABLE 6.4
ASM VIAL MARKERS

VIAL	PEG POSITION		INDICATION
	INNER*	OUTER**	
No	No	No	Skip Position
Yes	No	No	Sample
Yes	Yes	No	Blank
Yes	No	Yes	Standard
Yes	Yes	Yes	Rinse Sample***
No	No	Yes	Terminate Run

* Peg hole closer to center of sample tray.

** Peg hole closer to sample vial.

*** Sample is used for rinse only (no analysis).

NOTE: If the printer runs out of paper or jams during a run "Print last run" (**MAIN 2 3**) will reprint the run data from a buffer. This allows data otherwise lost to be retreived. The buffer which retains the data is not large enough, however, to hold a complete run of data in all cases. This buffer has sufficient capacity to hold data from approximately 32 vials with 3 replicates per vial in modes where each replicate requires one line to print (TC, IC, or NPOC). In the TOC mode, each replicate requires three lines to print. In this mode, the buffer will only hold approximately 10 vials with 3 replicates per vial. The buffer is filled on a first in first out basis so that the data remaining at the end of the run will be the last data point back until the buffer is full.

6.5 OPERATION OF THE RSM OPTION

The RSM option allows the continuous sampling of a sample stream which is tapped to flow through the RSM sample cell. The ASM will perform the designated number of replicates on the sample stream and then wait for a designated time period. The sampling cycle is then repeated. The TC, IC, and TOC analysis modes may be performed using the RSM option. However, if the sample stream IC and TC levels are not constant, the accuracy of the TOC analysis may suffer due to the time lag between the IC and TC portions of the analysis.

* Adjust the sample flow rate to the sample cell by slowly opening the needle valve (counter clockwise) until the water level stabilizes slightly above the drain port of the sample cell.

* If it is desired to save the current operating parameters before making any changes, select a new Set-up number (see Section 6.8).

* Select **TC**, **IC**, or **TOC** (see Section 6.2 for selection guidelines) and then **RSM** to set the analysis mode. Verify that the operating parameters are set to the desired values. Use the guidelines in Table 6.5.

* Calibrate the DC-190 according to the RSM calibration procedure in Section 6.8.

* Press **START** to begin the analysis. The RSM will continue until manually stopped.

* To stop the analysis, press **STOP** (same button as **START**). This will stop the DC-190 at the end of an analysis in progress or immediately during the time between runs. To stop the run immediately during an analysis, press the **STOP** button 5 times.

TABLE 6.5
RSM OPERATING PARAMETER GUIDELINES

Sample volume	See Figure 6.1
# of repeats	Select a number that is statistically comfortable. The allowed range is 1 - 5 repeats with 3 being the default.
Time between runs	This is the time from the conclusion of the last replicate of a group to the beginning of the first replicate of the next group. The allowable range is 0 to 54 minutes with a default of 0 minutes.

6.6 OPERATION OF THE BOAT OPTION

Use the boat sampler for slurries, sludges, solids, and suspensions. Operate in either the **TC** or **NPOC** mode. Refer to "Installation and Operation of the 183 Boat Sampling Module" (P/N 915-240) for sample introduction instructions (Section V, Parts 5A and 5B). The DC-190 calculates ppmC from liquids or solids.

SAMPLE TYPE	SAMPLE INTRODUCTION	CONCENTRATION UNITS
Liquids, light slurries, suspensions	See 183 Instructions for **Liquids**	mg/L
Solids, heavy slurries	See 183 Instructions for **Solids**	ug/g

* If it is desired to save the current operating parameters before making any changes, select a new Set-up number (see Section 6.8).

* Press **BOAT TC** or **NPOC** .

* Press **1** until the appropriate units are displayed.

* Introduce the sample into the boat - see "Installation and Operation of the 183 Boat Sampling Module".

* Press **START** and follow the 183 instructions.

* If ug/g units are selected, enter the sample weight when asked - "Sample weight (mg)?".

* **SOLIDS ONLY:** Enter the sample weight when asked - "Sample weight (mg)?".

6.7 CALIBRATION

The DC-190 offers a choice of either one point or two point calibration. Two point calibration is equivalent to subtracting the blank value automatically. The DC-190 system always calculates a two point linear calibration. If only a single point calibration is desired, the System Blank may be set to 0 before updating the Calibration Factor. In this case the System Blank will remain 0 after updating the Calibration Factor resulting in a single point calibration. Since the system blank for IC is normally insignificant, its value is set to zero and IC analysis always has one point calibration. When two-point calibration is used, both calibration factor and system blank are recalculated each time either the calibration factor or system blank is updated. In TOC mode, the system uses TC value for calibration and blank update.

The DC-190 system provides a common calibration set (calibration factor and system blank) for SYRINGE, ASM, and RSM modes. POC and BOAT modes have their own calibration sets. When changing inlet mode from SYRINGE to ASM or RSM, calibration stays the same. When changing inlet mode from SYRINGE, ASM, or RSM to POC or Boat. calibration changes accordingly. The multiple set-up function (see Section 6.8) provides capability to store and retrieve up to 5 calibration sets.

Since SYRINGE and ASM/RSM calibrations are not necessarily the same, calibration for these modes should be done separately. Use the multiple set-up function to store the different calibration sets.

SUMMARY
1. System Blank 2. Calibrating Syringe, POC, or Boat Modes 3. Calibrating The ASM Mode 4. Calibrating The RSM Option 5. Omitting Outlier Data 6. Calibration Equations

SYSTEM BLANK

System blank is defined as the response contributed by the analyzer when carbon-free water sample is injected and analyzed. In reality, it is very difficult to produce and preserve the carbon-free water. Thus the true system blank and the carbon content of the water sample cannot be accurately distinguished. However, the carbon content of high purity water can be below the defection limit (.2ppmC) and the response with such water may be assumed as the system blank. When it exists, the blank value is subtracted from every analysis except in IC mode where blank is always assumed to be zero.

The system blank becomes increasingly important for analyses below 10 mgC/L as shown :

MODE	VOLUME	TYPICAL BLANK (mgC/L)
TC NPOC	400 ul	.10 - .40
IC	400ul	0*
POC	10ml	0 - .03
BOAT	40ul	2.0 - 4.0

Factors affecting the blank :

- Cleanliness of syringes, spargers and IC chamber.
- Sample handling.
- Age and sample history of TC and boat combustion tubes.
- Dehumidifier temperature.

CALIBRATING THE SYRINGE, POC, or BOAT INLET MODES

See "SYSTEM BLANK" earlier in this section for guidelines to determine whether a two point calibration is needed for the samples to be analyzed.

* Analyze a standard in the analysis mode to be used. An average of at least two determinations is recommended. Respond **NO** to the prompt "Continue yes/no?" when satisfied with the results.

* Outlier data can be omitted at this point if desired. See the section "OMITTING OUTLIER DATA" at the end of this section for details on how to do this.

* Press **CALIBRATE** to review the calibration menu :

1. Calibration factor	1
2. System blank	0
3. Sample size	50
4. Std. concentration	1000
5. Update cal-factor	
6. Update system blank	
7. Other actions	

* Verify that the sample size and standard concentration shown on the "Calibration" menu are correct. If a one point calibration (no subtraction of the blank) is desired, make sure the System Blank is set to 0. Make any necessary changes.

* Press **5** to update the Calibration Factor. The new calibration factor will be calculated and displayed on the menu.

* To complete a two point calibration, if desired, repeat the above procedure with a blank sample. Use the cleanest reagent water available (less than 0.150 mgC/L). Press **6** to update the System Blank.

* The DC-190 is now calibrated for the selected analysis mode.

Analyze a check standard with each sample set. If the reported value deviates from the expected value by more than 2%, re-calibrate the system.

Note To Boat Users: It is easy to use a liquid standard to calibrate the DC-190 even when using "ug/g" units to analyze solid samples. For example, to obtain 10 mg of sample, simply inject 10 ul of standard. This relationship holds as long as the density of the standard is 1 g/mL, which will be true for most water-based standards.

CALIBRATING THE ASM INLET MODE

* Select the ASM operating paramaters as described in Section 6.4 and press **START** to begin analyzing the standard.

* Place the vials of standard in the first tray positions. It is recommended that two vials of standard be placed next to each other at the beginning of the ASM sample tray. Place a peg in the outer hole next to the second vial to mark it as a standard for calibration (see Table 6.4).

* If blanks are to be determined, place two or three vials of blank immediately following the vials of standard. In most circumstances, two vials are sufficient. For best accuracy at low levels, three vials are recommended. Place a peg in the inner hole next to the last of the two or three blank vials to instruct the DC-190 to determine a new blank value (see Table 6.4).

* Press **CALIBRATE** to review the calibration menu:

1. Calibration factor	1
2. System blank	0
3. Sample size	50
4. Std. concentration	1000
5. Update cal-factor	
6. Update system blank	
7. Other actions	

* Verify that the sample size and standard concentration shown on the "Calibration" menu are correct. If a one point calibration (no subtraction of the blank) is desired. make sure the System Blank is set to 0. Make any necessary changes.

* Place the sample vials in the sample tray following the standard and blank vials, and run the analysis according to the operation instructions in Section 6.4. The DC-190 will automatically calculate and use the calibration factor and blank value.

CALIBRATING THE RSM OPTION

The RSM mode is easiest to calibrate using a vial of the desired standard rather than by pumping the standard through the RSM sample cell. This method is described in the following steps:

* Lift the sample cell from its holder and secure it in the clip located to the left of the black cell holder.

* Place an ASM vial (P/N 080-140) containing the standard solution into the black cell holder.

* Select the RSM operating paramaters as described in Section 6.5 and press **START** to begin analyzing the standard.

* Since the RSM does not stop automatically, it is necessary to manually stop it by pressing **STOP** (the same button as **START**) during the last desired replicate of the standard. The DC-190 will then stop at the end of the current analysis.

* Outlier data can be omitted at this point if desired. See the Section "OMITTING OUTLIER DATA" at the end of this Section for details on how to do this.

* Press **CALIBRATE** to review the calibration menu:

1. Calibration factor	1
2. System blank	0
3. Sample size	50
4. Std. concentration	1000
5. Update cal-factor	
6. Update system blank	
7. Other actions	

* Verify that the sample size and standard concentration shown on the "Calibration" menu are correct. If subtraction of the blank is not desired, make sure the System Blank is set to 0. Make any necessary changes.

* Press **5** "Update cal factor" to calculate and store a new calibration factor.

* Repeat the above procedure with a blank sample and press **6** "Update system blank" on the "Calibration" menu if an update of the system blank is desired.

OMITTING OUTLIER DATA

The DC-190 provides the ability to reject outlier data when operated in the manual modes (Syringe, Boat, and POC) and the RSM mode (no provision for outlier rejection is made in the ASM mode). A new average and standard deviation are calculated after the data is rejected. This feature saves having to re-run a data set due to a bad data point when updating the Calibration Factor or System Blank. The DC-190 will not allow the number of replicates to be reduced to less than 2 as a result of data rejection. Data rejection is accomplished by the following steps:

* Complete the run by responding **NO** in one of the manual modes or **STOP** in the RSM mode (see the calibration instructions for the mode in use) to the prompt at the end of the analysis. Three or more replicates must have been generated.

* Select the "Auxiliary functions" menu (**MAIN 2**) and press **1** "Omit an outlier".

* At the prompt, enter the number of replicates to reject. Each replicate to be rejected will be prompted for separately. Enter a replicate number after each prompt.

* New statistics will be displayed on the screen and printer. An update of the Calibration Factor or System Blank will now be based on the new average value.

* If the "Omit an outlier" menu item is selected again after the current data set has been edited, the DC-190 will start the data rejection over and ignore the previous data editing.

CALIBRATION EQUATIONS

The following equations are used in the DC-190 system.

The equation for determining a calibrated result is:

$$y = (Fx - b) / V$$

where:
- y = Concentration (calibrated) of sample.
- x = NDIR peak with background subtracted. Normally invisible to the user. The displayed value, y, may be made to equal x by setting F, b, and V to the appropriate values (1, 0, and 1, respectively)
- F = "Calibration Factor". This is the slope of the linear fit line.
- b = Intercept. This is an internal parameter which is invisible to the user.
- SB = "System Blank" = b/V.
- V = Sample volume (or mass).

The quantities F and SB are the ones displayed on the calibration menu and are the ones which can be edited directly.

The Calibration Factor and Blank are calculated by:

$$F_n = F_o (C_s / y_s)$$

$$b_n = b_o (F_n / F_o)$$

where:
- C_s = Concentration of the standard.
- o = Old value.
- n = New value.
- s = Value for Standard.

These are the equations used internally by the DC-190 system. Both Fn and bn are re-calculated each time either the Calibration Factor or the System Blank is updated. It should be noted that if the old value bo is already 0, the new value bn and therefore SB will also be 0. This provides a means to have the system effectively do a one point calibration update when it calculates a new Calibration Factor. These equations may also be used to manually calculate the values and enter them on the "Calibration" menu directly.

6.8 USING THE MULTIPLE PARAMETER SETS

The DC-190 provides the capability to store 5 complete sets of operating parameters. This capability allows the user to return to a previously defined set of operating parameters without having to re-enter the parameters. The parameter set includes the inlet mode, the analysis mode, the parameters appropriate to the analysis/inlet mode as well as the Calibration Factor and System Blank..

One of the parameter sets is always the "working" set-up. This is the parameter set associated with the current set-up number. Any run started will now use the parameter values currently contained in the working parameter set. As changes are made to the operating parameters, these changes are made to the working set-up.

When a new set-up number is selected, the parameter values in the previous set-up are saved as they were at the time of the new selection. The working parameter set now takes the values associated with the new set-up number. Any run started will use the new parameter values and any parameter changes are now made to the new parameter set.

Returning to the previous set-up number will restore the operating parameters to the state they were in when the set-up number was last used.

If it is desired to save the current set of parameter values for future re-use, a new set-up number should be selected before starting to define a new parameter set.

To determine the set-up number:	Display the "System status" menu (**MAIN** 1) . Line 5 "Analysis set-up" indicates the current Set-up number.
To change to another set-up number:	Select the "System status" menu (**MAIN** 1) and then "Analysis set-up" (**5**) and enter the new Set-up number. This saves the current parameter set.

To print the current parameter set:

Press the analysis mode button with the lit LED and then select the "Print set-up" option on the displayed operating parameter menu.

To print all the parameter sets:

Display the "System status" menu (**MAIN** 1). Press 6 "Print set-up selections".

USING THE CLIPBOARD

A clipboard is provided in the DC-190 system which allows the Calibration Factor and System Blank to be copied from one parameter set to another. This feature can save time and effort when changing from parameter set to another after the system has been calibrated. Use the following steps:

* Select the "Other actions" section of the "Calibration" menu (**CALIBRATE 7**).

* Verify that the "Analysis set-up" shown on line 4 is the one from which to copy the calibration factors. If not..select **4** "Analysis set-up" and enter the desired set-up number.

* Select **2** to save the calibration factors.

* Enter the number of the new parameter set on line 4 and select **3** to copy the calibration factors.

The new parameter set now contains the same Calibration Factor and System Blank as the one copied.

DC-190 Operation Guide

DAILY START-UP

1. Gas @ 30 Psig.

2. Check that the acid bottle is 1/3 full.

3. Confirm that the IC chamber is 1/2 full **(gas off)**

4. Fill IC chamber by using the prime acid function.

5 Press **CARRIER** Check that gas is flowing in IC chamber.

6. Ensure there is water in the dehumidifier.

7. Observe green lights on carrier & furnace.

8. Check for: flow rate 180-220cc/min, dehumidifier temp. 0-10°C, and furnace temp. 680°C. (Most applications)

9. Confirm or change Set-up number on display. **(Section 6.8)**

10. Check analysis and inlet mode.

11. Print Set-up.

12. If using the Boat, connect Teflon tubing to inlet part of dehumidifier. **(Fig. 4.15)**

13. If using ASM, clean the rinse bottle and fill it with acidified DI water. **(Few Drops of H_3PO_4)**

14. Observe for stable baseline **(Peak to Peak <.2mV)** before starting analysis.

DAILY SHUT-DOWN

1. Check that system is <u>not</u> in the **RUN** mode.

2. Push **CARRIER** to turn off gas.

3. Leave furnace at operating temperature. **(Normally 680°C)**

4. Disconnect the Teflon tubing from the dehumidifier to boat at the boat inlet.

5. For total shut down turn **OFF** main power switch in the rear.

OPER. & CAL

1. Select analysis mode **(Table 6.1)**

2. Select inlet mode **(Table 6.2)**

3. Confirm or change volume. **(Fig. 6.1)**

4. For **CALIBRATION**, press **CAL** to confirm or change concentration. **(Section 6.7)**

5. For manual injection, see **Section 6.3** for injection technique.

6. For ASM, confirm or change other parameters. **(Table 6.3)**

7. Refer to **Table 6.3** for ASM vial markers.

8. For RSM, see **Section 6.5** .

9. To complete **CALIBRATION**, see **Section 6.7**.

MAINTENANCE

Daily checks:
1. Printer paper
2. Gas supplies
3. IC chamber 1/2 full & acidified
4. Water in dehumidifier tube
5. Acid bottle 1/3 full
6. Gas flow 180-220cc/min
7. Temp. at set point
8. Dehumidifier temp 0-10°C

Weekly checks:
1. Daily checks plus
2. Replace septum in POC sparger every 40 injections.
3. Inspect TC inlet valve
4. Inspect combustion tube. Wipe inside area near top with wet Q-tips if necessary.
5. Inspect IC inlet valve
6. Clean IC reactor
7. Drain dehumidifier water & replace with acidified water. Flush several times if necessary.

Monthly checks:
1. Daily & weekly and/or:
2. Inspect & replace LiOH if necessary.
3. After ~160 hrs of operation, rinse catalyst, and combustion tube, replace silver wool **(Section 7.1)**. Condition catalyst at 900°C for 1/2 hr with DI injections.
4. Inspect O-rings in TC inlet and bottom connector. Replace if necessary.

DO'S & DONT'S

1. **DO** Check the bottom connector when checking the combustion tube.

2. **DO** Use a Soap Film Bubble meter to check output gas flow rates.

3. **DO** leave furnace at 680°C except for long term shut down.

4. **DO** Condition new catalyst. 100ul of water every 5 min. for 2 hours at 900°C.

5. **DON'T** use Pyrex wool in the combustion tube.

6. **DO** clean combustion tube weekly if used heavily. DI injections @ 900°C for 1/2 hrs. Use good water–should stabilize at 1 to 3ppm or better.

7. **DO** check valve seal & O-rings monthly when inspecting TC & IC ports.

8. **DO** re-align TC & IC ports with ASM probe after inspections.

9. **DO** study flow diagram **Figs 8-1 & 8.2.**

10. **DO** acidify ASM rinse bottle

11. **DON'T** use ASM stirring time > 30 sec.

12. **DO** inject acidified water daily into TC port if non-acidified samples are analyzed. (3, 100ul inj. of pH1, HCl or HNO_3)

13. **DO** rinse **(section 7.1)** and condition catalyst **(section 5.3)** when catalyst is contaminated.

14. **DON'T** raise drain line higher than 1 1/2" above lab bench.

SECTION 10
STANDARDS PREPARATION AND SAMPLE HANDLING

10.1 STANDARDS PREPARATION

REAGENT WATER

Use:

Standards preparation, system blanks, sample dilution, cleaning, etc.

Requirements:

Deionized or distilled.

ASTM Type II reagent water or equivalent.

TOC level: Less than 0.2 mgC/L.

ACID SOLUTION

Use:

Automatic acid feed for IC chamber, sparge stations, autosampler.

Requirements:

Reagent water.

Phosphoric (H_3PO_4), sulfuric (H_2SO_4), or nitric (HNO_3) acid, concentrated, reagent grade.

Do not use hydrochloric acid (HCl).

Preparation:

Final volume: 100 ml.

20% Phosphoric Acid Solution:

Add 20 ml acid to 80 ml reagent water. Transfer to the acid bottle (4 oz borosilicate with open top screw cap).

If phosphoric acid is not available, 10% sulfuric acid or 5% nitric acid can be substituted.

Replace monthly.

TC and IC STOCK SOLUTIONS

Use:

Dilute to appropriate concentration for calibration or system check-out.

Requirements:

Reagent water.

Reagent-grade concentrated acid (H_3PO_4 or H_2SO_4) for TC stock only.

Standard compounds are reagent-grade, and must be dried to a constant weight. (See the table in the next page.)

Preparation:

Final volume: 100 mL.

Standard compound choice:
For system performance check and troubleshooting purposes, use a compound listed below. For routine analyses, use one of these, or any compound which might be more appropriate for your application.

Weigh the specified amount of the compound into a 100 ml volumetric flask. Add about 75 ml reagent water to dissolve the compound. Add about 0.1 ml acid to TC solutions to adjust pH below 3. Then fill to the mark.

Store stock solutions in amber borosilicate bottles with Teflon-lined closures at $4^{o}C$.

Replace monthly.

TC STOCK SOLUTIONS (Choose one):

Compound	Weight (g/100mL)	Concentration	Add Acid?
KHP ($C_8H_5KO_4$)	2.126	10,000 mgC/L	Yes
Sucrose ($C_{12}H_{22}O_{11}$)	2.375	10,000 mgC/L	Yes

IC STOCK SOLUTIONS (Choose one):

Compound	Weight (g/100mL)	Concentration	Add Acid?
Na_2CO_3 (Anhydrous)	0.883	1,000 mgC/L	No
$NaHCO_3$	0.699	1,000 mgC/L	No

Use this formula to determine the weight required to make 100 ml stock solutions using other compounds:

$$\text{g Compound} = \frac{\text{mw x \%C}}{\text{N x 12.01}}$$

where:

mw = molecular weight of compound
%C = concentration of standard in % carbon
N = number of carbon atoms per molecule
12.01 = atomic weight of carbon

For example

For a 1% (10,000 mgC/L) solution of sucrose (mw = 342.29):

$$\frac{342.29 \text{ x } 1\%}{12 \text{ x } 12.01} = 2.375 \text{ g.}$$

TC and IC
WORKING STANDARDS

Use:

Calibration or system check-out.
Choose the standard concentration to match the working range of your samples.

Requirements:

Reagent water.
Clean volumetric flasks and volumetric pipets.

Preparation:

Final volume: Depends on concentrations.

Use larger volumes as concentration decreases. Make 1 liter volume at 10 mgC/L. Do not make final volume smaller than 100 ml.

TC solutions only: Maintain at pH 3 or lower.

Store standard solutions in amber borosilicate bottles with Teflon-lined closures at $4^{o}C$. **Minimize exposure to atmosphere.**

Bottle volume: Between 100 - 200 mL, depending upon the concentration.

Replace weekly.

System Performance Check: (Initial Start-Up)

Make 100 ml of 1000 mgC/L TC standard and 100 ml of 100 mgC/L IC standard.

POC STANDARD

Use:

Calibrate POC sparger.

Requirements:

Very clean 1 liter volumetric flask.
Reagent water.
Stir plate and Teflon coated stirbar.
Reagent grade compound.

Preparation:

Final volume: 1000 ml.

Compound Choice:

Benzene or chloroform is strongly recommended. Other compounds can be used if reliable results can be demonstrated. Use only benzene or chloroform for system performance check and troubleshooting.

WARNING!

BENZENE	DANGER! Extremely flammable. Suspected human carcinogen. Harmful if swallowed, inhaled or absorded through the skin. May affect the blood system.
CHLOROFORM	Warning! Suspected human carcinogen. Harmful if inhaled or swallowed. Skin and eye irritant and may produce toxic vapors if burned.

Please consult material safety data sheets for more precautions regarding these compounds.

Fill the 1 liter flask to the mark with reagent water. Add the stir bar and gently agitate water on stirplate for 1 - 2 minutes to degas. Inject a microliter quantity of the compound. Use the table or formula in the following page to determine the proper quantity to inject. The syringe needle should be well immersed in the water. Cap the flask and gently agitate the solution until it comes to equilibrium (approximately 5 minutes).

COMPOUND	VOLUME TO INJECT	CONCENTRATION
Benzene (C_6H_6)	12 ul	9.92 mgC/L
Chloroforom ($CHCl_3$)	67 ul	9.72 mgC/L

To make other concentrations or standards, use this formula:

$$\textbf{Concentration of POC Standard } C = \frac{V \times D \times F}{L}$$

where:

C = Concentration of standard (mgC/L)
V = Microliters of POC solvent injected
D = Density of POC solvent (mg/ul)
F = Fraction of carbon per molecule by weight
L = Volume in liters of water

10.2 SAMPLE HANDLING

Good laboratory practice is important in obtaining reliable analysis for carbon content of samples. Since carbon is everywhere in nature, it is very easy to contaminate a sample. Follow these guidelines for sample handling during collection, pretreatment, and analysis.

Syringe Handling:

Dedicate a syringe to a particular carbon range. When the syringe gets contaminated (indicated by sample or standard not completely wetting the inner barrel), draw chromic acid into the syringe a few times, then rinse well with reagent water.

Sample Bottles:

It is preferable to store and collect samples in glass containers. Plastic bottles should only be used if it is established that the specific type of container to be used does not contribute contaminating organics.

The sample collection bottles should be cleaned well before collecting the sample. The amount of cleaning necessary is dependent on the expected concentration of carbon in the sample. As a rule of thumb, the following levels are suggested:

*** Greater than 100 mgC/L**

- Wash bottle in hot, soapy water.
- Rinse with clean water.
- Plastic cap may be used, but try to use Teflon-lined cap.
- Analyze samples within 2 weeks.
- Treat standard bottles and sparge vials the same way.

*** Less than 100 mgC/L**

- Use amber bottle.
- Wash in hot, soapy water.
- Rinse with clean water.
- Swirl with chromic/sulfuric acid cleaning solution.
- Rinse with reagent water.
- Use Teflon-lined cap.
- Store sample at $4^{o}C$.
- Analyze within two weeks.
- Treat standard bottles and sparge vials the same way.

Sample Pretreatment:

If a sample contains particulates larger than 0.5 mm or insoluble matter, homogenize with a blender or tissuemizer until the average particle size is less than 0.5 mm. Analyze these samples with the micropipettor or autosampler.

If the average particle size cannot be reduced to below 0.5 mm by homogenizing, dilute the sample with reagent water and blend again. or analyze the sample using the boat sampler.

*** Below 100 mgC/L:**

Minimize the sample handling and the blend time in order to minimize contamination and loss of volatiles. Analyze a blank with the same pretreatment as a sample.

Appendix B-16 – Explosives: AP-0062

AP-0062

Extraction, Preparation, and Analysis of Explosives and Their Degradation Products by HPLC

1.0 PURPOSE

This procedure is a method of determination for the identification and quantitation of nitroaromatics and nitroamines using High Performance Liquid Chromatography (HPLC).

2.0 SCOPE

This procedure applies to water, compost, compost leachate, soil, sediment, gravel, and plant samples. The following analytes (listed with their abbreviations as used in this document) can be identified and quantified with this procedure.

2,6-Diamino-4-nitrotoluene----------2,6-DANT
1,3,5-Trinitroso-1,3,5-triazacyclohexane----------Tri-RDX
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine-----HMX
2,4-Diamino-6-nitrotoluene----------2,4-DANT
1-Nitroso-3,5-dinitro-1,3,5-triazacyclohexane--------Mono-RDX
Hexahydro-1,3,5-trinitro-1,3,5-triazine----------RDX
1,3,5-Trinitrobenzene----------TNB
1,3-Dinitrobenzene----------1,3-DNB
3,5-Dinitroaniline----------3,5-DNA
2,4,6-Trinitrotoluene----------TNT
2-Amino-4,6-dinitrotoluene----------2-ADNT
4-Amino-2,6-dinitrotoluene----------4-ADNT
2,6-Dinitrotoluene----------2,6-DNT
2,4-Dinitrotoluene----------2,4-DNT
4,4',6,6'-Tetranitro-2,2'-azoxytoluene----------TN-2,2'-AZT
2,4',6,6'-Tetranitro-2',4-azoxytoluene----------TN-2',4-AZT
2,2',6,6',Tetranitro-4,4'-azoxytoluene----------TN-4,4'-AZT
2,2'-Dinitro-4,4'-azoxytoluene----------DN-4,4'-AZT

3.0 SUMMARY

Samples can be prepared for analysis with no prior extraction or concentration, or can be extracted and concentrated before analysis preparation. Sample extraction and concentration methods may also serve to remove substances which would interfere with analyte identification or quantitation. The resulting prepared sample is injection ready for HPLC analysis. Nitroaromatics and nitroamines in the prepared sample are chromatographically separated as they pass through a HPLC analytical

column. The nitroaromatic and nitroamine compounds are identified by comparing their retention times and UV spectra, generated on a photodiode array detector using commercial chromatography workstation software, with those of known standard compounds generated under similar conditions. The compounds are quantified by comparing their peak heights, generated on a single wavelength UV/VIS detector, with compound-specific calibration curves generated under identical conditions.

4.0 REFERENCES

4.1 "Method 8330 - Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC)." EPA Test Methods for Evaluating Solid Waste (SW-846), November 1992

4.2 Personal communications with Dr. Thomas Jenkins
U.S. Army Cold Regions Research and Engineering Laboratory
Hanover, NH

4.3 Personal communications with Philip G. Thorne
U.S. Army Cold Regions Research and Engineering Laboratory
Hanover, NH

4.4 Thorne, Philip G. "Hydrolytic Release of Bound Residues From Composted TNT-Contaminated Soil." 1996

4.5 Personal communications with Dr. Steve Larson
U.S. Army Corps of Engineers Waterways Experiment Station, Environmental Laboratory, Environmental Chemistry Branch

4.6 GLP-0018, "Method Detection Limits", Environmental Applications, Tennessee Valley Authority, Muscle Shoals, AL

5.0 RESPONSIBILITIES

5.1 It is the responsibility of the Supervisor of the Environmental Applications section, or his designee, to ensure that this procedure is followed during the handling, preparation, extraction and analysis of all samples for nitroaromatics and nitroamines by HPLC.

5.2 The Laboratory Group Leader, or his designee, shall delegate the performance of this procedure to personnel experienced with this procedure. Training of personnel inexperienced with this procedure shall be carried out by experienced personnel under the supervision of the Laboratory Group Leader.

5.3 The analyst shall follow this procedure and report any abnormal results or problems to the Laboratory Group Leader, or his designee.

6.0 REQUIREMENTS

6.1 Prerequisites

Method detection limits shall be determined as in GLP0018 (see Note 9.1)

6.2 Limitations and Actions

None

6.3. Materials/Apparatus/Equipment

6.3.1 HPLC system composed of a tertiary pump (Varian Model 9012 or equivalent), an autosampler (Varian Model 9300 or equivalent) and a single wavelength UV/Vis detector (Varian Model 9050 or equivalent) or a photodiode array detector (Varian Model 9065 or equivalent).

6.3.2 HPLC guard column - Ultracarb ODS (20), 30 X 4.6 mm, manufactured by Phenomenex - (or equivalent).

6.3.3 HPLC analytical column - Ultracarb ODS (20), 250 X 4.6 mm, manufactured by Phenomenex - (or equivalent).

6.3.4 Tissue homogenizer - Omni Mixer ES, manufactured by Omni International (or equivalent).

6.3.5 25 mm sawtooth generator probe for use with tissue homogenizer - Part # 15035, manufactured by Omni International (or equivalent).

6.3.6 Freeze Dryer - Model 77520 (6L-Benchtop), manufactured by Labconco - (or equivalent).

6.3.7 Sonicator bath - Bransonic 52, manufactured by Bransom of Smith/Kline (or equivalent).

6.3.8 Temperature controlled circulating bath - Model 2095 Bath and Circulator, manufactured by Forma Scientific - (or equivalent).

6.3.9 300 ml size freeze dry flask with rubber top and glass adapter - Assembly # 75406, manufactured by Labconco - (or equivalent).

6.3.10 Glass Class A volumetric pipets (various sizes).

6.3.11 Glass graduated cylinders (various sizes).

6.3.12 Glass Class A volumetric flasks (various sizes).

6.3.13 Glass separatory funnels 125 ml and 250 ml size.

6.3.14 Stainless steel spatulas.

6.3.15 Teflon coated stir bars (various sizes).

6.3.16 Heavy duty aluminum foil - Part # 0-10900, manufactured by Reynolds Aluminum Co. - (or equivalent).

6.3.17 12-port vacuum manifold - Cat. # 5-7030, manufactured by Supelco Inc - (or equivalent).

6.3.18 Sep-Pak Vac Adapters - Part # WAT054260, manufactured by Waters Corp. - (or equivalent).

6.3.19 60 ml Sep-Pak reservoir - Part # WAT024659, manufactured by Waters Corp. - (or equivalent).

6.3.20 Explosion-proof refrigerator - Model Cryo-Fridge, manufactured by Scientific Products Inc. - (or equivalent).

6.3.21 8ml and 16 ml glass vials with Teflon lined closures - Cat # 75008-SB and 75016-SB respectively, manufactured by Scientific Resources Inc. (SRI) - (or equivalent).

6.3.22 12 X 32 amber autosampler vials with Teflon lined closures - Cat. # 99575-A - (or equivalent).

6.3.23 250 ml tall form, wide mouth glass bottle with Teflon lined closures - Part # 131-08C/TL/WS, manufactured by Eagle Picher - (or equivalent).

6.3.24 60 ml pre-cleaned amber bottle with Teflon lined closure - Part # 120-02A, manufactured by Eagle Picher - (or equivalent).

6.3.25 40 ml vial with Teflon lined closure - Part # 141-40A, manufactured by Eagle Picher - (or equivalent).

6.3.26 10 ml disposable plastic syringe - Part # 309604, manufactured by Becton and Dickinson - (or equivalent).

6.3.27 25 mm, PTFE syringe filters having 0.2 or 0.45 µm pore size - Cat. # 42225-NP and 44525-PC respectively, manufactured by SRI - (or equivalent).

6.3.28 Alumina-A solid phase extraction cartridges 1, 5, and 10 gram sizes - Part # WAT054580, WAT054670 and WAT054710 respectively, manufactured by Waters Corp. - (or equivalent).

6.3.29 Porapak-Rdx solid phase extraction cartridge (500mg size) - Part # WAT047220, manufactured by Waters Corp. - (or equivalent).

6.3.30 Vacuum manifold for solid phase extraction cartridges - Cat. # 5-7030, manufactured by Supelco Inc. - (or equivalent).

6.3.31 Refrigerated centrifuge - Model CRU-5000, manufactured by IEC Inc. - (or equivalent).

6.3.32 Benchtop centrifuge - Model SS-4 Manual, manufactured by Sorvall - (or equivalent).

6.3.33 Magnetic stirrer - Cat. # 14-511-1A, manufactured by Fisher Scientific Co. - (or equivalent).

6.3.34 Analytical balance - Model A200S, manufactured by Sartorius - (or equivalent).

6.3.35 Glass vacuum desiccator with indicating desiccant.

6.3.36 Ceramic mortar and pestle.

6.3.37 Glass conical bottom centrifuge tubes (12 ml size).

6.3.38 30 mesh sieve.

6.3.39 Pasteur pipets - Cat. # P5201-1, manufactured by Scientific Products - (or equivalent).

6.3.40 Parafilm "M" - Laboratory Film, manufactured by American National Can (or equivalent).

6.3.41 Ultrapure nitrogen - compressed gas.

6.4 Reagents and Standards

6.4.1 Water (HPLC grade) - Part # WX0004-1, manufactured by E M Science - (or equivalent).

6.4.2 Methanol, CH_3OH (HPLC grade) - Part # MX0488-1, manufactured by E M Science - (or equivalent).

6.4.3 Acetonitrile, CH_3CN (HPLC grade) - Part # AX0142-1, manufactured by E M Science - (or equivalent).

6.4.4 Sodium chloride, NaCl (reagent grade) - Part # SX0420-1, manufactured by E M Science - (or equivalent).

6.4.5 Calcium chloride, $CaCl_2$ (reagent grade) - Part # C1096, manufactured by Spectrum Chemical - (or equivalent).

6.4.6 Sodium phosphate dibasic, $Na_2HPO_4 \cdot 7H_2O$ (reagent grade) - Part # SX0175-1, manufactured by E M Science - (or equivalent).

6.4.7 Concentrated sulfuric acid, H_2SO_4 (reagent grade) - Part # 5557, manufactured by Mallinckrodt Inc. - (or equivalent).

6.4.8 Blank soil - U.S. Army Environmental Center Standard Soil.

6.4.9 Neat explosive analyte standards - either provided by the U.S. Army Environmental Center, or purchased from Accustandard Inc, Stanford Research Institute International or Chem Service Inc.

6.4.9.1 Stock Standard Solutions (single analyte)

Each neat solid analyte standard is dried to a constant weight in a vacuum dessicator at room temperature in the dark. Each neat liquid analyte standard is transferred using glass Pasteur pipets or glass gas-tight syringes with Teflon tipped plungers. Approximately 0.1 g (weighed to 0.0001 g) of a single neat analyte is placed into a 100 ml volumetric flask and diluted to volume with acetonitrile. A stir bar is added to the flask which is then placed on a magnetic stirrer and stirred until the analyte has totally dissolved or mixed. During mixing, the flask is covered with an aluminum foil hood. The stir bar is removed, the flask is stoppered and wrapped in aluminum foil. The concentration of the stock solution is calculated from the actual weight of the analyte used, the purity of the analyte and the volume of the solution (nominal

concentration is 1,000 mg/L). These solutions should be stored, stoppered and sealed with Parafilm, in an explosion-proof refrigerator at 4° C.

6.4.9.2 Intermediate Standard Solutions (single or multiple analyte)

These solutions, at approximately 20 µg/ml per analyte, are prepared by dilutions of the stock standard solutions with acetonitrile in volumetric flasks. The flasks are wrapped with aluminum foil and stored, stoppered and sealed with Parafilm, in an explosion-proof refrigerator at 4° C. These solutions are used to prepare calibration standards.

6.4.9.3 Calibration Standard Solutions (multiple analyte)

These solutions, at a minimum of five levels covering the concentration range of interest (approximately 6 µg/ml to approximately 25 ng/ml), are usually prepared by dilutions of the Intermediate standard solutions with acetonitrile in volumetric flasks. The flasks are wrapped with aluminum foil and stored, stoppered and sealed with Parafilm, in an explosion-proof refrigerator at 4°C. Before analysis, these solutions are equilibrated to room temperature, diluted 1:1 with water, allowed to stand for 20 minutes and passed through a 25 mm PTFE syringe filter with 0.45 µm pore size.

6.4.10 Aqueous explosives spike solution (8 component) at 2 µg/ml each analyte.

Spike made from Accustandard solution containing HMX, RDX, (1,3,5-TNB), (1,3-DNB), nitrobenzene, TNT, 2-ADNT and 2,4-DNT at 1000 µg/ml each analyte. The spike solution is prepared by first diluting 1.0 ml of the 1000 µg/ml standard to 25.0 ml with acetonitrile. A 5.0 ml aliquot of this solution is diluted to 100.0 ml with HPLC water to yield a solution whose concentration is 2.0 ug/ml per analyte.

6.4.11 Spike solution for use with compost leachate extraction

Solution(s) whose concentration is approximately 3 ug/ml per analyte for the following compounds: (2,6-DANT), HMX, (2,4-DANT), RDX, (1,3,5-TNB), TNT, 4-ADNT, 2-ADNT, (2,6-DNT) and 2,4-DNT. Solution matrix is acetonitrile.

6.4.12 Spike solution for use with plant extraction

Prepare a spiking solution at approximately 100 ug/ml per analyte for the following analytes: (2,6-DANT), HMX, (2,4-DANT), RDX, (1,3,5-TNB), TNT, 4-ADNT, 2-ADNT, (2,6-DNT) and 2,4-DNT. The mixed analyte solution matrix should be acetonitrile. The solution should be prepared

from stock standard solutions (section 6.4.9.1) and should be stored in an aluminum foil wrapped flask, stoppered and sealed with Parafilm in an explosion-proof refrigerator at 4° C.

6.4.13 Sulfuric acid solution (1+1)

In a suitable container which is sitting on ice, place a known volume of HPLC water, add to this slowly and with swirling an equal volume of concentrated sulfuric acid. Allow to equilibrate to room temperature before using.

6.4.14 Sodium phosphate dibasic at 1.07 M concentration

Place appropriate quantity of sodium phosphate dibasic heptahydrate in a glass beaker and place in a forced air oven at 35°C and leave for at least 24 hr. Remove from oven and allow to cool. For about 1 L of solution, weigh out 322 g of the dried compound and place in a large Erlenmeyer flask. Add 1000 mL of HPLC water to the flask. Add a stir bar and place on a magnetic stirrer / hot plate on low heat and moderate stirring until all solids are dissolved. Cool to less than 30°C before use. This should not be kept for use for more than 2 days.

6.4.15 Saturated aqueous sodium chloride solution

Weigh out 325 g of sodium chloride and place in a 1000 ml volumetric flask. Add about 950ml of HPLC water. Add a stir bar and place on magnetic stirrer until most of the salt has dissolved. Remove the stir bar from solution, use HPLC water to make solution to volume, then return the stir bar to the solution and stir for at least 30 minutes (salt will still be present in bottom of flask).

6.4.16 Aqueous calcium chloride solution

Weigh out 6.67 g of calcium chloride dihydrate and place in a 1000 ml volumetric flask. Make to volume with HPLC water, add a stir bar and place on a magnetic stirrer until all solids are dissolved.

6.4.17 Acetonitrile based explosives spike solution (8 components) at 2 ug/ml for each analyte.

Spike made from Accustandard solution containing HMX, RDX, (1,3,5-TNB), (1,3-DNB), nitrobenzene, TNT, 2-ADNT and 2,4-DNT at 1000 µg/ml each analyte. The spike solution is prepared by first diluting 1.0 ml of the 1000 µg/ml standard to 25.0 ml with acetonitrile. A 5.0 ml aliquot of

this solution is then diluted to 100.0 ml with more acetonitrile to yield a solution whose concentration is 2.0 ug/ml per analyte.

6.4.18 1:1 mixture of acetonitrile/calcium chloride.

Combine equal volumes of HPLC grade acetonitrile (step 6.4.3) and 5 g/L calcium chloride solution (step 6.4.16). Allow solution to equilibrate to room temperature before using.

6.5 Quality Control Sample Requirements

6.5.1 Every batch of samples (20 members or less) whose matrix is water, soil, compost, sediment, or gravel, shall have the following QA/QC samples extracted and/or prepared at the same time in identical fashion: matrix spike, matrix spike duplicate, method blank and laboratory control sample (LCS).

Every batch of samples (20 members or less) whose matrix is compost leachate shall have the following QA/QC samples extracted at the same time and in identical fashion: matrix spike, method blank and LCS.

Every batch of samples (20 members or less) whose matrix is plant tissue shall have the following QA/QC samples extracted at the same time and in identical fashion: matrix spike, LCS, and method blank.

6.5.2 Daily Calibration Check of the UV/VIS detector system.

Midpoint calibration standards for each analyte of interest are analyzed in duplicate at the beginning of the analytical run, singly after every 10 sample vials and singly after the last sample of the run. The calculated concentration of each analyte of interest in each midpoint standard throughout the analytical run shall agree with its known value within +/- 15%. If this criterion is not met, samples following the previous acceptable standard and prior to the next acceptable standard may be reanalyzed, or all or part of the sample data may be "qualified" and flagged with a "Q" designation in the database. The decision to reanalyze or qualify samples shall be made by the Laboratory Group Leader.

7.0 PROCEDURE

7.1 Calibration

Initial Calibration of the UV/VIS detector system.

From one to three injections of each calibration standard over the concentration range of interest are sequentially injected into the HPLC in random order. Using commercial chromatography software, peak heights are obtained for each analyte. Calibration curves are generated using spreadsheets which utilize linear regression equations of the form $y = mx$, $y = a + bx$, or $y = a + bx + cx^2$. Selection of the equation form to use is made by assessing the data for goodness of fit and how closely back-calculation of the fit data reproduces the known concentrations of the calibration solutions.

7.2 Procedure Instructions

NOTE: Because some of the analytes of interest in the following procedures are photosensitive and thermolabile, standards, samples, extracts, filtrates, eluants, etc. should be exposed to light or heat as little as possible during the performance of the procedures. This is especially true during standing or storage periods.

7.2.1 Preparation of water samples (with no preconcentration) for qualitative / quantitative analysis.

NOTE: Batches of samples undergoing this preparation shall contain the following QA/QC samples: matrix spike, matrix spike duplicate, LCS, and method blank.

7.2.1.1 Retrieve samples and allow them to equilibrate to room temperature if necessary.

7.2.1.2 Obtain the appropriate sample worksheet (Attachment 1 - "Preparation of Liquids for Explosives Analysis"). Record on the worksheet, the laboratory number of the samples to be prepared for HPLC analysis, the date, analyst's name, the serial number and concentration of the spiking solution to be used.

Select one sample out of the batch for use in the creation of the matrix spike and matrix spike duplicate. Record the number of this sample in the appropriate area in the QC section of the worksheet.

7.2.1.3 If sample contains no solids, proceed to step 7.2.16. If sample contains solids, place appropriate volume of sample in a properly labeled, 40 ml vial and seal with a Teflon-lined closure.

7.2.1.4 Centrifuge the sample at 2000 rpm or greater for at least 15 minutes.

7.2.1.5 Decant the supernatant to a properly labeled, glass vial and seal with Teflon-lined closure.

7.2.1.6 Prepare the batch QA/QC samples as listed below. Record all critical data in the appropriate areas in the QC section of the sample worksheet.

Matrix spike and matrix spike duplicate - To a 10 ml volumetric flask, add 5.0 ml of selected sample, then add 1.0 ml of the aqueous spiking solution (see section 6.4.10). Make flask to volume with HPLC water, stopper and mix thoroughly by inversion. Store in dark until needed.

Laboratory control sample - To a 10 ml volumetric flask, add 5.0 ml of HPLC water, then add 1.0 ml of the aqueous spiking solution (see section 6.4.10). Make flask to volume with HPLC water, stopper and mix thoroughly by inversion. Store in dark until needed.

Method blank - To a 10 ml volumetric flask, add 10.0 ml of HPLC water, stopper the flask and mix thoroughly by inversion.

7.2.1.7 Using precisely measured volumes, place equal amounts of sample (regular and QA/QC) and HPLC grade acetonitrile (usually 2 ml of each component) in a glass vial, cap with a Teflon-lined closure and mix thoroughly by inversion.

7.2.1.8 Let mixture stand in dark at room temperature for 20 minutes.

7.2.1.9 Pass the mixture through a PTFE syringe filter with 0.45 μm pore size. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being apportioned as follows:

If only qualitative or quantitative analysis of the sample is required, then only one autosampler vial containing filtrate is required. If qualitative and quantitative analysis of the sample are required, then two autosampler vials containing filtrate are required.

7.2.1.10 Let the filtrate stand in the dark until ready to load onto the autosampler. If filtrates cannot be loaded onto the autosampler the same day they are

prepared for analysis, they should be stored in an explosion-proof refrigerator at 4° C.

7.2.1.11 If it becomes necessary during the analysis process to dilute the sample, record on the sample worksheet the aliquots and dilution volumes used.

7.2.2 **Preconcentration of water samples by Solid Phase Extraction (SPE) for qualitative/quantitative analysis by HPLC.**

NOTE: Batches of samples undergoing this extraction shall contain the following QA/QC samples: LCS and method blank

7.2.2.1 Retrieve the samples and allow them to equilibrate to room temperature.

7.2.2.2 Obtain the appropriate sample worksheet (Attachment 2 - "Preconcentration of Liquids by SPE for Explosives Analysis"). Record on the worksheet, the laboratory number of the samples to be concentrated and prepared for HPLC analysis, the date, analyst's name, the serial number and concentration of the spiking solution to be used.

7.2.2.3 Prepare the batch QA/QC samples as listed below. Record all critical data in the appropriate areas in the QC section of the sample worksheet.

Laboratory control sample - To a 100 ml Erlenmeyer flask, add approximately 50 ml of HPLC water, then add 1.0 ml of the aqueous spiking solution (see section 6.4.10) and swirl to mix. Seal with Parafilm and let stand in dark until needed.

Method blank - To a 100 ml Erlenmeyer flask, add 50 ml of HPLC water. Seal with Parafilm and place in dark until needed.

7.2.2.4 If sample does not contain solids, proceed to step 7.2.2.6 (be sure to read NOTE before step 7.2.2.6). If sample contains solids, fractionate an appropriate volume of sample to 40 ml vials with Teflon lined closures and centrifuge for at least 15 minutes at 2000 rpm or greater.

7.2.2.5 Remove the supernatant and place in an Erlenmeyer flask, seal with Parafilm, and let stand in dark until needed. This supernatant is the sample fraction that will be concentrated by SPE.

NOTE: Do not allow the resin bed of the Porapak-Rdx cartridge to become dry during the conditioning step, between the conditioning and the sample loading steps or during the sample loading.

7.2.2.6 Attach an adapter and a 60 ml reservoir to a Porapak-Rdx cartridge (500 mg size) and connect the cartridge to the vacuum manifold. Condition the cartridge by passing 15 ml of acetonitrile through it (gravity flow after starting flow with vacuum), followed by 30 ml of water at a rate of approximately 10 ml/minute using vacuum.

NOTE: In the following step, if the extract from this procedure is to be used for analyte identification only, approximate sample volumes can be used (minimum of 60ml). If the extract is to be used for analyte quantitation, it is necessary to use a known volume aliquoted with an accurate measuring device, such as a graduated cylinder (rinse device three times with HPLC water and add this to reservoir for the appropriate sample).

7.2.2.7 Pass the appropriate volume of sample through the SPE cartridge at a flow rate of approximately 10 ml/min. Record this known or approximate volume on the worksheet.

7.2.2.8 After the sample has been totally pulled through the cartridge, continue to apply a vacuum to the cartridge for about 5 minutes to remove residual moisture.

7.2.2.9 Remove the SPE cartridge from the vacuum manifold and remove the adapter and 60 ml reservoir from the cartridge.

7.2.2.10 Position the SPE cartridge over a properly labeled 5 ml volumetric flask (other sizes of volumetric flasks can be used if deemed necessary) with the Luer tip of the cartridge extending into the mouth of the flask.

7.2.2.11 Add 5 ml of acetonitrile to the SPE cartridge.

7.2.2.12 Apply a lightly pressurized flow of ultrapure nitrogen to the top of the cartridge in order to initiate solvent flow through the cartridge and into the volumetric flask. Once the solvent flow begins, remove the nitrogen source and allow the solvent flow to be by gravity alone.

7.2.2.13 After the solvent stops dripping from the cartridge, reapply the pressurized nitrogen to the top of the cartridge to force any trapped solvent into the flask.

7.2.2.14 Make the flask to volume with acetonitrile, stopper and mix thoroughly. Record the eluant volume on the worksheet.

7.2.2.15 Using accurately measured volumes, place equal amounts of eluant and HPLC grade water (usually 2 ml of each component) in a glass vial, cap with a Teflon-lined closure and mix thoroughly by inversion.

7.2.2.16 Let the mixture stand in the dark at room temperature for 20 minutes.

7.2.2.17 Pass the mixture through a PTFE syringe filter with 0.45 µm pore size. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being apportioned as follows:

If only qualitative or quantitative analysis of the sample is required, then only one autosampler vial containing filtrate is required. If qualitative and quantitative analysis of the sample are required, then two autosampler vials containing filtrate are required.

7.2.2.18 Allow the autosampler vial containing the filtrate to stand in the dark until ready to load onto the autosampler. If filtrates cannot be loaded onto autosampler the same day they are prepared for analysis, they should be stored in an explosion-proof refrigerator at 4° C.

7.2.3 **Preparation of soil or sediment samples for qualitative/quantitative analysis**

NOTE: Batches of samples undergoing this extraction/preparation shall contain the following QA/QC samples: matrix spike, matrix spike duplicate, LCS, and method blank.

7.2.3.1 Spread enough sample either onto acetonitrile rinsed ceramic dishes or clean aluminum foil for sample duplicates, matrix spikes, matrix spike duplicates, and percent moisture procedure.

7.2.3.2 Place the samples in the air flow of a darkened hood at room temperature and allow to dry for 12 - 18 hours (no visible moisture should be present).

7.2.3.3 Set aside enough air dried sample, for each regular sample, to perform a percent moisture determination as described in section 9.2 of this procedure. The start of this determination must be prompt so that sample moisture is not lost.

7.2.3.4 If the air dried sample is loose and free flowing without large clumps, no grinding will be required. But if the sample does contain large clumps, grind an appropriate quantity of sample in a dry, acetonitrile rinsed mortar. Place the sample (unground or ground) into a glass vial with Teflon-lined

closures. Store the sample in the dark at freezer temperatures (-10° C) until ready for use.

7.2.3.5 Obtain the appropriate sample worksheet (Attachment 3 - "Preparation of Solids for Explosives Analysis"). Record on the worksheet, the laboratory number of the samples to be extracted and prepared for HPLC analysis, the date, analyst's name, the sample matrix, the serial number and concentration of the spiking solution to be used.

7.2.3.6 Select one sample out of the batch for use in the creation of the matrix spike and matrix spike duplicate. Record the laboratory number of this sample in the appropriate areas in the QC section of the sample worksheet.

7.2.3.7 Into a properly labeled 40 ml glass vial, weigh out 2 grams of air dried sample. Record the sample weight to the nearest 0.0001 g on the worksheet in the appropriate area.

7.2.3.8 Prepare the batch QA/QC samples as listed below. Record all critical data in the appropriate areas in the QC section of the sample worksheet.

Matrix spike and matrix spike duplicate - For each spiked sample, weigh into a properly labeled 40 ml glass vial, 2 grams of air dried sample. Record the weight to the nearest 0.0001 g. Add 1.0 ml of the organic spiking solution (see section 6.4.17) to the sample, evaporate the acetonitrile of the spike using a flow of ultra high purity nitrogen or the air flow of an operating hood. Then add 10.0 ml of acetonitrile to the vial. Recap the vial and vortex for 1 minute. Place vial in the dark until ready for step 7.2.3.10.

Laboratory control sample - Into a properly labeled 40 ml glass vial, weigh out 2 g of standard soil (see section 6.4.8). Record the weight to the nearest 0.0001 g. Add 1.0 ml of the organic spiking solution (see section 6.4.17) to the sample, evaporate the acetonitrile of the spike using a flow of ultra high purity nitrogen or the air flow of an operating hood. Then add 10.0 ml of acetonitrile to the vial. Recap the vial and vortex for 1 minute. Place vial in the dark until ready for step 7.2.3.10.

Method blank - Into a properly labeled 40 ml glass vial, weigh out 2 g of standard soil (see section 6.4.8). Record the weight to the nearest 0.0001 g. Add 10.0 ml of acetonitrile to the vial. Recap the vial and vortex for 1 minute. Place vial in the dark until ready for step 7.2.3.10.

7.2.3.9 To all regular samples (non-QA/QC samples), add 10.0 ml of HPLC grade acetonitrile to the vial, replace the Teflon-lined closure, and vortex for 1

minute. Record this volume on the worksheet in the appropriate area. Place vial in the dark until ready for next step.

7.2.3.10 Suspend the extraction vials (regular samples and QA/QC samples) in a sonicator bath regulated between 10° C and 15° C and sonicate under low light conditions for 18 hours. The water level in the sonicator should be above the solvent level in the sample bottles.

7.2.3.11 Remove the vials from the sonicator bath and let stand in the dark at room temperature for 30 - 60 minutes. This allows particulates to settle and a supernatant to form.

If, at the end of the standing period, particulates make pipetting impossible, it will be necessary to centrifuge the sample at 2000 rpm or greater for at least 15 minutes before proceeding to next step.

7.2.3.12 With a volumetric pipet, remove an appropriate quantity of supernatant and mix it at 1:1 ratio with the calcium chloride solution (see section 6.4.16). Let the mixture stand in the dark for 20 minutes.

7.2.3.13 Remove the supernatant from the sample (avoid the flocculated particulates on the bottom) and filter through a 0.2 µm Teflon syringe filter. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being apportioned as follows:

If only qualitative or quantitative analysis of the sample is required, then only one autosampler vial containing filtrate is required. If qualitative and quantitative analysis of the sample are required, then two autosampler vials containing filtrate are required.

7.2.3.14 Allow the autosampler vial containing the filtrate to stand in the dark until ready to load onto the autosampler. If filtrates cannot be loaded onto autosampler the same day they are prepared for analysis, they should be stored in an explosion-proof refrigerator at 4° C.

7.2.4 **Preparation of gravel samples for qualitative/quantitative analysis**

NOTE: Batches of samples undergoing this extraction/preparation shall contain the following QA/QC samples: matrix spike, matrix spike duplicate, LCS, and method blank.

7.2.4.1 Spread enough sample either onto acetonitrile rinsed ceramic dishes or clean aluminum foil for sample duplicates, matrix spikes, matrix spike duplicates, and percent moisture procedure.

7.2.4.2 Place the samples in the air flow of a darkened hood at room temperature and allow to dry for 12 - 18 hours (no visible moisture should be present).

7.2.4.3 Set aside enough air dried sample, for each regular sample, to perform a percent moisture determination as described in section 9.2 of this procedure. The start of this determination must be prompt so that sample moisture is not lost.

7.2.4.4 Obtain the appropriate sample worksheet (Attachment 3 - "Preparation of Solids for Explosives Analysis"). Record on the worksheet, the laboratory number of the samples to be prepared for HPLC analysis, the date, analyst's name, the sample matrix type the serial number and concentration of the spiking solution to be used.

Select one sample out of the batch for use in the creation of the matrix spike and matrix spike duplicate. Record the number of this sample in the appropriate area in the QC section of the worksheet.

7.2.4.5 Weigh enough air dried sample into a pre-cleaned, 250 ml, wide-mouth, tall-form bottle to reach the base of the bottle's neck (usually over 200 g). Record this weight on the worksheet in the appropriate area.

7.2.4.6 Prepare the batch QA/QC samples as listed below. Record all critical data in the appropriate areas in the QC section of the sample worksheet.

Matrix spike and matrix spike duplicate - For each spiked sample, weigh out into a properly labeled 250 ml bottle, enough air dried sample to reach the base of the bottle's neck and cap the bottle with a Teflon-lined closure. Record the weight to the nearest 0.1 g. Add 5.0 ml of the aqueous spiking solution (see section 6.4.10) to the sample, recap the bottle and shake for 1 minute, then let the sample stand in the dark for 1 hour. Then add 95.0 ml of acetonitrile to the vial. Recap the bottle tightly and shake vigorously for 1 minute. Place the bottle in the dark until ready for step 7.2.4.9.

Laboratory control sample - Into a properly labeled 250 ml bottle, pipet 5.0 ml of the aqueous spiking solution (see section 6.4.10), recap the bottle and shake for 1 minute, then let the bottle stand in the dark for 1 hour. Then add 95.0 ml of acetonitrile to the bottle. Recap the bottle tightly and shake vigorously for 1 minute. Place the bottle in the dark until ready for step 7.2.4.9.

Method blank - Into a properly labeled 250 ml bottle, place 100.0 ml of acetonitrile. Recap the bottle tightly and shake vigorously for 1 minute. Place the bottle in the dark until ready for step 7.2.4.9.

7.2.4.7 For any regular sample (non-QA/QC samples) add 100.0 ml of acetonitrile to the bottle and replace the cap, taking care to ensure a tight fit. Record this volume on the worksheet in the appropriate area.

7.2.4.8 Shake the bottle vigorously for one minute.

7.2.4.9 Place bottle in a sonicator bath regulated between 10° C and 15° C and sonicate for 18 hours. The water level in the sonicator should be even with the solvent level in the sample bottles, but should not be high enough to float the bottles or touch the lids of the sample bottles.

7.2.4.10 Remove the bottle from sonicator bath and shake vigorously for one minute.

7.2.4.11 Let the bottle stand in the dark and equilibrate to room temperature.

7.2.4.12 Mix, in a glass vial, an appropriate volume of the acetonitrile sample extract at a 1:1 ratio with a calcium chloride solution (see section 6.4.16) and let stand in the dark for 20 minutes.

7.2.4.13 Remove the supernatant from the calcium chloride treated sample (avoid the flocculated particulates on the bottom) and filter through a 0.2 µm Teflon syringe filter. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being apportioned as follows:

If only qualitative or quantitative analysis of the sample is required, then only one autosampler vial containing filtrate is required. If qualitative and quantitative analysis of the sample are required, then two autosampler vials containing filtrate are required.

7.2.4.14 Allow the autosampler vial containing the filtrate to stand in the dark until ready to load onto the autosampler. If filtrates cannot be loaded onto autosampler the same day they are prepared for analysis, they should be stored in an explosion-proof refrigerator at 4° C.

7.2.5 **Preparation of compost leachate for qualitative/quantitative analysis**

NOTE: Batches of samples undergoing this extraction/preparation shall contain the following QA/QC samples: matrix spike, LCS, and method blank.

7.2.5.1 Prepare a sodium chloride solution as per section 6.4.15 of this procedure.

7.2.5.2 Retrieve the samples and allow to equilibrate to room temperature if necessary.

7.2.5.3 Obtain the appropriate sample worksheet (Attachment 4 - "Preparation of Compost Leachates for Explosives Analysis"). Record on the worksheet, the laboratory number of the samples to be extracted and prepared for HPLC analysis, the date, analyst's name, the serial number and concentration of the spiking solution to be used.

Select one sample out of the batch for use in the creation of the matrix spike. Record the number of this sample in the appropriate area in the QC section of the worksheet.

7.2.5.4 Fractionate at least 40 ml of the leachate sample (80 ml of the sample selected for matrix spike) into 40 ml glass vials with Teflon lined closures. Centrifuge the vials for 30 minutes at 2000 rpm or greater. After centrifugation, decant the supernatant for each sample into a clean 40 ml vial(s).

7.2.5.5 For each sample add 12.56 g of sodium chloride to a 500 ml separatory funnel. Measure out 38 ml of sample (use HPLC water for the method blank and LCS) and transfer to the separatory funnel containing the salt. Record these weights and volumes on the worksheet in the appropriate areas.

7.2.5.6 To the matrix spike and LCS, add 1.0 ml of the appropriate spiking solution (see section 6.4.11). Record the critical data on the worksheet in the appropriate areas.

7.2.5.7 Stopper the separatory funnel and shake until all the salt has dissolved (about 5 minutes).

7.2.5.8 Using volumetric pipets, add 9.0 ml of acetonitrile to the separatory funnel of each sample which has been spiked and 10.0 ml of acetonitrile to the funnel of each unspiked sample.

7.2.5.9 Stopper the funnel and shake for 5 minutes, venting as needed to relieve pressure, then let the samples stand for 10 minutes to allow phases to separate.

7.2.5.10 Drain off the salt layer (bottom layer) except for the last 1-2 ml and discard properly. Drain the acetonitrile layer (top layer of approximately 1-2 ml) along with the remaining salt layer into a 250 ml separatory funnel.

7.2.5.11 Add 16 ml of HPLC grade acetonitrile to first separatory funnel and rinse the walls into the second separatory funnel.

7.2.5.12 Add 84 ml (measure with 100 ml graduated cylinder) of salt solution (see step 7.2.5.1) to the 250 ml separatory funnel.

7.2.5.13 Stopper the separatory funnel and shake for 5 minutes, then allow to stand for 10 minutes for phase separation.

7.2.5.14 Discard to waste most of the bottom layer (salt) and transfer the acetonitrile layer (top layer) plus the last 1-2 ml of the salt layer to a glass centrifuge tube. Rinse the separatory funnel with 1.0 ml of acetonitrile and transfer to the same centrifuge tube.

7.2.5.15 Centrifuge the extract for 10 minutes at 5000 rpm. Then remove the acetonitrile layer (top) and place in a 10 ml graduated cylinder. Measure the extract volume to the nearest 0.1 ml (should be 3-4 ml). Record this volume on the worksheet in the appropriate area.

7.2.5.16 Pipet 2 ml of the sample from the 10 ml graduated cylinder into a glass vial. Add 2 ml of calcium chloride solution (see section 6.4.16) to the vial, mix and let stand for 20 minutes.

7.2.5.17 Remove the supernatant from the calcium chloride treated sample (avoid the flocculated particulates on the bottom) and filter through a 0.2 μm Teflon syringe filter. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being apportioned as follows:

If only qualitative or quantitative analysis of the sample is required, then only one autosampler vial containing filtrate is required. If qualitative and quantitative analysis of the sample are required, then two autosampler vials containing filtrate are required.

7.2.5.18 Allow the autosampler vial containing the filtrate to stand in the dark until ready to load onto the autosampler. If filtrates cannot be loaded onto autosampler the same day they are prepared for analysis, they should be stored in an explosion-proof refrigerator at 4° C.

7.2.6 Preparation of plant tissue for qualitative/quantitative HPLC analysis

Three separate extractions are performed on each vegetation sample: two with acetonitrile and one with sulfuric acid. The three extracts can then analyzed separately, or combined and analyzed as one sample. Total

concentrations of explosives and degradation products are calculated mathematically.

NOTE: Turn on the freeze dry unit (if it is not already running) and allow the temperature and vacuum to equilibrate to normal running levels during the performance of the following steps.

NOTE: Batches of samples undergoing this preparation shall contain the following QA/QC samples: matrix spike, LCS, and method blank.

7.2.6.1 Retrieve samples and allow to equilibrate to room temperature in the dark and out of air currents.

7.2.6.2 Obtain the appropriate sample worksheet (Attachment 5 - "Preparation of Plant Tissue for Explosives Analysis"). Record on the worksheet, the laboratory number of the samples to be prepared for HPLC analysis, the date, analyst's name, the serial number and concentration of the spiking solution to be used.

Select one sample out of the batch for use in the creation of the matrix spike. Record the number of this sample in the appropriate area in the QC section of the worksheet.

7.2.6.3 Set aside enough fresh plant tissue, for each regular sample, to perform a percent moisture determination as described in section 9.2 of this procedure. The start of this determination must be prompt so that sample moisture is not lost.

7.2.6.4 Rinse the outside of the plant tissue with deionized water, then gently blot excess moisture from the tissue using lab wipes.

NOTE: The amount of tissue used in step 7.2.6.5 will vary depending on the percent moisture of the plant. Use enough tissue to have 1.5 - 2.0 grams of tissue left after freeze drying.

7.2.6.5 Into a large plastic boat, weigh out 20 g of plant tissue (the tissue will have to be cut or torn into pieces) and record this weight on the sample worksheet.

7.2.6.6 Place the plant tissue in an acetonitrile rinsed ceramic mortar of appropriate size.

7.2.6.7 Add enough liquid nitrogen to the mortar to create a pool in the bottom about ½ inch deep. The nitrogen should be poured over the surface of the tissue to facilitate rapid freezing.

7.2.6.8 Using an acetonitrile rinsed ceramic pestle of appropriate size, gently crush and grind the frozen plant tissue until it reaches the consistency of a loosely flowing powder.

NOTE: Do not allow the plant tissue to warm enough during this step for liquid water to become visible in the mortar. Add more liquid nitrogen if necessary.

7.2.6.9 Using a powder funnel and a stainless steel spatula, quickly transfer the ground tissue from the mortar to a freeze dry flask (300 ml size).

7.2.6.10 Rinse any adhering tissue from the mortar into the freeze dry flask using small amounts of HPLC water from a squrit bottle.

7.2.6.11 With the tissue in the freeze dry flask, use the 25 mm sawtooth generator probe on the homogenizer to grind the plant tissue to a liquid consistency. Use the speeds and times listed below as a guideline. Keep the generator probe deep enough in the tissue slurry to prevent any material from being ejected from the flask.

1000 rpm----------2 min.
2500 rpm----------1.5 min.
5000 rpm----------1.5 min.
6500 rpm----------1.5 min.

NOTE: The initial homogenization at 1000 rpm may require additional time in order to break up the frozen tissue slurry

7.2.6.12 Sparingly rinse any tissue adhering to the generator probe back into the freeze dry flask with HPLC water.

NOTE: After each plant sample is homogenized, remove the generator probe from the mixer unit, disassemble the probe entirely and wash the parts thoroughly with a detergent solution, followed by a rinse with distilled water, acetonitrile and then with HPLC water. Then reassemble the generator probe.

7.2.6.13 At this point, if the sample is a matrix spike, add 2.0 ml of the appropriate spiking solution (see section 6.4.12) to the flask. The freeze dry flask

should then be sealed with Parafilm, the contents swirled for several seconds and then placed in a darkened area for 30 minutes.

7.2.6.14 Place a rubber cap, with its filter and glass adapter in place, on the freeze dry flask.

7.2.6.15 Place the flask containing the tissue slurry in an acetone/dry ice bath and shell freeze the slurry to the walls of the flask. Ensure there are no large masses of frozen tissue in the bottom of the flask and, that the slurry is thoroughly frozen.

7.2.6.16 Immediately transport the frozen sample to the freeze dryer, place the sample flask on a free port, carefully apply vacuum to the flask and allow the instrument to equilibrate to its normal operating levels before adding additional samples.

7.2.6.17 Leave sample(s) on the freeze dryer until all tissue is thoroughly dry (probably 24-48 hours for five or more samples). Then carefully remove each sample and seal the top of the glass adapter with Parafilm.

7.2.6.18 Carefully remove the rubber cap from the freeze dry flask. Use a clean spatula and scrape any tissue adhering to the top back into the flask.

7.2.6.19 Use a spatula to push the tissue from the inside walls of the freeze dry flask, to its bottom. Then gently chop and stir the tissue mass until it is finely divided.

7.2.6.20 Carefully transfer the tissue mass (scraping out as much as possible) to a clean glass wide-mouth container. Seal with a Teflon-lined closure and let stand in the dark until ready to proceed.

7.2.6.21 Into a 40 ml wide-mouth glass vial, weigh out 0.5 g of tissue. Record this weight to 0.0001 g the on worksheet.

7.2.6.22 Add 15.0 ml of acetonitrile to the vial and seal with Teflon-lined closure. Record this volume on the worksheet.

7.2.6.23 Suspend the vial in a sonicator bath whose temperature is controlled between 10° C and 15° C such that the water level covers the level of solids/liquids inside the vial. Place cover on the sonicator bath to block out light. Individual vials should not touch each other or the walls of the bath.

7.2.6.24 Sonicate the samples for 18 hours.

7.2.6.25 Remove the sample vials from the sonicator and allow to stand in the dark for 15 minutes.

7.2.6.26 Centrifuge sample vials at 2000 rpm or greater for 30 minutes.

7.2.6.27 Remove as much supernatant as possible from the vial, leaving the tissue pellet undisturbed for further extraction.

7.2.6.28 Place the supernatant in a 250 ml Erlenmeyer flask containing 100 ml of HPLC water. Seal the flask with Parafilm, label the flask as fraction "A" and place flask in dark until ready to proceed with step 7.2.6.35.

7.2.6.29 Using the tissue pellet remaining from the previous step, add 15.0 ml of acetonitrile to the vial and seal with Teflon-lined closure. Record this volume on the worksheet.

7.2.6.30 Suspend the vial in a sonicator bath whose temperature is controlled between 10° C and 15° C such that the water level covers the level of solids/liquids inside the vial. Place cover on the sonicator bath to block out light. Individual vials should not touch each other or the walls of the bath.

7.2.6.31 Sonicate the samples for 18 hours.

7.2.6.32 Remove the sample vials from the sonicator and allow to stand in the dark for 15 minutes. Centrifuge sample vials at 2000 rpm or greater for 30 minutes.

7.2.6.33 Remove as much supernatant as possible from the vial, leaving the tissue pellet undisturbed for further extraction. Place the supernatant in a 250 ml Erlenmeyer flask containing 100 ml of HPLC water. Seal the flask with Parafilm label the flask as fraction "B" and place flask in dark until ready to proceed with step 7.2.6.35.

7.2.6.34 Place the uncapped vial containing the tissue pellet at a forward leaning angle (facing outward) in the front portion of a functioning darkened hood. Pull the hood sash partially down and allow the pellet to dry out thoroughly. This should be done in a darkened room, away from possible analyte contamination.

7.2.6.34.1 Pipette 10.0 ml of 1+1 sulfuric acid into the vial with and break up the tissue pellet with a stainless steel spatula, being careful to leave all the tissue in the vial when the spatula is removed. Record this volume on the worksheet.

7.2.6.34.2 Suspend the vial in a sonicator bath whose temperature is regulated at 25° - 30° C and sonicate for 6 hours. The sonicator shall have a cover which blocks out the light.

7.2.6.34.3 Remove the vial from the sonicator bath and centrifuge at 2000 rpm or greater for 30 minutes.

7.2.6.34.4 Remove 5.0 ml of the acidic supernatant and add to 100 ml of 1.07 M sodium phosphate dibasic solution (see section 6.4.14) in an Erlenmeyer flask and swirl. Record the volume of supernatant on the worksheet.

7.2.6.34.5 Seal the flask with Parafilm and place the neutralized supernatant in an explosion-proof refrigerator at 4° C for 12-15 hours.

7.2.6.34.6 Remove the neutralized extract from the refrigerator. If the beaker contains a precipitate or a fluffy suspension, draw off and save most of the free liquid into a clean 250 ml Erlenmeyer flask then proceed to step 7.2.6.34.8.

7.2.6.34.7 If the beaker contains no precipitate or suspension, proceed to step 7.2.6.35.

7.2.6.34.8 Pour the suspension or precipitate layer into a 40 ml glass vial. Rinse the Erlenmeyer flask with HPLC water and add to 40 ml vial. Seal vial with Teflon-lined closure.

7.2.6.34.9 Centrifuge 40 ml vial at 2000 rpm or greater for 30 minutes.

7.2.6.34.10 Remove the supernatant from the precipitate and add to the supernatant removed in step 7.2.6.34.6.

7.2.6.34.11 Seal the flask with Parafilm, and place the accumulated supernatant in the dark until ready to proceed with step 7.2.6.35. Label this as sample fraction "C".

7.2.6.35 For each of the three fractions prepared from each sample (two acetonitrile extracts and one sulfuric acid extract) connect in series from top to bottom (using appropriate adapters), one 60 ml reservoir, one 5 gram Alumina-A SPE cartridge, one 1 gram Alumina-A SPE cartridge and one Porapak-Rdx SPE cartridge (500 mg size). Place this cartridge train onto the vacuum manifold

NOTE: Do not allow the bed of the Porapak-Rdx cartridge to become dry during the conditioning step, between the conditioning and the sample loading steps or during the sample loading step.

7.2.6.36 Condition the cartridges by first pulling 20 ml of acetonitrile (at a flow rate of 2-4 ml/min.) through them, immediately followed by 50 ml of HPLC water at a flow rate of 30 ml/minute. Immediately follow the HPLC water with the sample solution. When the sample flask is empty, rinse it with HPLC water three times and add this to the cartridge reservoir.

NOTE: If the sample being loaded onto the SPE cartridges has as its matrix the 1.07 M sodium phosphate buffer, follow the sample solution with about 70 ml of HPLC water to wash any accumulated salts out of the Porapak-Rdx cartridge.

7.2.6.37 After the sample solution (and wash solution if necessary) has totally passed through the cartridge train, separate the 60 ml reservoir, Alumina-A cartridges, and adaptors from the Porapak-Rdx cartridge which will remain on the vacuum manifold. Dispose of the Alumina-A cartridges properly.

7.2.6.38 Now apply a strong vacuum to the Porapak-Rdx cartridge for about 5 minutes to remove residual water.

7.2.6.39 Remove the Porapak-Rdx cartridge from the vacuum manifold.

7.2.6.40 Position the SPE cartridge over a properly labeled 5 ml volumetric flask (other sizes of volumetric flasks can be used if deemed necessary) with the Luer tip of the cartridge extending into the mouth of the flask.

7.2.6.41 Add 5.0 ml (record on worksheet) of acetonitrile to the SPE cartridge. Apply a lightly pressurized flow of ultrapure nitrogen to the top of the cartridge to start the solvent flowing through the cartridge and into the volumetric flask. Once the solvent flow begins, remove the nitrogen source and allow the solvent flow to be by gravity alone.

7.2.6.42 After the solvent stops dripping from the cartridge, reapply the pressurized nitrogen to the top of the cartridge to force any trapped solvent into the flask.

7.2.6.43 Make the volumetric flask to volume with acetonitrile and mix thoroughly. This extract should be prepared for HPLC analysis on the same day it was generated, or should be transferred to a glass vial with a Teflon-lined closure and stored in an explosion-proof refrigerator at 4° C until needed.

7.2.6.44 At this point, if each of the three sample fractions are to be analysed separately, proceed to step 7.2.6.44.1 (see calculations section 7.3.4.1). If the three sample fractions are to combined for analysis, proceed to step 7.2.6.44.2 (see calculations section 7.3.4.2).

7.2.6.44.1 Using precisely measured volumes, place equal amounts of eluant and HPLC grade water (usually 2 ml of each component) in a glass vial, cap with a Teflon-lined closure and mix thoroughly by inversion.

7.2.6.44.2 Using precisely measured volumes, and in a 16 ml glass vial, combine the eluants from sample fractions A, B, and C at the ratio of 1:1:2. Then add a volume of HPLC water equal to the total volume of the combined sample fractions. Cap the vial and mix.

7.2.6.45 Let the mixture stand in the dark at room temperature for 20 minutes.

7.2.6.46 Pass the mixture through a PTFE syringe filter with 0.45 µm pore size. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being apportioned as follows:

If only qualitative or quantitative analysis of the sample is required, then only one autosampler vial containing filtrate is required. If qualitative and quantitative analysis of the sample are required, then two autosampler vials containing filtrate are required.

7.2.6.47 Allow the autosampler vial containing the filtrate to stand in the dark until ready to load onto the autosampler. If filtrates cannot be loaded onto autosampler the same day they are prepared for analysis, they should be stored in an explosion-proof refrigerator at 4° C.

7.2.7 **Preparation of compost samples for qualitative/quantitative analysis of explosives.**

NOTE: Batches of samples undergoing this extraction/preparation shall contain the following QA/QC samples: matrix spike, matrix spike duplicate, LCS, and method blank.

7.2.7.1 Spread sample on clean holder and place in the air flow of a darkened hood at room temperature and allow to dry for 12-18 hours (no visible moisture should be present).

7.2.7.2 Obtain the appropriate sample worksheet (Attachment 6). Record on the sheet, the laboratory number of the samples being prepared, the date, analyst's name, and the serial number and concentration of the spiking solution to be used.

7.2.7.3 Select one sample out of the batch for use in the creation of the matrix spike and matrix spike duplicate. Record the laboratory number of the sample in

the appropriate area in the QC section of the preparation worksheet (Attachment 6).

7.2.7.4 Into a properly labeled 40 ml glass vial, weigh out 2 grams of air dried sample. Record this weight to the nearest 0.0001 g on the preparation worksheet in the appropriate area.

NOTE: Carried out in conjunction with this procedure and using the same air dried samples, perform a percent moisture determination as described in section 9.2 of this procedure.

7.2.7.5 Prepare the batch QA/QC samples as listed below. Record all critical data in the appropriate areas of the QC section of the worksheet.

Matrix spike and matrix spike duplicate - For each spiked sample, weigh into a properly labeled 40 ml glass vial, 2 grams of air dried sample. Add 1.0 ml of acetonitrile based spiking solution (section 6.4.17) to the sample. Evaporate the acetonitrile from the sample, recap the vial and place in dark until ready for step 7.2.7.6

Laboratory control sample - Weigh into a properly labeled 40 ml glass vial, 2 grams of air dried sample. Add 1.0 ml of acetonitrile based spiking solution (section 6.4.17) to the sample. Evaporate the acetonitrile from the sample, recap the vial and place in dark until ready for step 7.2.7.6

Method blank - Weigh into a properly labeled 40 ml glass vial, 2 grams of AEC blank soil. Recap the vial and place in dark until ready for step 7.2.7.6

7.2.7.6 To each sample vial, add 10.0 ml of HPLC grade acetonitrile. Replace the vial closure and vortex for one minute. Place the vial in dark until ready for next step.

7.2.7.7 Suspend the vial(s) in a sonicator bath regulated between 10° - 15° C and sonicate under low light conditions for 18 hours. The water level in the bath should remain above the solvent level in the vials.

7.2.7.8 Remove the vials from the sonicator bath and centrifuge at 2000-2500 rpm for at least 15 minutes. Decant the supernatant into a properly labeled glass vial (label as fraction "A"). Save the remaining pellet of solids for further extraction in step 7.2.7.9

7.2.7.8.1 Mix at a 1:1 ratio, the sample supernatant with a 5 g/L calcium chloride solution (section 6.4.16) and let stand in dark for 20 minutes.

7.2.7.8.2 Filter the flocculated mixture through a 0.2 um Teflon syringe filter. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being split equally between two amber autosampler vials.

7.2.7.8.3 Allow the autosampler vials to stand in dark until ready for use. If filtrates cannot be used the same day they are prepared, they should be stored at 4°C.

7.2.7.9 Add l0 ml of a 1:1 mixture of acetonitrile/calcium chloride solution (step 6.4.18) to the compost pellet from step 7.2.7.8. Vortex the pellet for 1 minute, then centrifuge the vial at 2000-2500 rpm for at least 15 minutes. Decant the supernatant to waste.

7.2.7.10 Repeat step 7.2.7.9 three more times.

7.2.7.11 Place the washed compost pellets in the air flow of a hood to dry until only slightly damp.

7.2.7.12 Add 10 ml of 1:1 sulfuric acid (step 6.4.13) to the pellet, replace the vial closure and vortex for 1 minute.

7.2.7.13 Suspend the vials in a sonicator bath regulated between 25° - 30°C and sonicate for 6 hours.

7.2.7.14 Remove the vials from the sonicator. If the next steps cannot be performed immediately, place the vials in a refrigerator at 4°C until they can be performed (delay should not be longer than 48 hours).

7.2.7.15 Centrifuge vials at 2000-2500 rpm for at least 15 minutes. Remove 5.0 ml of acidic supernatant from the sample and place into a beaker containing 50 ml of 1.07M sodium phosphate dibasic solution (step 6.4.14). Using more of the phosphate solution and a pH meter, adjust the pH of the buffered extract to 5.0.

7.2.7.16 For each sample, connect in series from top to bottom (using appropriate adapters), one 60 ml reservoir, one 5 gram Alumina-A SPE cartridge, one 1 gram Alumina-A SPE cartridge and one Porapak-Rdx SPE cartridge (500 mg size). Place this cartridge train onto the vacuum manifold

NOTE: Do not allow the bed of the Porapak-Rdx cartridge to become dry during the conditioning step, between the conditioning and the sample loading steps or during the sample loading step.

7.2.7.16.1 Condition the cartridges by first pulling 20 ml of acetonitrile (at a flow rate of 2-4 ml/min.) through them, immediately followed by 50 ml of HPLC

water at a flow rate of 10 ml/minute. Immediately follow the HPLC water with the sample solution. When the sample flask is empty, rinse it with HPLC water three times and add this to the cartridge reservoir. Follow the buffer solution with about 70 ml of HPLC water to wash any accumulated salts out of the Porapak-Rdx cartridge.

7.2.7.16.2 After all solutions have totally passed through the cartridge train, separate the 60 ml reservoir, Alumina-A cartridges, and adapters from the Porapak-Rdx cartridge which will remain on the vacuum manifold. Dispose of the Alumina-A cartridges properly.

7.2.7.16.3 Now apply a strong vacuum to the Porapak-Rdx cartridge for about 5 minutes to remove residual water.

7.2.7.16.4 Remove the Porapak-Rdx cartridge from the vacuum manifold.

7.2.7.16.5 Position the SPE cartridge over a properly labeled (label as sample fraction "B") 5 ml volumetric flask (other sizes of volumetric flasks can be used if deemed necessary) with the Luer tip of the cartridge extending into the mouth of the flask.

7.2.7.16.6 Add 5.0 ml (record on worksheet) of acetonitrile to the SPE cartridge. Apply a lightly pressurized flow of ultrapure nitrogen to the top of the cartridge to start the solvent flowing through the cartridge and into the volumetric flask. Once the solvent flow begins, remove the nitrogen source and allow the solvent flow to be by gravity alone.

7.2.7.16.7 After the solvent stops dripping from the cartridge, reapply the pressurized nitrogen to the top of the cartridge to force any trapped solvent into the flask.

7.2.7.16.8 Make the volumetric flask to volume with acetonitrile and mix thoroughly. This extract should be prepared for HPLC analysis on the same day it was generated, or should be transferred to a glass vial with a Teflon-lined closure and stored in an explosion-proof refrigerator at 4° C until needed.

7.2.7.16.9 Using precisely measured volumes, place equal amounts of eluant and HPLC grade water (usually 2 ml of each component) in a glass vial, cap with a Teflon-lined closure and mix thoroughly by inversion.

7.2.7.16.10 Let the mixture stand in the dark at room temperature for 20 minutes.

7.2.7.16.11 Pass the mixture through a PTFE syringe filter with 0.45 µm pore size. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being apportioned as follows:

If only qualitative or quantitative analysis of the sample is required, then only one autosampler vial containing filtrate is required. If qualitative and quantitative analysis of the sample are required, then two autosampler vials containing filtrate are required.

7.2.7.16.12 Allow the autosampler vial containing the filtrate to stand in the dark until ready to load onto the autosampler. If filtrates cannot be loaded onto autosampler the same day they are prepared for analysis, they should be stored in an explosion-proof refrigerator at 4° C.

7.2.8 HPLC procedure

After preparation is completed and autosampler vials are filled; load the autosampler, enter the parameters noted below, and start the analysis.

7.2.8.1 Tertiary pump parameters

Pump flow rate: 0.8 ml/min.

Run length: 50.00 minutes

Method end action: Equilibrate at end
Equilibration time: 5.00 minutes

Mobile phase gradient (where phase A is water and phase B is methanol)
Time = 0.00 min.; phase A = 83%; phase B = 17%
Time = 8.00 min.; phase A = 63%; phase B = 37%
Time = 10.00 min.; phase A = 42%; phase B = 58%
Time = 23.00 min.; phase A = 42%; phase B = 58%
Time = 28.00 min.; phase A = 0%; phase B = 100%
Time = 35.00 min.; phase A = 0%; phase B = 100%
Time = 40.00 min.; phase A = 84%; phase B = 16%

(NOTE: Mobile phase percentages and flow rates may be altered prior to an initial calibration to provide the best peak resolution and placement)

7.2.8.2 Autosampler parameters

Sample loop volume: 100 µl

Syringe volume: 1000 µl
Wash cycle volume: 500 µl
Tube volume: 13.0 µl
Viscosity factor: 1
Pre-injection delay: 10 sec.
Post-injection wash: Yes
Automixing volume %: 100%
Automixing type-air mixing: No
Stop: momentary: No
Expel tube volume to vial: No
Pulsed start output: Yes

7.2.8.3 Photodiode array detector parameters

Detector information
Bunch rate: 8 points (2.0 Hz)
Monitor length: 64 bunched points (32.0 seconds)

Polychrom parameters
Screen width: 50.00 minutes
Scan frequency: 16 Hz
Autoprint: Off
Ch A output: Absorbance
Ch A bandwidth: 4 nm
Ch A peak use: Upper-half
Ch A time constant: 0.500 seconds
Ch A offset: 10%

7.3 Calculations and Recording Data

7.3.1 Peak identification is made by commercial chromatography workstation software. Data are stored in individual files for each injection with the extension RP1.

7.3.2 Copy files from a run onto a diskette. Extract and concatenate data into a single file with QBASIC program 16.BAS (or its most recent revision). 16.BAS applies calibration curve factors to peak heights and calculates raw concentration.

7.3.3 Edit the output file from 16.BAS to eliminate mis-identified peaks. Add in peaks which were not identified by the software but were found in manual review of chromatograms.

7.3.4.1 For vegetation when the extracts are analyzed separately: Enter the weight of the vegetation sample, extraction volumes, and the measured concentration of each target compound for the three fractions into the spreadsheet 'PLANT_TMPLATE.xls' to calculate total concentrations.

Example: A 0.5041 g sample gives readings X1, X2, and X3 on the three fractions for analyte X.

Total X in micgrograms/gram = (X1*5.0 + X2*5.0 +X3*10.0)/0.5041

7.3.4.2 For vegetation when the extracts are combined before analysis:

If X grams of tissue are extracted into 5 ml of solvent for fraction "A"
and
The second extraction is made to 5 ml for fraction "B"
and
The third extraction initially uses 10 ml of acid, with 5 ml of the acid extract being used for the SPE step and having final volume of 5 ml for fraction "C"
and
The fractions are combined at a 1:1:2 ratio that when analyzed give a concentration of (Rc)
then
The Final concentration = Rc*20/X

7.3.5 Interface the edited file with the EBS database. Add weights, volumes, dilution factors, concentration factors, and unit conversion factors to EBS.

Review percent recoveries and relative percent differences as calculated by EBS.

7.3.6 Review data and resolve all discrepancies. Print a final copy of the customer report and route it to the supervisor along with the data package for final review.

7.3.7 Store chromatograms, preparation worksheets, EBS printouts, run narratives, notes, logbooks, final reports, and other information as quality assurance records.

8.0 SAFETY

8.1 Care should be taken when handling neat HMX, RDX and TNT since these compounds are classified as explosives. Safety glasses and vinyl gloves should be worn during the use of these compounds. Quantities should be minimized as far as possible.

8.2 Standard laboratory safety precautions should be followed when handling the organic solvents used in this procedure. Safety glasses shall be worn at all times in the laboratory and gloves, appropriate for the solvent being handled, should be worn.

9.0 NOTES

9.1 Method Detection Limit determination in GLP-0018 is done in accordance with Title 40, Code of Federal Regulations, Part 136, Appendix B, "Definition and Procedure for the Determine of the Method Detection Limit" - Revision 1.11.

9.2 Percent moisture by Oven Drying

9.2.1 Obtain the appropriate worksheet (Attachment 7 - "Determination of Percent Moisture by Oven Drying"). Record on the worksheet, laboratory numbers, sample description, and your name.

NOTE: For each sample, the steps (9.2.2-9.2.9) will be identical

9.2.2 Obtain an appropriately sized aluminum weighing boat and label with laboratory number of sample.

9.2.3 Weigh the boat to 0.0001 g and record this as the tare weight (TW) in the appropriate area of the worksheet.

9.2.4 Add the appropriate weight of sample (see list below) to the boat. (Weights may vary depending on the amount of sample material available)

Soil----------5 g
Sediment----5 g
Gravel-------30 g
Plant----------2 g
Compost-----5 g

9.2.5 Record the weight of the boat plus sample to 0.0001 g and record this as the gross weight (GW) in the appropriate area of the worksheet.

9.2.6 Place the boat containing the sample in an oven at 105° C and leave for 12-15 hours. Record on the worksheet, the date and time the samples were placed in the oven and its temperature at that time.

9.2.7 Remove the boat and allow to equilibrate to room temperature in a dessicator. Record on the worksheet, the date and time the samples were removed from the oven and its temperature at that time.

9.2.8 Remove from the dessicator and weigh the boat and dried sample. Record this weight to 0.0001 g as the dried weight (DW) in the appropriate area of the worksheet.

9.2.9 Calculate the percent moisture of the sample as shown on the worksheet and record the results in the appropriate areas. The formula for the calculation is:

$$\% \text{ Moisture} = \frac{(GW - DW) * 100}{(GW - TW)}$$

9.3 Approximate analyte retention times:

ANALYTE	MINUTES
2,6-Diamino-4-nitrotoluene	12.11
1,3,5-Trinitroso-1,3,5-triazacyclohexane	12.07
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	13.05
2,4-Diamino-6-nitrotoluene	13.53
1-Nitroso-3,5-dinitro-1,3,5-triazacyclohexane	16.11
Hexahydro-1,3,5-trinitro-1,3,5-triazine	15.78
1,3,5-Trinitrobenzene	18.30
1,3-Dinitrobenzene	20.44
3,5-Dinitroaniline	21.82
2,4,6-Trinitrotoluene	23.56
2-Amino-4,6-dinitrotoluene	24.58
4-Amino-2,6-dinitrotoluene	25.43
2,6-Dinitrotoluene	25.85
2,4-Dinitrotoluene	26.44
4,4',6,6'-Tetranitro-2,2'-azoxytoluene	32.69
2,4',6,6'-Tetranitro-2',4-azoxytoluene	32.96
2,2',6,6',Tetranitro-4,4'-azoxytoluene	33.21
2,2'-Dinitro-4,4'-azoxytoluene	34.33

ATTACHMENT 1

Preparation of Liquids for Explosives Analysis

Start Date:_____ **Spike s/n:**_____

End Date:_____ **Spike concentraton:**_____

Analyst:_____ **Matrix:** Aqueous Organic

Laboratory Number	Aliquot (ml)	Dilution Vol. (ml)	Aliquot (ml)	Dilution Vol. (ml)	1:1 Mixer Matrix		PTFE Filter	
					Water	AcCN	0.2 um	0.45um

QA/QC Samples

	Sample Vol. (ml)	Spike Vol. (ml)	Final Vol. (ml)		1:1 Mixer Matrix		PTFE Filter	
					Water	AcCN	0.2 um	0.45um
Method Blank								
LCS								
Matrix Spike								
Martix Spike Dupl.								

ATTACHMENT 2

Preconcentration of Liquids by SPE for Explosives Analysis

Start Date:_____ **Spike s/n:**_____

End Date:_____ **Spike concentraton:**_____

Analyst:_____

Laboratory Number	Sample Vol. (ml)	Eluant Vol. (ml)	Aliquot (ml)	Dilution Vol. (ml)	1:1 Mixer Matrix		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um

QA/QC Samples

	Spike Vol. (ml)	Final Vol. (ml)	Eluant Vol. (ml)		1:1 Mixer Matrix		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um
Method Blank								
LCS								

ATTACHMENT 3

Preparation of Solids for Explosives Analysis

Start Date:_____ **Spike s/n:**_____

End Date:_____ **Spike concentraton:**_____

Analyst:_____ **Matrix:** Soil Sediment Gravel

Laboratory Number	Sample Wt. (g)	Extraction Vol. (ml)	Aliquot (ml)	Dilution Vol. (ml)	1:1 Mixer Matrix		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um

QA/QC Samples

	Sample Wt. (g)	Spike Vol. (ml)	Final Vol. (ml)		1:1 Mixer Matrix		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um
Method Blank								
LCS								
Matrix Spike								
Martix Spike Dupl.								

ATTACHMENT 4

Preparation of Compost Leachates for Explosives Analysis

Start Date:_____ **Spike s/n:**_____

End Date:_____ **Spike concentraton:**_____

Analyst:_____

Laboratory Number	Sample Vol. (ml)	Salt Wt. (g)	Extract Vol. (ml)	Dilution Vol. (ml)	1:1 Mixer Matrix		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um

QA/QC Samples

	Sample Vol. (ml)	Salt Wt. (g)	Spike Vol. (ml)	Extract Vol. (ml)	1:1 Mixer Matrix		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um
Method Blank								
LCS								
Matrix Spike								

Preparation of Plants for Explosives Analysis

Start Date:_____

End Date:_____

Analyst:_____

Spike s/n:_____

Spike concentraton:_____

Spike Vol. (ml): MS_____ LCS_____

			Sonication Extraction								Acid Digestion				
			Sonication #1				Sonication #2								
Laboratory Number	Wet Wt. (g)	Dry Wt. (g)	AcCN Vol. (ml)	Eluant Vol. (ml)	Mixer Water	Filter 0.45um	AcCN Vol. (ml)	Eluant Vol. (ml)	Mixer Water	Filter 0.45um	Acid (ml)	Extract Used (ml)	Eluant (ml)	Mixer Water	Filter 0.45um

QA/QC Samples

			Sonication Extraction								Acid Digestion				
			Sonication #1				Sonication #2								
	Wet Wt. (g)	Dry Wt. (g)	AcCN Vol. (ml)	Eluant Vol. (ml)	Mixer Water	Filter 0.45um	AcCN Vol. (ml)	Eluant Vol. (ml)	Mixer Water	Filter 0.45um	Acid (ml)	Extract Used (ml)	Eluant (ml)	Mixer Water	Filter 0.45um
Method Blank															
LCS															
Matrix Spike															

Preparation of Compost for Explosives Analysis

Start Date:_____ Spike s/n:_____

End Date:_____ Spike concentraton:_____

Analyst:_____ Spike Vol. (ml): MS_____ LCS_____

			Sonication Extraction						Acid Digestion						
Laboratory Number	Sample Wt. (g)		AcCN Vol. (ml)		1:1 Mixer		PTFE Filter		Acid (ml)	Extract Used (ml)	Eluant (ml)	1:1 Mixer		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um				Water	$CaCl_2$	0.2 um	0.45um

QA/QC Samples

			Sonication Extraction						Acid Digestion						
	Sample Wt. (g)		AcCN Vol. (ml)		1:1 Mixer		PTFE Filter		Acid (ml)	Extract Used (ml)	Eluant (ml)	1:1 Mixer		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um				Water	$CaCl_2$	0.2 um	0.45um
Method Blank															
LCS															
Matrix Spike															
Matrix Spike dupl.															

ATTACHMENT 7

Percent Moisture by Oven Drying

Analyst:_____ **Sample Type:**_____

Initial date / time:_____ **Initial oven temp.:**_____

Final date / time:_____ **Final oven temp.:**_____

Laboratory Number	Tare Wt. (TW) Grams	Gross Wt. (GW) Grams	Dried Wt. (DW) Grams	Percent Moisture

Percent Moisture = $\frac{(GW - DW) * 100}{(GW - TW)}$