10301

DEMONSTRATION RESULTS OF PHYTOREMEDIATION OF EXPLOSIVES-CONTAMINATED GROUNDWATER USING CONSTRUCTED WETLANDS AT THE MILAN ARMY AMMUNITION PLANT, MILAN, TENNESSEE

Volume III of IV

Prepared for
U.S. ARMY ENVIRONMENTAL CENTER
Aberdeen Proving Ground, Maryland 21010-5401

Funded Through

ESTCP

Prepared by
Tennessee Valley Authority
Resource Management
Muscle Shoals, Alabama 35662-1010

December 1998

TVA Contract No. TV-88826V
Report No. SFIM-AEC-ET-CR-97059

Demonstration Results of
Phytoremediation of
Explosives-Contaminated Groundwater
Using
Constructed Wetlands
At The
Milan Army Ammunition Plant,
Milan, Tennessee

Volume III of IV

Prepared for
U.S. Army Environmental Center
Pollution Prevention and Environmental Technology Division
Aberdeen Proving Ground, MD 21010-5401
POC: Ms. Darlene F. Bader

Funded Through
U.S. Department of Defense
Environmental Security
Technology Certification Program

Prepared by

F. J. Sikora, R. A. Almond, L. L. Behrends, J. J. Hoagland,
D. A. Kelly, W. D. Phillips, W. J. Rogers, R. K. Summers,
F. C. Thornton, and J. R. Trimm
of the
Tennessee Valley Authority
Resource Management
Muscle Shoals, AL 35662-1010

and

Darlene F. Bader
of the
U.S. Army Environmental Center
Pollution Prevention and Environmental Technology Division
Aberdeen Proving Ground, MD 21010-5401

December 1998

SFIM-AEC-ET-CR-97059
TVA Contract No. TV-88826V

REPORT DOCUMENTATION PAGE	*Form Approved* *OMB No. 0704-0188*

. REPORT DATE *(DD-MM-YYYY)* December 1998	**2. REPORT TYPE** Final	**3. DATES COVERED** *(From - To)*

4. TITLE AND SUBTITLE
Demonstration Results of Phytoremediation of Explosives-Contaminated Groundwater Using Constructed Wetlands at the Milan Army Ammunition Plant, Milan, Tennessee

5a. CONTRACT NUMBER
TVA Contract No. TV-88826V

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
F. J. Sikora, R. A. Almond, L. L. Behrends, J. J. Hoagland, D. A. Kelly, W. D. Phillips, W. J. Rogers, R. K. Summers, F. C. Thornton, J. R. Trimm, and D. F. Bader

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Tennessee Valley Authority
TVA Reservation
Attn: CEB 4C-M
P. O. Box 1010
Muscle Shoals, Alabama 35662-1010

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
. S. Army Environmental Center
ttn: SFIM-AEC-ETD
5179 Hoadley Road
Aberdeen Proving Ground, MD 21010-5401

10. SPONSOR/MONITOR'S ACRONYM(S)
USAEC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
SFIM-AEC-ET-CR-97059

12. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution Unlimited, Approved for Public Release

13. SUPPLEMENTARY NOTES
4 Volumes

14. ABSTRACT
This report describes a demonstration of phytoremediation methods to remediate explosives-contaminated groundwater. The demonstration was conducted at the Milan Army Ammunition Plant near Milan, Tennessee, using a gravel-based subsurface flow wetland and a lagoon-based surface flow wetland. The report provides demonstration results as they are related to the effectiveness of the wetlands; and provides cost and other information necessary for the transfer of the technology to the user community. The report concludes that gravel-based wetlands could be used to remediate groundwater contaminated with a variety of explosives including TNT and RDX and that, based on the demonstration results, the use of lagoon-based wetlands could not be recommended.

15. SUBJECT TERMS
Phytoremediation of Explosives-Contaminated Groundwater in Constructed Wetlands

. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Darlene F. Bader
U	U	U	UU	1172	**19b. TELEPHONE NUMBER** *(Include area code)* 410-436-6861

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

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APPENDIX A

METHODS AND PROCEDURES

APPENDIX A-1

Procedure for Explosives: Method AP-0062

Tennessee Valley Authority

Specialty Laboratory of Land and Water Sciences and Remediation
Environmental Research Center
Muscle Shoals, AL 35662

Procedure Number : AP-0062

Title: Extraction, Preparation, and Analysis of Explosives and Their Degradation Products

Signature	Title	Date
Prepared by: W. David Phillips W. David Phillips	Chemist	4/14/98
Concurred: Eugene A. Zarate Eugene A. Zarate	Laboratory Section Leader	4/14/98
Concurred: William J. Rogers	QA Officer	4/13/98
Concurred:		
Approved: Kathy Taro for Joe Hoagland Joseph J. Hoagland	Manager	4/16/98

Revision	R0	R1				
Control Date	17-Oct-97	13-Apr-98				

Copy No: ___ has been issued to holder on ________

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 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 swirled 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 spike solution (8 component) at 2 µg/ml each analyte for use with water, soil, and sediment.

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

Use the calibration standard 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.

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 place 50.0 ml of HPLC water, add to this slowly and with swirling 50.0 ml 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 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 room temperature before use. This should not be kept for use for more than 2 days.

6.4.15 Aqueous sodium chloride solution

Weigh out 325 g of sodium chloride and place in a 1000 ml volumetric flask. Make flask to volume with HPLC water. Add a stir bar and place on magnetic stirrer until the solution is saturated (usually 30 minutes).

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.

6.5 Quality Control Sample Requirements

6.5.1 Every batch of samples (20 members or less) whose matrix is water, soil, 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

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, your name, the serial number of the spiking solution to be used, and the concentration of the spiking solution.

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 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 30 minutes.

7.2.1.5 Decant the supernatant to a properly labeled, glass vial (40 ml size) 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. Wrap flask with aluminum foil.

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. Wrap flask with aluminum foil.

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 is 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, your name, the serial number of the spiking solution to be used, and the concentration of the spiking solution.

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 50 ml volumetric flask, add approximately 25 ml of HPLC water, then add 1.0 ml of the aqueous spiking solution (see section 6.4.10). Make the flask to volume with HPLC water, stopper and mix thoroughly by inversion. Let stand in dark until needed.

Method blank - To a 50 ml volumetric flask, add 50 ml of HPLC water. Stopper and mix thoroughly by inversion. Place in dark until needed.

7.2.2.4 Fractionate an appropriate volume of sample to 40 ml vials with Teflon lined closures and centrifuge for 30 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 and connect the cartridge to the vacuum manifold. Condition the cartridge by passing 15 ml of acetonitrile through it (drip by gravity), followed by 30 ml of water at a rate of about 10 ml/minute using vacuum.

NOTE: In the following step, if the extract from this procedure is to be used for analyte identification only, approximately 60 ml of sample is used. If the extract is to be used for analyte quantitation, it is necessary to use a known volume aliquoted with a graduated cylinder (rinse cylinder 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 about 10 ml/min. Record this known or approximate volume in the appropriate area 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 water.

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 5.0 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 flask volume in the appropriate area on the worksheet.

7.2.2.15 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.

7.2.2.16 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 is 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 Obtain a "Percent Moisture" worksheet (Attachment 6). Record on this worksheet the laboratory number of the samples for this determination, the date, and your name.

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 Grind an appropriate quantity of air dried sample in an acetonitrile rinsed mortar. Pass the ground sample through a clean 30 mesh sieve onto clean aluminum foil. Place the sieved sample into a glass vial with Teflon-lined closures. Store the sample in the dark at room temperature 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, your name, the sample matrix, the serial number of the spiking solution to be used, and the concentration of the spiking solution.

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 16 ml glass vial, weigh out 2 grams of air dried, ground and sieved 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 16 ml glass vial, 2 grams of air dried, ground and sieved sample. Record the weight to the nearest 0.0001 g. Add 1.0 ml of the aqueous spiking solution (see section 6.4.10) to the sample, recap the vial and let the sample stand in the dark for 1 hour. Then add 9.0 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 16 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 aqueous spiking solution (see section 6.4.10) to the sample, recap the vial and let the sample stand in the dark for 1 hour. Then add 9.0 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 16 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 HPLC water to the sample, recap the vial and let the sample stand in the dark for 1 hour. Add 9.0 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 25° 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 pipetable supernatant to form.

If, at the end of the standing period, there is still a large volume of suspended particulates, it will be necessary to transfer the sample to a 40 ml vial and centrifuge it at 2000 rpm or greater for 30 minutes.

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 is 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 Obtain a "Percent Moisture" worksheet (Attachment 6). Record on this worksheet the laboratory number of the samples for this determination, the date, and your name.

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, your name, the sample matrix type the serial number of the spiking solution to be used, and the concentration of the spiking solution.

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 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 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 vial and 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 25° 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 be high enough to float the bottles or touch the lids of the sample bottles.

7.2.4.10 Remove 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 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 is 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 Retrieve the samples and allow to equilibrate to room temperature if necessary.

7.2.5.2 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, your name, the serial number of the spiking solution to be used, and the concentration of the spiking solution.

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.

7.2.5.3 Fractionate approximately 160 ml of the leachate sample into 40 ml glass vials with Teflon lined closures. Centrifuge the vials for 30 minutes at 2000 rpm or greater. After centrifugation, decant the total supernatant for one sample into a 250 ml Erlenmeyer flask.

7.2.5.4 Prepare a sodium chloride solution as per section 6.4.15 of this procedure.

7.2.5.5 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 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, 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 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 into second separatory funnel.

7.2.5.12 Add 84 ml (measure with 100 ml graduated cylinder) of salt solution (see step 7.2.5.4) to the second separatory funnel.

7.2.5.13 Shake the separatory funnel for 5 minutes and 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 to an 8 ml glass vial. Add 2 ml of calcium chloride solution (see section 6.4.16) to the vial, shake 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 is 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 fractions are injected and analyzed separately. 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, your name, the serial number of the spiking solution to be used, and the concentration of the spiking solution.

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 Obtain a "Percent Moisture" worksheet (Attachment 6). Record on this worksheet the laboratory number of the samples for this determination, the date, and your name.

7.2.6.4 Using acetonitrile rinsed scissors, or clean gloved hands, cut or tear approximately 30 g of plant tissue into coarse pieces onto clean aluminum foil.

7.2.6.5 Into a plastic boat, weigh out 20 g of plant tissue for the explosives extraction and record this weight on a sample worksheet.. Record this weight on the worksheet in the appropriate place.

7.2.6.6 Set aside enough 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.

7.2.6.7 Place the 20 grams of plant tissue into an acetonitrile rinsed and dried 400 ml glass beaker.

7.2.6.8 Add approximately 200 ml of HPLC water to the beaker. Swirl the plant tissue around for several seconds and then carefully decant the water into a proper waste receptacle. Do not allow any of the plant tissue to be lost.

7.2.6.9 Repeat step 7.2.6.8 twice more, then empty the contents of the beaker onto clean paper towels. Allow the tissue to adequately drain to the point that the tissue can be easily separated from the towels.

7.2.6.10 Place the washed and drained plant tissue in an acetonitrile rinsed ceramic mortar of appropriate size.

7.2.6.11 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.

7.2.6.12 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.

7.2.6.13 Using a powder funnel and a stainless steel spatula, quickly transfer the ground tissue from the mortar to an acetonitrile rinsed freeze dry flask (300 ml size).

7.2.6.14 Add 20 ml of HPLC water to the mortar and use to rinse any adhering tissue into the freeze dry flask.

7.2.6.15 Use a HPLC water wash-bottle and rinse all tissue adhering to the mortar, pestle and funnel into the freeze dry flask. Minimize the volume of water used for this step.

7.2.6.16 Use a stainless steel spatula and mix the water and frozen tissue in the freeze dry flask. This is done to keep the tissue mass from freezing into a block.

7.2.6.17 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 breakup the frozen tissue slurry.

7.2.6.18 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 probe totally and wash the parts thoroughly with detergent, followed by a rinse with distilled water, acetonitrile and then with HPLC water. Then reassemble the generator probe.

7.2.6.19 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.

7.2.6.20 Place a rubber cap, with its filter and glass adapter in place, on the freeze dry flask.

7.2.6.21 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.

7.2.6.22 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.

7.2.6.23 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.

7.2.6.24 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.

7.2.6.25 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.

7.2.6.26 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.

7.2.6.27 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.

7.2.6.28 Add 15.0 ml of acetonitrile to the vial and seal with Teflon-lined closure. Record this volume on the worksheet.

7.2.6.29 Suspend the vial in a sonicator bath whose temperature is controlled between 10° C and 25° 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.

7.2.6.30 Sonicate the samples for 18 hours.

7.2.6.31 Remove the sample vials from the sonicator and allow to stand in the dark for 15 minutes.

7.2.6.32 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.

7.2.6.34 Place the supernatant in a 250 ml Erlenmeyer flask containing 100 ml of HPLC water. Seal the flask with Parafilm and place flask in dark until ready to proceed with step 7.2.6.37.1.

7.2.6.35 Using the tissue pellet remaining from the previous step, repeat steps 7.2.6.28 through 7.2.6.34. Treat this as a separate fraction. Place the supernatant in a separate Erlenmeyer flask from the first fraction.

7.2.6.36 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.

7.2.6.36.1 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.

7.2.6.36.2 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.

7.2.6.36.3 Remove the vial from the sonicator bath and centrifuge at 2000 rpm or greater for 30 minutes.

7.2.6.36.4 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.

7.2.6.36.5 Seal the flask with Parafilm and place the neutralized supernatant in an explosion-proof refrigerator at 4° C for 12-15 hours.

7.2.6.36.6 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 7.2.6.36.8.

7.2.6.36.7 If the beaker contains no precipitate or suspension, proceed to step 7.2.6.37.

7.2.6.36.8 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.

7.2.6.36.9 Centrifuge 40 ml vial at 2000 rpm or greater for 30 minutes.

7.2.6.36.10 Remove the supernatant from the precipitate and add to the supernatant removed in step 7.2.6.36.6.

7.2.6.36.11 Place the accumulated supernatant in the dark until ready to proceed with step 7.2.6.37.

7.2.6.37 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, two Alumina-A SPE (each a 1 g size), 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.

7.2.6.38 Condition the cartridges by first pulling 20 ml of acetonitrile (at a flow rate of 2-4 ml/min.) through them, immediately followed by 30 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.

7.2.6.39 After the sample solution (and wash solution if necessary) has totally passed through the cartridge train, separate the 60 ml reservoir and Alumina-A cartridges from the Porapak-Rdx cartridge which will remain on the vacuum manifold. Dispose of the Alumina-A cartridges properly.

7.2.6.40 Now apply a strong vacuum to the Porapak-Rdx cartridge for about 5 minutes to remove residual water.

7.2.6.41 Remove the Porapak-Rdx cartridge from the vacuum manifold.

7.2.6.42 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.

7.2.6.43 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.

7.2.6.44 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.

7.2.6.45 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.

7.2.6.46 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.

7.2.6.47 Let the mixture stand in the dark at room temperature for 20 minutes.

7.2.6.48 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 is required, then two autosampler vials containing filtrate are required.

7.2.6.49 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 HPLC procedure

After preparation is completed and autosampler vials are filled; load the autosampler, enter the parameters noted below, and start the analysis.

7.2.7.1 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)

7.2.7.2 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 output momentary: No
Expel tube volume to vial: No
Pulsed start output: Yes

7.2.7.3 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 For vegetation: 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

7.3.5 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 5 - "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 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

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

10.0 ATTACHMENTS AND APPENDICES

10.1 Attachment 1
Worksheet - "Preparation of Liquids for Explosives Analysis"

Preparation of Liquids for Explosives Analysis

Start Date:____________________
End Date:____________________
Analyst:____________________

Spike s/n:____________________
Spike concentraton:____________________
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.								

10.2 Attachment 2
Worksheet - "Preconcentration of Liquids by SPE for Explosives Analysis"

Preconcentration of Liquids by SPE for Explosives Analysis

Start Date:____________________

End Date:____________________

Analyst:____________________

Spike s/n:____________________

Spike concentraton:____________________

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								

10.3 Attachment 3
Worksheet - "Preparation of Solids for Explosives Analysis"

Preparation of Solids for Explosives Analysis

Start Date:____________________
End Date:____________________
Analyst:____________________

Spike s/n:____________________
Spike concentraton:____________________
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.								

10.4 Attachment 4
Worksheet - "Preparation of Compost Leachates for Explosives Analysis"

Preparation of Compost Leachates for Explosives Analysis

Start Date:____________________
End Date:____________________
Analyst:____________________

Spike s/n:____________________
Spike concentraton:____________________

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								
Martix Spike Dupl.								

10.5 Attachment 5
Worksheet - "Preparation of Plant Tissue for Explosives Analysis"

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						
Laboratory Number	Wet Wt. (g)	Dry Wt. (g)	AcCN Vol. (ml)	Eluant 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						
	Wet Wt. (g)	Dry Wt. (g)	AcCN Vol. (ml)	Eluant 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															

10.6 Attachment 6
Worksheet - "Percent Moisture by Oven Drying"

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 $\frac{(GW - DW) * 100}{(GW - TW)}$

End of Procedure

APPENDIX A-2

Procedures for Metals: Method 200 Series

WP-0031
EPA Method 200.7 for Milan Samples
Metals Analysis by Inductively Coupled Plasma Spectroscopy

1.0 Procedure

1.1 Analyze samples from the Milan project for dissolved metals according to the attached procedure, EPA Method 200.7. The sample collector will have filtered and acidified the metals in the field.

1.2 Examine samples prior to analysis to determine if any precipitate was formed after filtration. If a precipitate is found, coordinate sample preparation and documentation with the metals area team leader.

1.3 The following minor changes to Method 200.7 apply.

1.3.1 Since there was no sample preparation, use no reagent blank.

1.3.2 Read calibration blanks, which are in the same matrix as the samples.

1.3.3 Analyze and report any field blanks submitted by the consignor as routine samples.

1.4 Prepare the interference check sample (IFC) to correspond to the sample matrix for the Milan samples. Determine this sample's composition by running a metals scan on typical samples. Analyze the interference check sample at the beginning of each analytical run.

Note: The only element present that was not part of the routine request list is sulfur. Match this amount in the IFC.

2.0 Recordkeeping

Maintain copies of machine printouts, calibration details, use of standard solutions, details of mixing quality control samples, percent recovery calculations, and any sample preparation worksheets from samples with precipitates.

2/20/98

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Inductively Coupled Plasma—Atomic Emission Spectrometric Method for Trace Element Analysis of Water and Wastes—Method 200.7

1. Scope and Application

1.1 This method may be used for the determination of dissolved, suspended, or total elements in drinking water, surface water, domestic and industrial wastewaters.

1.2 Dissolved elements are determined in filtered and acidified samples. Appropriate steps must be taken in all analyses to ensure that potential interference are taken into account. This is especially true when dissolved solids exceed 1500 mg/L. (See 5.)

1.3 Total elements are determined after appropriate digestion procedures are performed. Since digestion techniques increase the dissolved solids content of the samples, appropriate steps *must* be taken to correct for potential interference effects. (See 5.)

1.4 Table 1 lists elements for which this method applies along with recommended wavelengths and typical estimated instrumental detection limits using conventional pneumatic nebulization. Actual working detection limits are sample dependent and as the sample matrix varies, these concentrations may also vary. In time, other elements may be added as more information becomes available and as required.

1.5 Because of the differences between various makes and models of satisfactory instruments, no detailed instrumental operating instructions can be provided. Instead, the analyst is referred to the instructions provided by the manufacturer of the particular instrument.

2. Summary of Method

2.1 The method describes a technique for the simultaneous or sequential multielement determination of trace elements in solution. The basis of the method is the measurement of atomic emission by an optical spectroscopic technique. Samples are nebulized and the aerosol that is produced is transported to the plasma torch where excitation occurs. Characteristic atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma (ICP). The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes. The photocurrents from the photomultiplier tubes are processed and controlled by a computer system. A background correction technique is required to compensate for variable background contribution to the determination of trace elements. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result. The possibility of additional interferences named in 5.1 (and tests for their presence as described in 5.2) should also be recognized and appropriate corrections made.

3. Definitions

3.1 Dissolved — Those elements which will pass through a 0.45 μm membrane filter.

3.2 Suspended — Those elements which are retained by a 0.45 μm membrane filter.

3.3 Total — The concentration determined on an unfiltered sample following vigorous digestion (9.3), or the sum of the dissolved plus suspended concentrations. (9.1 plus 9.2.)

3.4 Total recoverable — The concentration determined on an unfiltered sample following treatment with hot, dilute mineral acid (9.4).

3.5 Instrumental detection limit — The concentration equivalent to a signal, due to the analyte, which is equal to three times the standard deviation of a series of ten replicate measurements of a reagent blank signal at the same wavelength.

3.6 Sensitivity — The slope of the analytical curve, i.e. functional relationship between emission intensity and concentration.

3.7 Instrument check standard — A multielement standard of known concentrations prepared by the analyst to monitor and verify instrument performance on a daily basis. (See 7.6.1)

3.8 Interference check sample — A solution containing both interfering and analyte elements of known concentration that can be used to verify background and interelement correction factors. (See 7.6.2)

3.9 Quality control sample — A solution obtained from an outside source having known, concentration values to be used to verify the calibration standards. (See 7.6.3)

3.10 Calibration standards — a series of know standard solutions used by the analyst for calibration of the instrument (i.e., preparation of the analytical curve). (See 7.4)

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

3.12 Reagent blank — A volume of deionized, distilled water containing the same acid matrix as the calibration standards carried through the entire analytical scheme. (See 7.5.2)

3.13 Calibration blank — A volume of deionized, distilled water acidified with HNO_3 and HCl. (See 7.5.1)

3.14 Method of standard addition — The standard addition technique involves the use of the unknown and the unknown plus a known amount of standard. (See 10.6.1)

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified (14.7, 14.8 and 14.9) for the information of the analyst.

5. Interferences

5.1 Several types of interference effects may contribute to inaccuracies in the determination of trace elements. They can be summarized as follows:

5.1.1 Spectral interferences can be categorized as 1) overlap of a spectral line from another element; 2) unresolved overlap of molecular band spectra; 3) background contribution from continuous or recombination phenomena; and 4) background contribution from stray light from the line emission of high concentration elements. The first of these effects can be compensated by utilizing a computer correction of the raw data, requiring the monitoring and measurement of the interfering element. The second effect may require selection of an alternate wavelength. The third and fourth effects can usually be compensated by a background correction adjacent to the analyte line. In addition, users of simultaneous multielement instrumentation must assume the responsibility of verifying the absence of spectral interference from an element that could occur in a sample but for which there is no channel in the instrument array. Listed in Table 2 are some interference effects for the recommended wavelengths given in Table 1. The data in Table 2 are intended for use only as a rudimentary guide for the indication of potential spectral interferences. For this purpose, linear relations between concentration and intensity for the analytes and the interferents can be assumed.

The interference information, which was collected at the Ames Laboratory,[1] is expressed at analyte concentration eqivalents (i.e. false analyte concentrations) arising from 100 mg/L of the interferent element. The suggested use of this information is as follows: Assume that arsenic (at 193.696 nm) is to be determined in a sample containing approximately 10 mg/L of aluminum. According to Table 2, 100 mg/L of aluminum would yield a false signal for arsenic equivalent to approximately 1.3 mg/L. Therefore, 10 mg/L of aluminum would result in a false signal for arsenic equivalent to approximately 0.13 mg/L. The reader is cautioned that other analytical systems may exhibit somewhat different levels of interference than those shown in Table 2, and that the interference effects must be evaluated for each individual system.

Only those interferents listed were investigated and the blank spaces in Table 2 indicate that measurable interferences were not observed for the interferent concentrations listed in Table 3. Generally, interferences were discernible if they produced peaks or background shifts corresponding to 2-5% of the peaks generated by the

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[1] Ames Laboratory, USDOE, Iowa State University, Ames Iowa 50011

analyte concentrations also listed in Table 3.

At present, information on the listed silver and potassium wavelengths are not available but it has been reported that second order energy from the [illegible]sium 383.231 nm wavelength [illegible]res with the listed potassium line at [illegible]6.491 nm.

5.1.2 Physical interferences are generally considered to be effects associated with the sample nebulization and transport processes. Such properties as change in viscosity and surface tension can cause significant inaccuracies especially in samples which may contain high dissolved solids and/or acid concentrations. The use of a peristaltic pump may lessen these interferences. If these types of interferences are operative, they must be reduced by dilution of the sample and/or utilization of standard addition techniques. Another problem which can occur from high dissolved solids is salt buildup at the tip of the nebulizer. This affects aersol flow-rate causing instrumental drift. Wetting the argon prior to nebulization, the use of a tip washer, or sample dilution have been used to control this problem. Also, it has been reported that better control of the argon flow rate improves instrument performance. This is accomplished with the use of mass flow controllers.

[illegible] *Chemical Interferences* are characterized by molecular compound formation, ionization effects and solute vaporization effects. Normally these effects are not pronounced with the ICP technique, however, if observed they can be minimized by careful selection of operating conditions (that is, incident power, observation position, and so forth), by buffering of the sample, by matrix matching, and by standard addition procedures. These types of interferences can be highly dependent on matrix type and the specific analyte element.

5.2 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 5.2.1 through 5.2.4, will ensure the analyst that neither positive nor negative interference effects are operative on any of the analyte elements thereby distorting the accuracy of the reported values.

[illegible] *Serial dilution*—If the analyte [illegible]ntration is sufficiently high (minimally a factor of 10 above the instrumental detection limit after dilution), an analysis of a dilution should agree within 5 % of the original determination (or within some acceptable control limit (14.3) that has been established for that matrix). If not, a chemical or physical interference effect should be suspected.

5.2.2 Spike addition—The recovery of a spike addition added at a minimum level of 10X the instrumental detection limit (maximum 100X) to the original determination should be recovered to within 90 to 110 percent or within the established control limit for that matrix. If not, a matrix effect should be suspected. The use of a standard addition analysis procedure can usually compensate for this effect. *Caution:* The standard addition technique does not detect coincident spectral overlap. If suspected, use of computerized compensation, an alternate wavelength, or comparison with an alternate method is recommended. (See 5.2.3)

5.2.3 Comparison with alternate method of analysis—When investigating a new sample matrix, comparison tests may be performed with other analytical techniques such as atomic absorption spectrometry, or other approved methodology.

5.2.4 Wavelength scanning of analyte line region—If the appropriate equipment is available, wavelength scanning can be performed to detect potential spectral interferences.

6. Apparatus

6.1 Inductively Coupled Plasma-Atomic Emission Spectrometer.

6.1.1 Computer controlled atomic emission spectrometer with background correction.

6.1.2 Radiofrequency generator.

6.1.3 Argon gas supply, welding grade or better.

6.2 Operating conditions — Because of the differences between various makes and models of satisfactory instruments, no detailed operating instructions can be provided. Instead, the analyst should follow the instructions provided by the manufacturer of the particular instrument. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be investigated and established for each individual analyte line on that particular instrument. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions used satisfy the analytical requirements and to maintain quality control data confirming instrument performance and analytical results.

7. Reagents and standards

7.1 Acids used in the preparation of standards and for sample processing must be ultra-high purity grade or equivalent. Redistilled acids are acceptable.

7.1.1 Acetic acid, conc. (sp gr 1.06).

7.1.2 Hydrochloric acid, conc. (sp gr 1.19).

7.1.3 Hydrochloric acid, (1+1): Add 500 mL conc. HCl (sp gr 1.19) to 400 mL deionized, distilled water and dilute to 1 liter.

7.1.4 Nitric acid, conc. (sp gr 1.41).

7.1.5 Nitric acid,(1+1): Add 500 mL conc. HNO_3 (sp. gr 1.41) to 400 mL deionized, distilled water and dilute to 1 liter.

7.2 *Dionized, distilled water:* Prepare by passing distilled water through a mixed bed of cation and anion exchange resins. Use deionized, distilled water for the preparation of all reagents, calibration standards and as dilution water. The purity of this water must be equivalent to ASTM Type II reagent water of Specification D 1193 (14.6).

7.3 *Standard stock solutions* may be purchased or prepared from ultra high purity grade chemicals or metals. All salts must be dried for 1 h at 105°C unless otherwise specified. (CAUTION: Many metal salts are extremely toxic and may be fatal if swallowed. Wash hands thoroughly after handling.) Typical stock solution preparation procedures follow:

7.3.1 Aluminum solution, stock, 1 mL = 100 µg Al: Dissolve 0.100 g of aluminum metal in an acid mixture of 4 mL of (1+1) HCl and 1 mL of conc. HNO_3 in a beaker. Warm gently to effect solution. When solution is complete, transfer quantitatively to a liter flask, add an additional 10 mL of (1+1) HCl and dilute to 1,000 mL with deionized, distilled water.

7.3.2 Antimony solution stock, 1 mL = 100 µg Sb: Dissolve 0.2669 g $K(SbO)C_4H_4O_6$ in deionized distilled water, add 10 mL (1+1) HCl and dilute to 1000 mL with deionized, distilled water.

7.3.3 Arsenic solution, stock, 1 mL = 100 µg As: Dissolve 0.1320 g of As_2O_3 in 100 mL of deionized, distilled water containing 0.4 g NaOH. Acidify the solution with 2 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.4 Barium solution, stock, 1 mL = 100 µg Ba: Dissolve 0.1516 g $BaCl_2$ (dried at 250°C for 2 hrs) in 10 mL deionized, distilled water with 1 mL (1+1) HCl. Add 10.0 mL (1+1) HCl and dilute to 1,000 mL with deionized, distilled water.

7.3.5 Beryllium solution, stock, 1 mL = 100 µg Be: *Do not dry.* Dissolve 1.966 g $BeSO_4 \cdot 4\ 4H_2O$, in deionized, distilled water, add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.6 Boron solution, stock, 1 mL = 100 µg B: *Do not dry.* Dissolve 0.5716 g anhydrous H_3BO_3 in deionized distilled water dilute to 1,000 mL. Use a reagent meeting ACS specifications, keep the bottle tightly stoppered and store in a desiccator to prevent the entrance of atmospheric moisture.

7.3.7 Cadmium solution, stock, 1 mL = 100 µg Cd: Dissolve 0.1142 g CdO in a minimum amount of (1+1) HNO_3. Heat to increase rate of dissolution. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.8 Calcium solution, stock, 1 mL = 100 µg Ca: Suspend 0.2498 g $CaCO_3$ dried at 180°C for 1 h before weighing in deionized, distilled water and dissolve cautiously with a minimum amount of (1+1) HNO_3. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.9 Chromium solution, stock, 1 mL = 100 µg Cr: Dissolve 0.1923 g of CrO_3 in deionized, distilled water. When solution is complete, acidify with 10 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.10 Cobalt solution, stock, 1 mL = 100 µg Co: Dissolve 0.1000 g of cobalt metal in a minimum amount of (1+1) HNO_3. Add 10.0 mL (1+1) HCl and dilute to 1,000 mL with deionized, distilled water.

7.3.11 Copper solution, stock, 1 mL = 100 µg Cu: Dissolve 0.1252 g CuO in a minimum amount of (1+1) HNO_3 Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.12 Iron solution, stock, 1 mL = 100 µg Fe: Dissolve 0.1430 g Fe_2O_3 in a warm mixture of 20 mL (1+1) HCl and 2 mL of conc. HNO_3. Cool, add an additional 5 mL of conc. HNO_3 and dilute to 1000 mL with deionized, distilled water.

7.3.13 Lead solution, stock, 1 mL = 100 µg Pb: Dissolve 0.1599 g $Pb(NO_3)_2$ in minimum amount of (1+1) HNO_3. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.14 Magnesium solution, stock, 1 mL = 100 µg Mg: Dissolve 0.1658 g MgO in a minimum amount of (1+1) HNO_3. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.15 Manganese solution, stock, 1 mL = 100 µg Mn: Dissolve 0.1000 g of manganese metal in the acid mixture 10 mL conc. HCl and 1 mL conc. HNO_3, and dilute to 1,000 mL with deionized, distilled water.

7.3.16 Molybdenum solution, stock, 1 mL = 100 µg Mo: Dissolve 0.2043 g $(NH_4)_2MoO_4$ in deionized, distilled water and dilute to 1,000 mL.

7.3.17 Nickel solution, stock, 1 mL = 100 µg Ni: Dissolve 0.1000 g of nickel metal in 10 mL hot conc. HNO_3, cool and dilute to 1,000 mL with deionized, distilled water.

7.3.18 Potassium solution, stock, 1 mL = 100 µg K: Dissolve 0.1907 g KCl, dried at 110°C, in deionized, distilled water dilute to 1,000 mL.

7.3.19 Selenium solution, stock, 1 mL = 100 µg Se: *Do not dry.* Dissolve 0.1727 g H_2SeO_3 (actual assay 94.6%) in deionized, distilled water and dilute to 1,000 mL.

7.3.20 Silica solution, stock, 1 mL = 100 µg SiO_2: *Do not dry.* Dissolve 0.4730 g $Na_2SiO_3 \cdot 9H_2O$ in deionized, distilled water Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.21 Silver solution, stock, 1 mL = 100 µg Ag: Dissolve 0.1575 g $AgNO_3$ in 100 mL of deionized, distilled water and 10 mL conc. HNO_3. Dilute to 1,000 mL with deionized, distilled water

7.3.22 Sodium solution, stock, 1 mL = 100 µg Na: Dissolve 0.2542 g NaCl in deionized, distilled water. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water

7.3.23 Thallium solution, stock, 1 mL = 100 µg Tl: Dissolve 0.1303 g $TlNO_3$ in deionized, distilled water. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.24 Vanadium solution, stock, 1 mL = 100 µg V: Dissolve 0.2297 NH_4VO_3 in a minimum amount of conc. HNO_3. Heat to increase rate of dissolution. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.25 Zinc solution, stock, 1 mL = 100 µg Zn: Dissolve 0.1245 g ZnO in a minimum amount of dilute HNO_3. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.4 Mixed calibration standard solutions—Prepare mixed calibration standard solutions by combining appropriate volumes of the stock solutions in volumetric flasks. (See 7.4.1 thru 7.4.5) Add 2 mL of (1+1) HCl and dilute to 100 mL with deionized, distilled water. (See Notes 1 and 6.) 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 that the elements are compatible and stable. Transfer the mixed standard solutions to a FEP fluorocarbon or unused polyethylene bottle for storage. Fresh mixed standards should be prepared as needed with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample and monitored weekly for stability (See 7.6.3). Although not specifically required, some typical calibration standard combinations follow when using those specific wavelengths listed in Table 1.

7.4.1 Mixed standard solution I—Manganese, beryllium, cadmium, lead, and zinc.

7.4.2 Mixed standard solution II—Barium, copper, iron, vanadium, and cobalt.

7.4.3 Mixed standard solution III—Molybdenum, silica, arsenic, and selenium

7.4.4 Mixed standard solution IV—Calcium, sodium, potassium, aluminum, chromium and nickel

7.4.5 Mixed standard solution V—Antimony, boron, magnesium, silver, and thallium.

NOTE 1: If the addition of silver to the recommended acid combination results in an initial precipitation, [illegible] 15 mL of deionized distilled [illegible]r and warm the flask until the solution clears. Cool and dilute to 100 mL with deionized, distilled 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.

7.5 Two types of blanks are required for the analysis. The calibration blank (3.13) is used in establishing the analytical curve while the reagent blank (3.12) is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

7.5.1 The calibration blank is prepared by diluting 2 mL of (1+1) HNO$_3$ and 10 mL of (1+1) HCl to 100 mL with deionized, distilled water. (See Note 6.) Prepare a sufficient quantity to be used to flush the system between standards and samples.

7.5.2 The reagent blank must con-contain all the reagents and in the same volumes as used in the pro[illegible]ing of the samples. The reagent [illegible] must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

7.6 In addition to the calibration standards, an instrument check standard (3.7), an interference check sample (3.8) and a quality control sample (3.9) are also required for the analyses.

7.6.1 The *instrument check standard* is prepared by the analyst by combining compatible elements at a concentration equivalent to the midpoint of their respective calibration curves. (See 12.1.1)

7.6.2 The *interference check sample* is prepared by the analyst in the following manner. Select a representative sample which contains minimal concentrations of the analytes of interest by known concentration of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at the [illegible]oximate concentration of either [illegible] μg/L or 5 times the estimated detection limits given in Table 1. (For effluent samples of expected high concentrations, spike at an appropriate level.) If the type of samples analyzed are varied, a synthetically prepared sample may be used if the above criteria and intent are met. A limited supply of a synthetic interference check sample will be available from the Quality Assurance Branch of EMSL-Cincinnati. (See 12.1.2)

7.6.3 The *quality control sample* should be prepared in the same acid matrix as the calibration standards at a concentration near 1 mg/L and in accordance with the instructions provided by the supplier. The Quality Assurance Branch of EMSL-Cincinnati will either supply a quality control sample or information where one of equal quality can be procured. (See 12.1.3)

8. Sample handling an preservation

8.1 For the determination of trace elements, contamination and loss are of prime concern. Dust in the laboratory environment, impurities in reagents and impurities on laboratory apparatus which the sample contacts are all sources of potential contamination. Sample containers can introduce either positive or negative errors in the measurement of trace elements by (a) contributing contaminants through leaching or surface desorption and (b) by depleting concentrations through adsorption. Thus the collection and treatment of the sample prior to analysis requires particular attention. Laboratory glassware including the sample bottle (whether polyethylene, polyproplyene or FEP-fluorocarbon) should be thoroughly washed with detergent and tap water; rinsed with (1+1) nitric acid, tap water, (1+1) hydrochloric acid, tap and finally deionized, distilled water in that order (See Notes 2 and 3).

NOTE 2: Chromic acid may be useful to remove organic deposits from glassware; however, the analyst should be be cautioned that the glassware must be thoroughly rinsed with water to remove the last traces of chromium. This is especially important if chromium is to be included in the analytical scheme. A commercial product, NOCHROMIX, available from Godax Laboratories, 6 Varick St., New York, NY 10013, may be used in place of chromic acid. Chomic acid should not be used with plastic bottles.

NOTE 3: If it can be documented through an active analytical quality control program using spiked samples and reagent blanks, that certain steps in the cleaning procedure are not required for routine samples, those steps may be eliminated from the procedure.

8.2 Before collection of the sample a decision must be made as to the type of data desired, that is dissolved, suspended or total, so that the appropriate preservation and pretreatment steps may be accomplished. Filtration, acid preservation, etc., are to be performed at the time the sample is collected or as soon as possible thereafter.

8.2.1 For the determination of dissolved elements the sample must be filtered through a 0.45-μm membrane filter as soon as practical after collection. (Glass or plastic filtering apparatus are recommended to avoid possible contamination.) Use the first 50-100 mL to rinse the filter flask. Discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) HNO$_3$ to a pH of 2 or less. Normally, 3 mL of (1+1) acid per liter should be sufficient to preserve the sample.

8.2.2 For the determination of suspended elements a measured volume of unpreserved sample must be filtered through a 0.45-μm membrane filter as soon as practical after collection. The filter plus suspended material should be transferred to a suitable container for storage and/or shipment. No preservative is required.

8.2.3 For the determination of total or total recoverable elements, the sample is acidified with (1+1) HNO$_3$ to pH 2 or less as soon as possible, preferable at the time of collection. The sample is not filtered before processing.

9. Sample Preparation

9.1 For the determinations of dissolved elements, the filtered, preserved sample may often be analyzed as received. The acid matrix and concentration of the samples and calibration standards must be the same. (See Note 6.) If a precipitate formed upon acidification of the sample or during transit or storage, it must be redissolved before the analysis by adding additional acid and/or by heat as described in 9.3.

9.2 For the determination of suspended elements, transfer the membrane filter containing the insoluble material to a 150-mL Griffin beaker and add 4 mL conc. HNO$_3$. Cover the beaker with a watch glass and heat gently. The wam acid will soon dissolve the membrane.

Increase the temperature of the hot plate and digest the material. When the acid has nearly evaporated, cool the beaker and watch glass and add another 3 mL of conc. HNO_3. Cover and continue heating until the digestion is complete, generally indicated by a light colored digestate. Evaporate to near dryness (2 mL), cool, add 10 mL HCl (1+1) and 15 mL deionized, distilled water per 100 mL dilution and warm the beaker gently for 15 min. to dissolve any precipitated or residue material. Allow to cool, wash down the watch glass and beaker walls with deionized distilled water and filter the sample to remove insoluble material that could clog the nebulizer. (See Note 4.) Adjust the volume based on the expected concentrations of elements present. This volume will vary depending on the elements to be determined (See Note 6). The sample is now ready for analysis. Concentrations so determined shall be reported as "suspended."

NOTE 4: In place of filtering, the sample after diluting and mixing may be centrifuged or allowed to settle by gravity overnight to remove insoluble material.

9.3 For the determination of total elements, choose a measured, volume of the well mixed acid preserved sample appropriate for the expected level of elements and transfer to a Griffin beaker. (See Note 5.) Add 3 mL of conc. HNO_3. Place the beaker on a hot plate and evaporate to near dryness cautiously, making certain that the sample does not boil and that no area of the bottom of the beaker is allowed to go dry. Cool the beaker and add another 5 mL portion of conc. HNO_3. Cover the beaker with a watch glass and return to the hot plate. Increase the temperature of the hot plate so that a gentle reflux action occurs. Continue heating, adding additional acid as necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change in appearance with continued refluxing.) Again, evaporate to near dryness and cool the beaker. Add 10 mL of 1+1 HCl and 15 mL of deionized, distilled water per 100 mL of final solution and warm the beaker gently for 15 min. to dissolve any precipitate or residue resulting from evaporation. Allow to cool, wash down the beaker walls and watch glass with deionized distilled water and filter the sample to remove insoluble material that could clog the nebulizer. (See Note 4.) Adjust the sample to a predetermined volume based on the expected concentrations of elements present. The sample is now ready for analysis (See Note 6). Concentrations so determined shall be reported as "total."

NOTE 5: If low determinations of boron are critical, quartz glassware should be use.

NOTE 6: If the sample analysis solution has a different acid concentration from that given in 9.4, but does not introduce a physical interference or affect the analytical result, the same calibration standards may be used.

9.4 For the determination of total recoverable elements, choose a measured volume of a well mixed, acid preserved sample appropriate for the expected level of elements and transfer to a Griffin beaker. (See Note 5.) Add 2 mL of (1+1) HNO_3 and 10 mL of (1+1) HCl to the sample and heat on a steam bath or hot plate until the volume has been reduced to near 25 mL making certain the sample does not boil. After this treatment, cool the sample and filter to remove insoluble material that could clog the nebulizer. (See Note 4.) Adjust the volume to 100 mL and mix. The sample is now ready for analysis. Concentrations so determined shall be reported as "total."

10. Procedure

10.1 Set up instrument with proper operating parameters established in 6.2. The instrument must be allowed to become thermally stable before beginning. This usually requires at least 30 min. of operation prior to calibration.

10.2 Initiate appropriate operating configuration of computer.

10.3 Profile and calibrate instrument according to instrument manufacturer's recommended procedures, using the typical mixed calibration standard solutions described in 7.4. Flush the system with the calibration blank (7.5.1) between each standard. (See Note 7.) (The use of the average intensity of multiple exposures for both standardization and sample analysis has been found to reduce random error.)

NOTE 7: For boron concentrations greater than 500 µg/L extended flush times of 1 to 2 min. may be required.

10.4 Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than ± 5 percent (or the established control limits whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this condition.

10.5 Begin the sample run flushing the system with the calibration blank solution (7.5.1) between each sample. (See Note 7.) Analyze the instrument check standard (7.6.1) and the calibration blank (7.5.1) each 10 samples.

10.6 If it has been found that method of standard addition are required, the following procedure is recommended.

10.6.1 The standard addition technique (14.2) involves preparing new standards in the sample matrix by adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal thus producing a different slope from that of the calibration standards. It will not correct for additive interference which causes a baseline shift. The simplest version of this technique is the single-addition method. The procedure is as follows. Two identical aliquots of the sample solution, each of volume V_x, are taken. To the first (labeled A) is added a small volume V_s of a standard analyte solution of concentration c_s. To the second (labeled B) is added the same volume V_s of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration c_x is calculated:

$$c_X = \frac{S_B V_S c_S}{(S_A - S_B)\, V_X}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_S and c_S should be chosen so that S_A is roughly twice S_B on the average. It is best if V_S is made much less than V_X, and thus c_S is much greater than c_X, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results from this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond the same as the analyte in the sample.

3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

[illegible] Calculation

[illegible] Reagent blanks (7.5.2) should be subtracted from all samples. This is particularly important for digested samples requiring large quantities of acids to complete the digestion.

11.2 If dilutions were performed, the appropriate factor must be applied to sample values.

11.3 Data should be rounded to the thousandth place and all results should be reported in mg/L up to three significant figures.

12. Quality Control (Instrumental)

12.1 Check the instrument standardization by analyzing appropriate quality control check standards as follow:

12.1.1 Analyze an appropriate instrument check standard (7.6.1) containing the elements of interest at a frequency of 10%. This check standard is used to determine instrument drift. If agreement is not [illegible]in ±5% of the expected values or [illegible] the established control limits, [illegible]ever is lower, the analysis is out of control. The analysis should be terminated, the problem corrected, and the instrument recalibrated.

Analyze the calibration blank (7.5.1) at a frequency of 10%. The result should be within the established control limits of two standard deviations of the mean value. If not, repeat the analysis two more times and average the three results. If the average is not within the control limit, terminate the analysis, correct the problem and recalibrate the instrument.

12.1.2 To verify interelement and background correction factors analyze the interference check sample (7.6.2) at the beginning, end, and at periodic intervals throughout the sample run. Results should fall within the established control limits of 1.5 times the standard deviation of the mean value. If not, terminate the analysis, correct the problem and recalibrate the instrument.

12.1.3 A quality control sample (7.6.3) obtained from an outside [illegible]ce must first be used for the [illegible]l verification of the calibration standards. A fresh dilution of this sample shall be anlayzed every week thereafter to monitor their stability. If the results are not within ±5% of the true value listed for the control sample, prepare a new calibration standard and recalibrate the instrument. If this does not correct the problem, prepare a new stock standard and a new calibration standard and repeat the calibration.

Precision and Accuracy

13.1 In an EPA round robin phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been dosed with various metal concentrates. Table 4 lists the true value, the mean reported value and the mean % relative standard deviation.

References

1. Winge, R.K., V.J. Peterson, and V.A. Fassel, "Inductively Coupled Plasma-Atomic Emission Spectroscopy: Prominent Lines." EPA-600/4-79-017.

2. Winefordner, J.D., "Trace Analysis: Spectroscopic Methods for Elements," *Chemical Analysis,* Vol. *46*, pp. 41-42.

3. Handbook for Analytical Quality Control in Water and Wastewater Laboratories, EPA-600/4-79-019.

4. Garbarino, J.R. and Taylor, H.E., "An Inductively-Coupled Plasma Atomic Emission Spectrometric Method for Routine Water Quality Testing," Applied Spectroscopy *33*, No. 3(1979).

5. "Methods for Chemical Analysis of Water and Wastes," EPA-600/4-79-020.

6. Annual Book of ASTM Standards, Part 31

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

8. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

9. "Safety in Academic Chemistry Laboratories, American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979

Table 1. Recommended Wavelengths [1] and Estimated Instrumental Detection Limits

Element	Wavelength, nm	Estimated detection limit, μg/L[2]
Aluminum	308.215	45
Arsenic	193.696	53
Antimony	206.833	32
Barium	455.403	2
Beryllium	313.042	0.3
Boron	249.773	5
Cadmium	226.502	4
Calcium	317.933	10
Chromium	267.716	7
Cobalt	228.616	7
Copper	324.754	6
Iron	259.940	7
Lead	220.353	42
Magnesium	279.079	30
Manganese	257.610	2
Molybdenum	202.030	8
Nickel	231.604	15
Potassium	766.491	see[3]
Selenium	196.026	75
Silica (SiO_2)	288.158	58
Silver	328.068	7
Sodium	588.995	29
Thallium	190.864	40
Vanadium	292.402	8
Zinc	213.856	2

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[1] The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference. (See 5.1.1.).

[2] The estimated instrumental detection limits as shown are taken from "Inductively Coupled Plasma-Atomic Emission Spectroscopy-Prominent Lines, "EPA-600/4-79-017. They are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

[3] Highly dependent on operating conditions and plasma position.

Table 2. Analyte Concentration Equivalents (mg/L) Arising From Interferents at the 100 mg/L Level

Analyte	Wavelength, nm	Interferent									
		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	—	—	—	.25	0.45
[illegible]ic	193.696	1.3	—	0.44	—	—	—	—	—	—	1.1
Barium	455.403	—	—	—	—	—	—	—	—	—	—
Beryllium	313.042	—	—	—	—	—	—	—	—	0.04	0.05
Boron	249.773	0.04	—	—	—	0.32	—	—	—	—	—
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	—	—	—	—	—
Silicon	288.158	—	—	0.07	—	—	—	—	—	—	0.01
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	—	—

3. Interferent and Analyte Elemental Concentrations Used for Interference Measurements in Table 2.

Analytes	(mg/L)	Interferents	(mg/L)
Al	10	Al	1000
As	10	Ca	1000
B	10	Cr	200
Ba	1	Cu	200
Be	1	Fe	1000
Ca	1	Mg	1000
Cd	10	Mn	200
Co	1	Ni	200
Cr	1	Ti	200
Cu	1	V	200
Fe	1		
Mg	1		
Mn	1		
Mo	10		
Na	10		
Ni	10		
Pb	10		
Sb	10		
Se	10		
Si	1		
Tl	10		
V	1		
Zn	10		

Table 4. *ICP Precision and Accuracy Data*

Element	Sample # 1 True Value µg/L	Sample # 1 Mean Reported Value µg/L	Sample # 1 Mean Percent RSD	Sample #2 True Value µg/L	Sample #2 Mean Reported Value µg/L	Sample #2 Mean Percent RSD	Sample #3 True Value µg/L	Sample #3 Mean Reported Value µg/L	Sample #3 Mean Percent RSD
Be	750	733	6.2	20	20	9.8	180	176	5.2
Mn	350	345	2.7	15	15	6.7	100	99	3.3
V	750	749	1.8	70	69	2.9	170	169	1.1
As	200	208	7.5	22	19	23	60	63	17
Cr	150	149	3.8	10	10	18	50	50	3.3
Cu	250	235	5.1	11	11	40	70	67	7.9
Fe	600	594	3.0	20	19	15	180	178	6.0
Al	700	696	5.6	60	62	33	160	161	13
Cd	50	48	12	2.5	2.9	16	14	13	16
Co	500	512	10	20	20	4.1	120	108	21
Ni	250	245	5.8	30	28	11	60	55	14
Pb	250	236	16	24	30	32	80	80	14
Zn	200	201	5.6	16	19	45	80	82	9.4
Se	40	32	21.9	6	8.5	42	10	8.5	8.3

Not all elements were analyzed by all laboratories.

APPENDIX A-3

Procedure for Biochemical Oxygen Demand: Method 405.1

TENNESSEE VALLEY AUTHORITY

NO.: AP-0032

CHEMICAL AND ENVIRONMENTAL ANALYSIS SECTION

TITLE: BIOCHEMICAL OXYGEN DEMAND, 5-DAY

	Signature	Title	Date
Prepared by:	Sammie C. Smith Sammie C. Smith	Research Chemist	12/16/91
Concurred:	William J. Rogers William J. Rogers	QA/QC Coordinator	12/17/91
Concurred:			
Concurred:			
Concurred:			
Concurred:			
Approved:	B. Paul Bernauer B. Paul Bernauer	Supervisor, Chemical and Environmental Analysis Section	12-17-91

REVISION	RO	R1	R2		
CONTROL DATE:	05/31/90	01/04/91	12/20/91		

COPY NO.:__________ HAS BEEN ISSUED TO HOLDER ON __________

TVA 17207 (OPM-8-83)

FORMAT FOR RCFs

1.0 Reason for Change

2.0 Changes This Revision

Page	Action
15	Replace

3.0 Summary of Changes

Page	Action	Revision	Date
15	Replace 7.5	02	12/20/91

TITLE:	NO.:	PAGE:
BIOCHEMICAL OXYGEN DEMAND, 5-DAY	AP-0032 DATE: 12/20/91	1 of 18

1.0 PURPOSE

This procedure provides a method for determining the relative oxygen requirements of municipal and industrial wastewater.

2.0 SCOPE

The Biochemical Oxygen Demand (BOD5) test is an empirical bioassay-type procedure which measures the dissolved oxygen consumed by microbial life while assimilating and oxidizing the organic matter present. The standard test conditions include dark incubation at 20°C for five days.

3.0 SUMMARY

The sample of waste, or an appropriate dilution, is incubated for five days at 20°C in the dark. The reduction in dissolved oxygen (DO) concentration during the incubation period yields a measure of the (BOD5).

4.0 REFERENCES

4.1 U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastewater, EPA-600/4-79-020, pp. 405.1-1 - 405.1-2, 1983.

4.2 American Public Health Association, Standard Methods For The Examination of Water and Wastewater, Seventeenth Edition, pp. 5-2 - 5-10, 1989.

5.0 RESPONSIBILITIES

5.1 The Laboratory Supervisor, or his designee, shall ensure that this procedure is followed during the analysis of all samples for (BOD5).

5.2 The Laboratory Shift Supervisor/Laboratory Group Leader, or his designee, shall delegate the performance of this procedure to personnel experienced with this procedure.

5.3 The analyst shall follow this procedure and report any abnormal results or nonconformance to the Laboratory Shift Supervisor/Laboratory Group Leader.

6.0 PROCEDURE/REQUIREMENTS

6.1 Prerequisites

6.1.1 Store a container (no more than two-thirds full, with cotton plug not capped) of deionized distilled water, free of organic matter, in the incubator set at 20°C for approximately 24 hours before beginning the BOD_5 test.

6.1.2 Samples containing caustic alkalinity or acidity: Neutralize samples to pH of 6.5 to 7.5 with a solution of sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH) of such strength that the quantity of reagent does not dilute the sample by more than 0.5%.

6.1.3 Samples supersaturated with DO: Samples containing more than 9 mg DO/L at 20°C may be encountered in cold waters or in waters where photosynthesis occurs. To prevent loss of oxygen during incubation of such samples, reduce DO to saturated at 20°C by bringing sample to about 20°C in partially filled bottle while agitating by vigorous shaking or by aerating with clean, filtered compressed air.

6.1.4 Sample temperature adjustment: Bring samples to 20 ± 1°C before making dilutions.

6.2 Limitations and Actions

6.2.1 Grab samples: If analysis is begun within two hours of collection, cold storage is unnecessary . If analysis is not started within 2 hours of sample collection, keep sample at or below 4°C from the time of collection. Begin analysis within 6 hours of collection; when this is not possible because the sampling site is distant from the laboratory, store at or below 4°C and report length and temperature of storage with the results. In no case, start analysis more than 24 hours after grab sample collection. When samples are to be used for regulatory purposes make every effort to deliver samples for analysis within 6 hours of collection.

6.2.2 Composite samples: Keep samples at or below 4°C during compositing. Limit compositing period to 24 hours. Use the same criteria as for storage of grab samples, starting the measurement of holding time from end of compositing period. State storage time and conditions as part of the results.

6.3 Requirements

6.3.1 Apparatus/Equipment

NOTE: All new and used glassware must be thoroughly cleaned with a detergent, rinsed with deionized distilled water, and allowed to drain before use.

6.3.1.1 Incubator: Thermostatically controlled at 20 ± 1°C.

6.3.1.2 BOD bottles: 300 mL with stoppers and caps.

6.3.1.3 Graduated cylinders: Various sizes.

6.3.1.4 Volumetric pipets: Various sizes.

6.3.1.5 Dissolved oxygen meter with BOD probe.

6.3.1.6 Analytical balance: Capable of weighing 0.1 mg.

6.3.2 Reagents and Standards

<u>NOTE</u>: Discard any of the following reagents if there is any sign of biological growth in the stock bottle.

6.3.2.1 Buffer nutrient pillows: 3-mL size prepares 3 liters of dilution water. May be obtained from Hach Company (Catalog No. 14861-98).

6.3.2.2 Phosphate buffer solution: Dissolve 8.5-grams potassium phosphate, monobasic (KH_2PO_4), 21.75-grams potassium phosphate, diabasic ($KHPO_4$), 33.4-grams sodium phosphate, diabasic ($Na_2HPO_4 \cdot 7H_2O$), and 1.7-grams ammonium chloride (NH_4Cl) in about 500 mL of deionized distilled water and dilute with deionized distilled water to 1 liter. Mix well. The pH should be 7.2 without further adjustment.

6.3.2.3 Magnesium sulfate solution: Dissolve 22.5 grams of magnesium sulfate ($MgSO_4 \cdot 7H_2O$) in deionized distilled water and dilute to 1 liter.

6.3.2.4 Calcium chloride solution: Dissolve 27.5 grams of calcium chloride ($CaCl_2$) in deionized distilled water and dilute to 1 liter.

6.3.2.5 Ferric chloride solution: Dissolve 0.25 grams of ferric chloride ($FeCl_3 \cdot 6H_2O$) in deionized distilled water and dilute to 1 liter.

6.3.2.6 Sulfuric acid solution - H_2SO_4 -1N: Slowly and while stirring, add 28 mL of concentrated sulfuric acid (H_2SO_4) to deionized distilled water. Dilute to 1 liter.

6.3.2.7 Sodium hydroxide solution - NaOH-1N: Dissolve 40 grams of sodium hydroxide (NaOH) in deionized distilled water. Dilute to 1 liter.

6.3.2.8 Sodium sulfite solution: Dissolve 1.575 grams of Na_2SO_3 in 1000-mL deionized distilled water. This solution is not stable; prepare daily.

6.3.2.9 Glucose-glutamic acid solution: Dry reagent-grade glucose ($C_6H_{12}O_6$) and reagent-grade glutamic acid $\{C_3H_5NH_2(COOH)_2\}$ at 103°C for one hour. Add 150-mg glucose and 150-mg glutamic acid to deionized distilled water and dilute to 1 liter. Prepare fresh immediately before use.

6.3.2.10 Polyseed solution: Place the contents of one Polyseed capsule (discard the gelatin capsule) in 500 mL of dilution water (6.5.1.2). Aerate and stir the Polyseed solution for 60 minutes. CONTINUE to stir and aerate the Polyseed solution while adding to BOD bottles.

NOTE 1: For best results, the Polyseed solution should be used within 6 hours of rehydration.

NOTE 2: Bran, which acts as the carrier for the micro-organisms, will neither dissolve, nor will it inhibit microbial activity.

NOTE 3: Polyseed is a blend of bacteria having a broad spectrum of capabilities for degradation of both industrial and municipal wastes. May be obtained from Baxter Scientific Products (Catalog No. W2802-1).

6.3.2.11 Sulfuric acid solution - H_2SO_4 - 1 + 50: Slowly and while stirring, add 10 mL of concentrated sulfuric acid (H_2SO_4) to deionized distilled water. Dilute to 500 mL.

6.3.2.12 Potassium iodide solution - KI: Dissolve 100 grams of potassium iodide (KI) in deionized distilled water. Dilute to 1 liter.

6.3.2.13 Starch indicator solution: To 5 grams of starch (potato, arrowroot, or soluble), add a little cold deionized distilled water and grind in a mortar to a thin paste. Pour 1 liter of boiling deionized distilled water, stir, and let settle overnight. Use clear supernate. Preserve with 1.25-grams salicylic acid or 4-grams zinc chloride.

6.4 Calibration

<u>NOTE 1</u>: Replace probe membrane if a large bubble forms in the electrolyte or if the membrane becomes fouled or damaged. Inspect the gold cathode when changing the membrane; it should be right and untarnished.

<u>NOTE 2</u>: Calibrate before each use.

6.4.1 Dissolved oxygen meter: YSI model 58 with YSI BOD bottle probe model 5720A.

6.4.1.1 Set the function switch to % mode.

6.4.1.2 Place the BOD probe in a BOD bottle containing about one inch of water to provide a 100% relative humidity calibration environment.

<u>NOTE</u>: The highest accuracy of measurement is achieved when the probe is zeroed and calibrated at a temperature as close as possible to the temperature of the sample to be measured.

6.4.1.3 Set the function switch to ZERO and readjust display to read 0.00. Switch back to % air saturation mode.

6.4.1.4 When the display reading has stabilized, unlock the O2 CALIB control locking ring and adjust the display to the CALIB VALUE indicated in the PRESSURE/ALTITUDE chart in Attachment 2 (also printed in the instructions on the back of the meter). Relock the locking ring to prevent inadvertent changes.

6.4.1.5 Record the barometric pressure and the CALIB VALUE on the analysis worksheet (Attachment 1).

6.5 Procedure Instructions

NOTE: Begin analysis within 6 hours of collection.

6.5.1 Preparation of dilution water

6.5.1.1 Approximately 24 hours before analysis is to begin, place a desired volume of deionized distilled water, free of organic matter, in a suitable container (clean plastic or glass, no more than two-thirds full, with cotton plug not capped) in the incubator set at 20°C. This water will be used for dilution water. Add oxygen by shaking for at least 30 seconds.

6.5.1.2 Dilution water: Add one 3-mL buffer nutrient pillow (6.3.2.1) to deionized water (6.5.1.1) for each 3 liters of dilution water. [Alternately add 1 mL of each of the following solutions for each liter of dilution water: phosphate buffer solution (6.3.2.2), magnesium sulfate solution (6.3.2.3), calcium chloride solution (6.3.2.4), and ferric chloride solution (6.3.2.5)]. Mix and add oxygen by shaking for at least 30 seconds. Before using, bring dilution water temperature to 20°C.

6.5.2 Sample dechlorination

6.5.2.1 Adjust sample pH if necessary between 6.5 and 7.5 using 1N H_2SO_4 (6.3.2.6) or 1N NaOH (6.3.2.7).

6.5.2.2 Aliquot 100 mL of neutralized sample into a white porcelain casserole.

6.5.2.3 Add 10 mL of 1 + 50 sulfuric acid (6.3.2.11), 10 mL of potassium iodide solution (6.3.2.12), about 1 mL of starch solution (6.3.2.13), and titrate with sodium sulfite solution (6.3.2.8) to the starch-iodine end point.

6.5.2.4 Determine the volume of neutralized sample needed to perform the BOD test.

6.5.2.5 Add to neutralized sample, the relative volume of sodium sulfite solution (6.3.2.8) determined by the above test plus a 2-mL excess (for example: to dechlorinate 1 liter of neutralized sample, add 10 times the sodium sulfide titration volume plus 2 mL). Mix, and after 10 to 20 minutes check sample for residual chloride.

NOTE: Excess sodium sulfite exerts an oxygen demand and reacts slowly with certain organic chloramine compounds that may be present in chlorinated samples.

6.5.3 Sample dilution technique

NOTE 1: For domestic wastewater, unchlorinated or otherwise undisinfected effluents from biological waste treatment plants, and surface waters receiving wastewater discharges containing satisfactory microbial populations, the addition of seeding material to the samples (6.5.3.9) and the glucose-glutamic acid check samples (6.5.3.5), and determining the seed BOD (6.5.3.11) is not necessary.

NOTE 2: Dilutions that result in a residual DO of at least 1 mg/L and a DO uptake of at least 2 mg/L after 5 days incubation produce the most reliable results. Make several dilutions of prepared sample to obtain DO uptake in this range.

6.5.3.1 Adjust sample temperature to 20 ± 1°C before making dilutions.

6.5.3.2 Label the BOD bottles for sample identification and record bottle number on analysis worksheet.

6.5.3.3 Dilution water blank: Fill one BOD bottle to overfill with the aerated dilution water (6.5.1.2). Add the water slowly without agitation by running the water down the side of the bottle. Examine the bottle for any air bubbles. Remove trapped air bubbles by tapping side of bottle with stopper. Stopper carefully. Examine again for air bubbles.

6.5.3.4 Seeded dilution water blank: Fill one BOD bottle about one-half full with aerated dilution water (6.5.1.2). Add 2 mL of polyseed solution (6.3.2.10) and finish filling with aerated dilution water. Add the water slowly without agitation by running the water down the side of the bottle. Examine the bottle for any air bubbles. Remove trapped air bubbles by tapping side of bottle with stopper. Stopper carefully. Examine again for air bubbles. The DO uptake of seeded dilution water should be between 0.6 and 1.0 mg/L.

6.5.3.5 Select a range of dilutions that result in a residual DO of at least 1 mg/L and DO uptake of at least 2 mg/L after 5 days incubation.

6.5.3.6 Using a wide-tip volumetric pipet, add the desired dechlorinated sample volume to individual BOD bottles. Add the sample slowly without agitation by running the sample down the side of the bottle.

6.5.3.7 Add 2 mL of the polyseed solution (6.3.2.10) to each bottle slowly without agitation by running the solution down the side of the bottle.

6.5.3.8 Fill bottles with enough dilution water so that insertion of stopper will displace all air, leaving no bubbles. Add the water slowly without agitation by running the water down the side of the bottle. Examine the bottle for any air bubbles. Remove trapped air bubbles by tapping side of bottle with stopper. Stopper carefully. Examine again for air bubbles.

6.5.3.9 Seed control: Add 25 mL of the polyseed solution (6.3.2.10) to 3 BOD bottles slowly without agitation by running the solution down the side of the bottle. Fill the bottles with enough dilution water so that insertion of stopper will displace air, leaving no bubbles. Add the water slowly without agitation by running the water down the side of the bottle. Remove trapped air bubbles by tapping side of bottle with stopper. Stopper carefully. Examine again for air bubbles.

6.5.4 Glucose-glutamic acid check samples

NOTE 1: The glucose-glutamic acid check is intended to be a reference point for evaluation of dilution water quality, seed effectiveness, and analytical technique.

NOTE 2: The acceptable range for results on the glucose-glutamic acid check is between 163 mg/L and 237 mg/L. Samples must be recollected for BOD analysis if the glucose-glutamic acid check falls outside this range.

6.5.4.1 Pipet 6 mL of the glucose-glutamic acid solution (6.3.2.9) and 2 mL of the polyseed solution (6.3.2.10) into 3 BOD bottles.

6.5.4.2 Fill bottles with enough dilution water so that insertion of stopper will displace all air, leaving no bubbles. Add the water slowly without agitation by running the water down the side of the bottle. Examine the bottle for any air bubbles. Remove trapped air bubbles by tapping side of bottle with stopper. Stopper carefully. Examine again for air bubbles.

6.5.5 Determination of initial DO

6.5.5.1 Calibrate the DO meter (see 6.4).

6.5.5.2 Determine the DO of each sample, check samples, seed control, and dilution water. Insert the BOD probe slowly into the BOD bottle to prevent trapping air bubbles. Turn probe stirrer on. Let sample stir at least 30 seconds before taking readings.

6.5.5.3 Turn the DO meter function switch to O2 ZERO and adjust if necessary.

6.5.5.4 Turn the DO meter function switch to TEMP and record the temperature on the analysis worksheet.

6.5.5.5 Turn the DO meter function switch to the 0.01 mg/L position and read the DO. Record the DO on the analysis worksheet.

6.5.5.6 Remove the BOD probe from the bottle slowly to prevent drawing air into the sample. Replace any displaced contents with dilution water.

6.5.5.7 Examine the bottle for any air bubbles. Remove trapped air bubbles by tapping side of bottle with stopper. Stopper carefully. Examine again for air bubbles.

6.5.5.8 Water-seal by filling the BOD bottle neck completely with dilution water.

6.5.5.9 Cap the bottle with the special BOD cap.

6.5.5.10 Incubate the sample dilutions, check samples, and dilution water blank at 20 ± 1°C for 5 days.

6.5.6 Determination of final DO

After 5 days remove all bottles from the incubator and determine the final DO of the sample dilutions, check samples, seed controls, and dilution water blanks by performing steps 6.5.5.1 through 6.5.5.5.

<u>NOTE</u>: The DO uptake of the dilution water blank should not be more than 0.2 mg/L and preferably not more than 0.1 mg/L.

6.5.7 Glassware cleaning

6.5.7.1 Wash all BOD bottles, stoppers, and caps in hot, soapy water. Rinse several times with deionized distilled water.

6.5.7.2 Invert and allow to drain until dry. When dry, stopper and store them in the incubator until needed.

6.6 Calculations and Recording Data

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

NOTE 2: In these calculations, do not make corrections for DO uptake by the dilution water blank during incubation. This correction is unnecessary if dilution water meets the blank criteria stipulated above. If the dilution water does not meet these criteria, proper corrections are difficult and results become questionable.

6.6.1 $$\text{BOD5, mg/L} = \frac{\{(D1 - D2) - [(B1 - B2) \times f]\} \times 300}{A}$$

where:

D1 = DO of diluted sample immediately after preparation, mg/L

D2 = DO of diluted sample after 5-day incubation at 20°C, mg/L

B1 = DO of seed control before incubation, mg/L

B2 = DO of seed control after 5-day incubation at 20°C, mg/L

f = Ratio of seed in diluted sample to seed in seed control
= (% seed in diluted sample)/(% seed in seed control)

300 = Volume of BOD bottles, mL

A = Volume of sample used, mL

6.6.2 Data Recording

Record all data and calculations on the appropriate worksheet (Attachment 1).

7.0 <u>QUALITY ASSURANCE PROVISION</u>

7.1 Responsibility of Inspection

7.1.1 The analysts shall inspect all numerical data reasonableness and shall observe the DO meter, the dilutions, and other phenomena associated with the procedure for unusual occurrences.

7.1.2 The Shift Supervisor, or the Control Laboratory Supervisor or his designee. shall inspect the results of each analysis.

7.1.3 The Control Laboratory Supervisor, or his designee. shall inspect the results of the procedure on a regular basis.

7.2 Acceptable Criteria

Critical measurements are made in the following steps:

6.5.1.1-- Addition of oxygen to deionized distilled water to be used for dilution water

6.5.1.2-- Preparation of dilution water

6.5.2.1-- Sample pH adjustment

6.5.2.3-- Determination of residual chloride

6.5.2.5-- Dechlorination of sample to be used for BOD determination

6.5.3.6-- Pipeting sample

6.5.3.7-- Adding polyseed to samples

6.5.3.8-- Filling sample bottles with dilution water

6.5.3.9-- Preparation of seed control

6.5.4.1-- Pipeting glucose-glutamic acid solution

6.5.4.2-- Filling check samples bottles with dilution water

6.5.5.1-- Calibration of DO meter

6.5.5.2-- Determining and recording the initial DO

6.5.5.10- Incubation for 5 days

6.5.6 -- Determining and recording the final DO

6.6 -- Calculations and recording data

7.3 Material Monitoring

All reagents shall be of ACS reagent-grade quality.

7.4 Equipment Monitoring

7.4.1 Record incubator temperature daily on Record Of Temperatures, Standards Recovery, And Blanks Report Sheet (Attachment 3).

7.4.2 Observe condition of BOD probe membrane before reading DO.

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7.5 Certification

This procedure shall be certified by the triplicate analysis of a certified demand QC sample. The average results and each individual results shall be within the 95% confidence interval of the QC sample.

8.0 SAFETY

General laboratory safety rules shall be observed.

9.0 NOTES

None

10.0 ATTACHMENTS AND APPENDICES

10.1 Attachment 1--BOD Analysis Worksheet.

10.2 Attachment 2--Calibration Values for Various Atmospheric Pressures and Altitudes.

10.3 Attachment 3--Record Of Temperatures, Standards Recovery, And Blanks Report Sheet.

ATTACHMENT 1

BOD ANALYSIS WORKSHEET

SAMPLE IDENTIFICATION: ______________________________

BOD 5-DAY (Method 405.1)

DO METER Calib.: Bar. Press. = ______ mm Hg; Calib. Value = ______
SAMPLE Collected: Date: Began ______ Time: Began ______ COMPOSITE _____
End ______ End ______ GRAB _____
INCUBATION: Date: Began ______ Time: Began ______
5-DAY @ 20°C End ______ End ______
SAMPLE: Temp. = °C ; pH = @ °C ; DO = mg/l

SAMPLE DECHLORINATION		
Na_2SO_3 used to titrate 100 mL sample, mL	A	
Na_2SO_3 to dechlorinate 1 L of sample, mL, [(A x 10) + 2 mL]	B	
Na_2SO_3 to titrate 100 mL sample after addition of "B", mL	C	

SEED BOD5									
Bottle Number									
Volume seed analyzed, mL	D								
Initial temperature, °C									
Initail DO, mg/L	E								
Final temperature, °C									
Final DO, mg/L	F								
DO depletion, mg/L, (E-F)	G								

SAMPLE BOD5									
Bottle Number									
Volume sample analyzed, mL	H	DW	DW + seed						
Volume seed added, mL	J	xxxx	2	2	2	2	2	2	2
Initial temperature, °C									
Initail DO, mg/L	K								
Final temperature, °C									
Final DO, mg/L	L								
DO depletion, mg/L, (K-L)	M								
Seed correction (GxJ/D)	N								
BOD, (M-N)x(300/H)									

GLUCOSE-GLUTAMIC ACID CHECK									
Bottle Number									
Volume check solution, mL	P								
Volume seed added, mL	R	2	2	2	2	2	2	2	2
Initial temperature, °C									
Initail DO, mg/L	S								
Final temperature, °C									
Final DO, mg/L	T								
DO depletion, mg/L, (S-T)	U								
BOD, [U-(GxR/D)]x[300/P]									

Analyzed by ______________
Checked by ______________

FORMBOD5

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ATTACHMENT 2

PRESSURE			ALTITUDE		CALIB. VALUE
Inches Hg	mm Hg	kPa	FT.	m	
30.23	768	102.3	-276	-84	101
29.92	760	101.3	0	0	100
29.61	752	100.3	278	85	99
29.33	745	99.3	558	170	98
29.02	737	98.3	841	256	97
28.74	730	97.3	1126	343	96
28.43	722	96.3	1413	431	95
28.11	714	95.2	1703	519	94
27.83	707	94.2	1995	608	93
27.52	699	93.2	2290	698	92
27.24	692	92.2	2587	789	91
26.93	684	91.2	2887	880	90
26.61	676	90.2	3190	972	89
26.34	669	89.2	3496	1066	88
26.02	661	88.2	3804	1160	87
25.75	654	87.1	4115	1254	86
25.43	646	86.1	4430	1350	85
25.12	638	85.1	4747	1447	84
24.84	631	84.1	5067	1544	83
24.53	623	83.1	5391	1643	82
24.25	616	82.1	5717	1743	81
23.94	608	81.1	6047	1843	80
23.62	600	80.0	6381	1945	79
23.35	593	79.0	6717	2047	78
23.03	585	78.0	7058	2151	77
22.76	578	77.0	7401	2256	76
22.44	570	76.0	7749	2362	75
22.13	562	75.0	8100	2469	74
21.85	555	74.0	8455	2577	73
21.54	547	73.0	8815	2687	72
21.26	540	71.9	9178	2797	71
20.94	532	70.9	9545	2909	70
20.63	524	69.9	9917	3023	69
20.35	517	68.9	10293	3137	68
20.04	509	67.9	10673	3253	67
19.76	502	66.9	11058	3371	66

Calibration Values for various atmospheric pressures and altitudes.
Normal barometric variations are equivalent to ~ ± 500 feet at sea level.

ATTACHMENT 3

TVA CONTROL LABORATORY

RECORD OF TEMPERATURES, STANDARDS RECOVERY, AND BLANKS

Month of ____________

Date	Oven Temp. °F	BOD INCUB. °C	O & G BATH °C	Std.- % Recovery				Blanks (mg/liter)				
				Titration		Electrode		Oil & Grease	Titration		Electrode	
				T.K.N.	NH3-N	T.K.N.	NH3-N		T.K.N.	NH3-N	T.K.N.	NH3-N
1												
2												
3												
4												
5												
6												
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BIOCHEMICAL OXYGEN DEMAND

Method 405.1 (5 Days, 20°C)

STORET NO. 00310
Carbonaceous 80082

1. Scope and Application
    - 1.1 The biochemical oxygen demand (BOD) test is used for determining the relative oxygen requirements of municipal and industrial wastewaters. Application of the test to organic waste discharges allows calculation of the effect of the discharges on the oxygen resources of the receiving water. Data from BOD tests are used for the development of engineering criteria for the design of wastewater treatment plants.
    - 1.2 The BOD test is an empirical bioassay-type procedure which measures the dissolved oxygen consumed by microbial life while assimilating and oxidizing the organic matter present. The standard test conditions include dark incubation at 20°C for a specified time period (often 5 days). The actual environmental conditions of temperature, biological population, water movement, sunlight, and oxygen concentration cannot be accurately reproduced in the laboratory. Results obtained must take into account the above factors when relating BOD results to stream oxygen demands.
2. Summary of Method
    - 2.1 The sample of waste, or an appropriate dilution, is incubated for 5 days at 20°C in the dark. The reduction in dissolved oxygen concentration during the incubation period yields a measure of the biochemical oxygen demand.
3. Comments
    - 3.1 Determination of dissolved oxygen in the BOD test may be made by use of either the Modified Winkler with Full-Bottle Technique or the Probe Method in this manual.
    - 3.2 Additional information relating to oxygen demanding characteristics of wastewaters can be gained by applying the Total Organic Carbon and Chemical Oxygen Demand tests (also found in this manual).
    - 3.3 The use of 60 ml incubation bottles in place of the usual 300 ml incubation bottles, in conjunction with the probe, is often convenient.
4. Precision and Accuracy
    - 4.1 Eighty-six analysts in fifty-eight laboratories analyzed natural water samples plus an exact increment of biodegradable organic compounds. At a mean value of 2.1 and 175 mg/1 BOD, the standard deviation was ±0.7 and ±26 mg/1, respectively (EPA Method Research Study 3).
    - 4.2 There is no acceptable procedure for determining the accuracy of the BOD test.

Approved for NPDES CBOD: pending approval for Section 304(h), CWA
Issued 1971
Editorial revision 1974

5. References

5.1 The procedure to be used for this determination is found in:
Standard Methods for the Examination of Water and Wastewater, 15th Edition, p. 483, Method 507 (1980).

5.2 Young, J. C., "Chemical Methods for Nitrification Control," J. Water Poll. Control Fed., 45, p. 637 (1973).

APPENDIX A-4

Procedure for Suspended Solids and Residuals: Method 160.2

Table of Contents – Appendix A-4

Residue	Method 160.1	Residue, Filterable
	Method 160.2	Residue, Non-Filterable
	Method 160.3	Residue, Total

1.0 Procedure

Perform residue analysis in accordance with any or all of procedures 160.1, 160.2, or 160.3 as requested.

2.0 Recordkeeping

Retain all machine printouts, worksheets, and notes.

3.0 Quality Control Samples

None

RESIDUE, FILTERABLE

Method 160.1 (Gravimetric, Dried at 180°C)

STORET NO. 70300

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
   1.2 The practical range of the determination is 10 mg/l to 20,000 mg/l.
2. Summary of Method
   2.1 A well-mixed sample is filtered through a standard glass fiber filter. The filtrate is evaporated and dried to constant weight at 180°C.
   2.2 If Residue, Non-Filterable is being determined, the filtrate from that method may be used for Residue, Filterable.
3. Definitions
   3.1 Filterable residue is defined as those solids capable of passing through a glass fiber filter and dried to constant weight at 180°C.
4. Sample Handling and Preservation
   4.1 Preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to 4°C, to minimize microbiological decomposition of solids, is recommended.
5. Interferences
   5.1 Highly mineralized waters containing significant concentrations of calcium, magnesium, chloride and/or sulfate may be hygroscopic and will require prolonged drying, desiccation and rapid weighing.
   5.2 Samples containing high concentrations of bicarbonate will require careful and possibly prolonged drying at 180°C to insure that all the bicarbonate is converted to carbonate.
   5.3 Too much residue in the evaporating dish will crust over and entrap water that will not be driven off during drying. Total residue should be limited to about 200 mg.
6. Apparatus
   6.1 Glass fiber filter discs, 4.7 cm or 2.1 cm, without organic binder, Reeve Angel type 934-AH, Gelman type A/E, or equivalent.
   6.2 Filter holder, membrane filter funnel or Gooch crucible adapter.
   6.3 Suction flask, 500 ml.
   6.4 Gooch crucibles, 25 ml (if 2.1 cm filter is used).
   6.5 Evaporating dishes, porcelain, 100 ml volume. (Vycor or platinum dishes may be substituted).
   6.6 Steam bath.
   6.7 Drying oven, 180°C ± 2°C.
   6.8 Desiccator.

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Issued 1971

6.9 Analytical balance, capable of weighing to 0.1 mg.

7. Procedure

7.1 Preparation of glass fiber filter disc: Place the disc on the membrane filter apparatus or insert into bottom of a suitable Gooch crucible. While vacuum is applied, wash the disc with three successive 20 ml volumes of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Discard washings.

7.2 Preparation of evaporating dishes: If Volatile Residue is also to be measured heat the clean dish to 550 ±50°C for one hour in a muffle furnace. If only Filterable Residue is to be measured heat the clean dish to 180 ±2°C for one hour. Cool in desiccator and store until needed. Weigh immediately before use.

7.3 Assemble the filtering apparatus and begin suction. Shake the sample vigorously and rapidly transfer 100 ml to the funnel by means of a 100 ml graduated cylinder. If total filterable residue is low, a larger volume may be filtered.

7.4 Filter the sample through the glass fiber filter, rinse with three 10 ml portions of distilled water and continue to apply vacuum for about 3 minutes after filtration is complete to remove as much water as possible.

7.5 Transfer 100 ml (or a larger volume) of the filtrate to a weighed evaporating dish and evaporate to dryness on a steam bath.

7.6 Dry the evaporated sample for at least one hour at 180 ±2°C. Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained or until weight loss is less than 0.5 mg.

8. Calculation

8.1 Calculate filterable residue as follows:

$$\text{Filterable residue, mg/l} = \frac{(A - B) \times 1{,}000}{C}$$

where:

A = weight of dried residue + dish in mg
B = weight of dish in mg
C = volume of sample used in ml

9. Precision and Accuracy

9.1 Precision and accuracy are not available at this time.

Bibliography

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 92, Method 208B, (1975).

RESIDUE, NON-FILTERABLE

Method 160.2 (Gravimetric, Dried at 103–105°C)

STORET NO. 00530

1. Scope and Application
   - 1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
   - 1.2 The practical range of the determination is 4 mg/1 to 20,000 mg/1.
2. Summary of Method
   - 2.1 A well-mixed sample is filtered through a glass fiber filter, and the residue retained on the filter is dried to constant weight at 103–105°C.
   - 2.2 The filtrate from this method may be used for Residue, Filterable.
3. Definitions
   - 3.1 Residue, non-filterable, is defined as those solids which are retained by a glass fiber filter and dried to constant weight at 103–105°C.
4. Sample Handling and Preservation
   - 4.1 Non-representative particulates such as leaves, sticks, fish, and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result.
   - 4.2 Preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to 4°C, to minimize microbiological decomposition of solids, is recommended.
5. Interferences
   - 5.1 Filtration apparatus, filter material, pre-washing, post-washing, and drying temperature are specified because these variables have been shown to affect the results.
   - 5.2 Samples high in Filterable Residue (dissolved solids), such as saline waters, brines and some wastes, may be subject to a positive interference. Care must be taken in selecting the filtering apparatus so that washing of the filter and any dissolved solids in the filter (7.5) minimizes this potential interference.
6. Apparatus
   - 6.1 Glass fiber filter discs, without organic binder, such as Millipore AP-40, Reeves Angel 934-AH, Gelman type A/E, or equivalent.
     NOTE: Because of the physical nature of glass fiber filters, the absolute pore size cannot be controlled or measured. Terms such as "pore size", collection efficiencies and effective retention are used to define this property in glass fiber filters. Values for these parameters vary for the filters listed above.
   - 6.2 Filter support: filtering apparatus with reservoir and a coarse (40–60 microns) fritted disc as a filter support.

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NOTE: Many funnel designs are available in glass or porcelain. Some of the most common are Hirsch or Buchner funnels, membrane filter holders and Gooch crucibles. All are available with coarse fritted disc.

6.3 Suction flask.

6.4 Drying oven, 103–105°C.

6.5 Desiccator.

6.6 Analytical balance, capable of weighing to 0.1 mg.

7. Procedure

7.1 Preparation of glass fiber filter disc: Place the glass fiber filter on the membrane filter apparatus or insert into bottom of a suitable Gooch crucible with wrinkled surface up. While vacuum is applied, wash the disc with three successive 20 ml volumes of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Remove filter from membrane filter apparatus or both crucible and filter if Gooch crucible is used, and dry in an oven at 103–105°C for one hour. Remove to desiccator and store until needed. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg). Weigh immediately before use. After weighing, handle the filter or crucible/filter with forceps or tongs only.

7.2 Selection of Sample Volume

For a 4.7 cm diameter filter, filter 100 ml of sample. If weight of captured residue is less than 1.0 mg, the sample volume must be increased to provide at least 1.0 mg of residue. If other filter diameters are used, start with a sample volume equal to 7 ml/cm^2 of filter area and collect at least a weight of residue proportional to the 1.0 mg stated above.

NOTE: If during filtration of this initial volume the filtration rate drops rapidly, or if filtration time exceeds 5 to 10 minutes, the following scheme is recommended: Use an unweighed glass fiber filter of choice affixed in the filter assembly. Add a known volume of sample to the filter funnel and record the time elapsed after selected volumes have passed through the filter. Twenty-five ml increments for timing are suggested. Continue to record the time and volume increments until fitration rate drops rapidly. Add additional sample if the filter funnel volume is inadequate to reach a reduced rate. Plot the observed time versus volume filtered. Select the proper filtration volume as that just short of the time a significant change in filtration rate occurred.

7.3 Assemble the filtering apparatus and begin suction. Wet the filter with a small volume of distilled water to seat it against the fritted support.

7.4 Shake the sample vigorously and quantitatively transfer the predetermined sample volume selected in 7.2 to the filter using a graduated cylinder. Remove all traces of water by continuing to apply vacuum after sample has passed through.

7.5 With suction on, wash the graduated cylinder, filter, non-filterable residue and filter funnel wall with three portions of distilled water allowing complete drainage between washing. Remove all traces of water by continuing to apply vacuum after water has passed through.

NOTE: Total volume of wash water used should equal approximately 2 ml per cm^2. For a 4.7 cm filter the total volume is 30 ml.

7.6 Carefully remove the filter from the filter support. Alternatively, remove crucible and filter from crucible adapter. Dry at least one hour at 103–105°C. Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg).

8. Calculations

8.1 Calculate non-filterable residue as follows:

$$\text{Non-filterable residue, mg/l} = \frac{(A - B) \times 1{,}000}{C}$$

where:

A = weight of filter (or filter and crucible) + residue in mg
B = weight of filter (or filter and crucible) in mg
C = ml of sample filtered

9. Precision and Accuracy

9.1 Precision data are not available at this time.

9.2 Accuracy data on actual samples cannot be obtained.

Bibliography

1. NCASI Technical Bulletin No. 291, March 1977. National Council of the Paper Industry for Air and Stream Improvement, Inc., 260 Madison Ave., NY.

RESIDUE, TOTAL

Method 160.3 (Gravimetric, Dried at 103–105°C)

STORET NO. 00500

1. Scope and Application
   - 1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
   - 1.2 The practical range of the determination is from 10 mg/1 to 20,000 mg/1.
2. Summary of Method
   - 2.1 A well mixed aliquot of the sample is quantitatively transferred to a pre-weighed evaporating dish and evaporated to dryness at 103–105°C.
3. Definitions
   - 3.1 Total Residue is defined as the sum of the homogenous suspended and dissolved materials in a sample.
4. Sample Handling and Preservation
   - 4.1 Preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to 4°C, to minimize microbiological decomposition of solids, is recommended.

Interferences
   - 5.1 Non-representative particulates such as leaves, sticks, fish and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result.
   - 5.2 Floating oil and grease, if present, should be included in the sample and dispersed by a blender device before aliquoting.

6. Apparatus
   - 6.1 Evaporating dishes, porcelain, 90 mm, 100 ml capacity. (Vycor or platinum dishes may be substituted and smaller size dishes may be used if required.)
7. Procedure
   - 7.1 Heat the clean evaporating dish to 103–105°C for one hour, if Volatile Residue is to be measured, heat at 550 ±50°C for one hour in a muffle furnace. Cool, desiccate, weigh and store in desiccator until ready for use.
   - 7.2 Transfer a measured aliquot of sample to the pre-weighed dish and evaporate to dryness on a steam bath or in a drying oven.
     - 7.2.1 Choose an aliquot of sample sufficient to contain a residue of at least 25 mg. To obtain a weighable residue, successive aliquots of sample may be added to the same dish.
     - 7.2.2 If evaporation is performed in a drying oven, the temperature should be lowered to approximately 98°C to prevent boiling and splattering of the sample.

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7.3 Dry the evaporated sample for at least 1 hour at 103–105°C. Cool in a desiccator and weigh. Repeat the cycle of drying at 103–105°C, cooling, desiccating and weighing until a constant weight is obtained or until loss of weight is less than 4% of the previous weight, or 0.5 mg, whichever is less.

8. Calculation

8.1 Calculate total residue as follows:

$$\text{Total residue, mg/l} = \frac{(A - B)\times 1{,}000}{C}$$

where:

A = weight of sample + dish in mg
B = weight of dish in mg
C = volume of sample in ml

9. Precision and Accuracy

9.1 Precision and accuracy data are not available at this time.

Bibliography

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 91, Method 208A, (1975).

APPENDIX A-5

Procedure for Chlorides and Bromides Method AP-0300

1.0 PURPOSE

This procedure provides instructions for the determination of anion concentrations in water by ion chromatography.

2.0 SCOPE

This procedure applies to aqueous samples and extracts with a total anion concentration at the parts per million level to lower parts per thousand level. It is an ion chromatography (IC) method for fluoride, chloride, nitrate, nitrite, bromide, ortho-phosphate, or sulfate analysis. Any combination or subset of the anions may be analyzed by this procedure.

3.0 SUMMARY

Anions of interest are separated on an ion chromatograph consisting of an eluent pump, injection valve, guard column, separation column, suppressor, and conductivity detector. Comparisons are made of peak area (or height) to a calibration curve made by measuring a series of standard solutions.

4.0 REFERENCES

4.1 "The Determination of Inorganic Anions in Water by Ion Chromatography," Method 300.0, EPA-600/4-84-017.

4.2 "4110 Determination of Anions by Ion Chromatography," *Standard Methods for The Examination of Water and Wastewater*, edited by Arnold E. Greenberg et al. , American Public Health Association.

4.3 "Standard Test Method for Anions in Water by Chemically Suppressed Ion Chromatography," ASTM D4327-91.

5.0 RESPONSIBILITIES

5.1 It is the responsibility of the Supervisor of the Land and Water Sciences and Remediation section, or his designee, to ensure that this procedure is followed during analysis of samples.

5.2 The Laboratory Team 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

6.1.1 Before an analysis configuration is utilized, solutions containing individual ions must be injected to determine retention times and to ensure there is no overlap of commonly expected anions.

6.2 Limitations and Actions

6.2.1 Metal cations which may complex with fluoride (aluminum and iron) will interfere with analysis of that anion by IC. An ion-specific electrode method should be employed for samples suspected to contain these ions.

6.2.2 Any substance with a retention time coinciding with an analyte of interest will cause an interference. High concentration of any ion may interferes with other nearby peaks. Sample dilution or gradient elution may be used to overcome such interferences. The method of standard additions may be useful in quantifying some compounds in the presence of interference.

6.2.3 Samples to be analyzed for nitrate, nitrite, or phosphate should be stored in a refrigerator until analysis.

6.2.4 If the water dip is too near the fluoride peak, it may be eliminated by the addition of 0.01 ml of stock carbonate-bicarbonate solution per ml of standard or sample.

6.2.5 Samples that contain particles larger than 0.45 microns should be filtered to prevent damage to columns and flow systems.

6.2.6 Samples falling higher than the upper limit of the calibration curve shall be diluted and reanalyzed.

6.2.7 The maximum hold time before analysis is 48 hours for refrigerated samples for nitrate, nitrite, and phosphate. Hold time is 28 days for fluoride, chloride, bromide, and sulfate. The required anion with the shortest hold time will determine the hold time for the sample.

6.3. Apparatus/Equipment

6.3.1 Analytical balance capable of weighing to 0.0001g

6.3.2 Ion chromatograph with data system. The ion chromatograph should be equipped with an eluant pump, injection valve, guard column, separation column, suppressor, and conductivity detector.

6.3.2.1 Guard column - Dionex HPIC-AG4A or similar column recommended by the manufacturer.

6.3.2.2 Separation column - Dionex HPIC-AS4A or similar column recommended by the manufacturer.

6.3.2.3 Suppressor - a chemical suppressor or electronic suppressor as recommended by the manufacturer.

6.3.3 Pipettes - various glass pipettes or mechanical autopipettes

6.3.4 Volumetric Flasks - Various sizes of volumetric flasks used in mixing standards and dilutions.

6.3.5 Autosampler vials - 12 x 32 mm - SRI Catalog Number 99575-A or similar.

6.3.6 Syringe filters - Titan nylon 25-mm syringe filters - 0.45 micron. SRI Catalog number 44525-NN or equivalent.

6.3.7 Syringes - 10 cc syringe with Luer Lok, B-D Part 309604 or equivalent. (Smaller volumes are acceptable)

6.4 Reagents and Standards

6.4.1 Water - deionized water

6.4.2 Eluant Stock - Dissolve 19.1 g Sodium carbonate and 14.3 g sodium bicarbonate in 1 liter of water.

Note: Other eluant systems as recommended by the manufacturer may be sutstituted (such as borate-gluconate).

6.4.3 Eluant - (1.7mM Sodium Carbonate and 1.8 mM Sodium Bicarbonate) -Dilute 20 ml of eluant stock to 2 liters.

6.4.4 Regenerant solution - 25 mN Sulfuric Acid - Dilute 2.8 mL of concentrated sulfuric acid to 4 liters with water. This is used only when a chemical suppression unit is used.

6.4.5 Helium gas - for sparging solutions and pressurizing flow systems.

6.4.6 Calibration Stock Solutions - Individual anion solutions at 1000 ppm may be purchased from reputable vendors. Observe shelf lives as assigned by the vendors. Alternately, stock solutions may be mixed from dry reagent grade chemicals as follows:

Chemical	Weight (g) per liter	Units
Sodium Chloride	1.6485	1000 mg Cl^-/L
Sodium Fluoride	2.2100	1000 mg F^-/L
Sodium Nitrate	6.0679	1000 mg NO_3^--N/L
Sodium Nitrite	4.9257	1000 mg NO_2^--N/L
Potassium Dihydrogen Phosphate	4.3937	1000 mg PO_4^{-3}-P/L
Potassium Sulfate	1.8141	1000 mg SO_4^{-2}/L
Sodium Bromide	1.2876	1000 mg Br^-/L

Note: Each of these should be dried at105° for 30 minutes except for sodium nitrite which should be dried to constant weight in a desiccator.

Note: These are target weights. When actual weights differ, the values should be recorded and actual concentrations should be calculated and entered in the data handling system.

Note: Some vendors provide nitrate, nitrite, or phosphate as 1000 ppm of the compound, not as the element. These may be utilized as long as units are properly entered in the data handling system.

6.4.7 Calibration Solutions - For each analyte of interest, prepare single or mixed calibration solutions of at least three concentrations in the range of interest. Accurately pipette volumes of 1000 ppm stock solutions into a volumetric flask and dilute to volume with water. Maintain records of all dilutions and use of stock solutions to make calibration standards in the system logbook. Update the concentrations and units in the data handling system to match each set of standards. Dilute standards of nitrate, nitrite, and phosphate have a shelf life of one month if refrigerated. Typical concentrations are 5, 10, 20, and 50 ppm with a 100-microliter sample loop for fluoride, chloride, nitrate, phosphate, and sulfate. Typical bromide concentrations have been 0.5, 5, 10, and 25 ppm. Other concentration ranges may be used provided calibration curves are fairly linear.

6.4.8 Laboratory Control Sample (LCS) - A laboratory control sample should be made from a separate stock than that used to make the calibration standards. It should be mixed so that components are concentrated enough to be well quantified. Due to the possibility of changes in peak retention time, it is useful to make two LCS solutions with different mixes of the analytes of interest (e.g. one with fluoride, nitrate, and sulfate and the other with chloride and phosphate) or with the analytes at varying concentrations for peaks which elute adjacent to each other (e.g. fluoride at 5, chloride at 10, nitrate at 15, phosphate at 20, and sulfate at 10). Note all details of mixing the LCS in the logbook.

6.4.9 Matrix Spike Solution - It is useful to make a spiking solution from the same stock used to make the LCS at approximately 100 ppm for all components of interest for making matrix spikes. Note all details of mixing this solution in the logbook.

6.5 Quality Control Sample Requirements

6.5.1 After calibration, at the end of the run, and every ten injections between (counting customer samples and other quality control samples), run a midpoint calibration standard and a blank.

6.5.2 For every 20 customer samples or subset thereof, run an LCS sample, a matrix spike, and a sample duplicate.

7.0 PROCEDURE

7.1 Calibration

7.1.1 Begin the run by loading a blank and the calibration standards in 2-ml vials in the autosampler. Ensure the run schedule lists "Blank", "Autocal1", "Autocal2", etc. No variations in spelling are allowed. Verify concentrations in the method file match those loaded on the machine.

7.1.2 Calibration is performed automatically at the start of each run when samples titled "Autocalx" are encountered.

7.2 Procedure Instructions

7.2.1 Filter any samples with particulate matter using a 0.45 micron syringe filter. Filter them directly into the sample vials. For samples without particulate matter, pour samples directly into the vials. Using a marker, code the vials.

7.2.2 Using the "Schedule" module, enter the calibration standards as in step 7.1.1 and a descriptive title for each sample and quality control sample. Dilution factors may be entered in this module or after the run in the calculation spreadsheet. (See 7.3.4). Select a unique file name for the schedule in the format YYMMDDx where x is used when more than one run is made in a day. Select the proper method file to be used in analysis.

7.2.3 Save the schedule file. Ensure the data file name for run files matches the schedule file name.

7.2.4 Print a copy of the schedule to be used in loading samples in the autosampler. Print a copy of the method file.

7.2.5 Load sample vials in the autosampler tray.

7.2.6 In the "Run" module, load the schedule file with the marker for "initiate run upon receipt of signal from interface" set.

7.2.7 Load the run parameters in the autosampler sample queue, allowing three minutes longer for the run than in the method.

7.3 Calculations and Recording Data

7.3.1 Chromatography workstation software will perform peak identification and integration. It will fit curves and apply factors to provide concentration.

7.3.2 Review printouts of sample schedule, chromatograms, peak identification, and calibration curves.

7.3.2.1 If peaks have migrated out of the retention time window, change the retention time parameter in the method file and rerun with the "Batch" module.

7.3.2.2 If peaks are larger than the highest calibration peak, dilute the sample appropriately and schedule it for reanalysis.

7.3.2.2 If peak overlap from large peaks causes interference, dilute the sample appropriately and schedule it for reanalysis. Alternately, discuss with factory representatives changes in operating conditions which might aid in separation of the peaks of interest. Make such changes and schedule the samples for reanalysis. Document all decisions in the system logbook.

7.3.3 Use the "Cal Plot" routine to produce a printout of the calibration curve. Observe this curve for linearity. Discuss any problems with the section supervisor . Adjust parameters and reanalyze as needed.

7.3.4 Dilution factors may be calculated by entering them in the sample run table so that chromatography workstation software makes the calculation. Alternately, the final data summary may be exported from the "Batch" module into an ASCII file and from there into a spreadsheet designed for calculation of dilution factors and percent recovery of quality control samples.

7.3.5 Note information about the run in workstation logbook (date, time, workorders included). Add notes about any problems encountered.

8.0 SAFETY

8.1 As with all electrical equipment with moving parts, beware of shock hazards. Do not place your hands in the autosampler when it is running.

8.2 The pump and sample lines may contain high pressure. Exercise care when disassembling components, performing routine maintenance, or when changing parts.

9.0 NOTES

None

10.0 ATTACHMENTS AND APPENDICES

None

End of Procedure

APPENDIX A-6

Procedure for YSI 600 Sonde

Table of Contents – Appendix A-6

1. INTRODUCTION

1.1 DESCRIPTION

The 600XL Environmental Monitoring System is a multiparameter, water quality measurement, and data collection system. It is intended for use in research, assessment, and regulatory compliance applications.

Measurement parameters include:

- Dissolved Oxygen
- Conductivity
- Specific Conductance
- Salinity
- Total Dissolved Solids
- Resistivity
- Temperature
- pH
- ORP
- Depth
- Level

The YSI Model 600XL is similar in appearance and performance to the original YSI Model 600 series, but is characterized by three significant enhancements. First, the Model 600XL offers field replaceable sensors. Second, the instrument can be configured with a factory-installed depth sensor module. Third, the unit is now available with an ORP sensor. Like the original Model 600, the 600XL has significant similarities to the YSI Model 6000 series, but also differs from that larger instrument in several ways. First, the Model 600XL does not have internal battery capability, and therefore must be powered from an external power source such as an AC adapter, battery pack, or terminal device. Second, the Model 600XL has no internal logging capability, and therefore the Model 600XL must be used with a terminal, data logger, data collection platform, or computer. Finally, several sensors, such as turbidity, nitrate, and ammonium, which are available on the Model 6000, cannot be used with the 600XL.

The Model 600XL is ideal for profiling and monitoring water conditions in industrial and waste water effluents, lakes, rivers, wetlands, estuaries, coastal waters, and monitoring wells. It can be left unattended for weeks at a time with measurement parameters sampled at your setup interval and data transmitted to your computer or logging device. The Model 600XL can be used 200 feet below the water's surface or in as little as a few inches of water. The fast sensor response of the Model 600XL makes it ideal for vertical profiling. Its very small size allows it to fit down 2 inch diameter monitoring wells.

The Model 600XL is equipped with YSI's patented Rapid Pulse Dissolved Oxygen Sensor which exhibits low stirring dependence, and therefore provides accurate results without an expensive and bulky stirrer. Because stirring is not required, external battery life is extended. In addition, because of the nature of the technology, sensor drift caused by passive fouling is minimized.

The Model 600XL communicates with an ASCII terminal or a computer with a terminal emulation program. Use of the 600XL with our 610 D and 610 DM display/loggers provides an ideal system for profiling or spot sampling.

Every Model 600XL comes with IBM-compatible PC based software for simple and convenient setup and data handling. Reports and plots are automatically generated and their presentation easily customized. Data is easily exported to any spreadsheet program for more sophisticated data processing.

The RS-232C and SDI-12 interfaces provide maximum versatility for system networking and real time data collection. Several Model 600XL units are easily installed as a network, providing valuable water quality data at a variety of locations. For real time results, the Model 600XL can interface to radio telemetry systems, satellite, modem and cellular phone data collection platforms.

The Model 600XL is available with an economical built-in cable of various lengths, or with a sonde-mounted connector. Optional interface cables in several lengths are available for interfacing with a computer or terminal. These cables are waterproof at the sonde end and can be used in the lab or field.

See Appendix D for a complete list of accessories and calibration reagents.

1.2 GENERAL SPECIFICATIONS

See also Section 1.3 Sensor Specifications.

Operating Environment
Medium: fresh, sea, or polluted water
Temperature: -5 to +45 °C
Depth: 0 to 200 (61 meters)

Storage Temperature: -40 to +60 °C

Material: PVC, Stainless Steel

Dimensions and weight with a 50 foot integral cable.
Diameter: 1.6 inches (4.06 cm)
Length: 14 inches (35.56 cm)
Weight: 4.9 pounds (2.22 kg)

Dimensions with depth sensor bulkhead installed and no attached cable.
Diameter: 1.6 inches (4.06 cm)
Length: 20.75 inches (52.7 cm)
Weight: 1.75 pounds (0.8 kg)

Computer Interface
RS-232C
SDI-12

Software
IBM PC compatible computer, 3 1/2 or 5 1/4 inch, high or low density floppy disk drive.
Minimum RAM requirement: 256 K bytes
Optional graphic adapter for plotting

Power
External 12 VDC (8 to 13.8 VDC)

1.3 SENSOR SPECIFICATIONS

The following are typical performance specifications for each sensor.

Depth - Medium
Sensor Type...........Stainless steel strain gauge
Range.....................0 to 200 ft (61 m)
Accuracy................+/- 0.4 ft (0.12 m)
Resolution..............0.001 ft (0.001 m)

Depth - Shallow
Sensor Type.............Stainless steel strain gauge
Range........................0-30 ft (9.1 m)
Accuracy .... +/- 0.06 ft (0.018 m)
Range........................0.001 ft (0.001 m)

Temperature
Sensor Type...........Thermistor
Range.....................-5 to 45 °C
Accuracy................+/- 0.15 °C (optional configuration at +/- 0.05 °C)
Resolution..............0.01 °C

Dissolved Oxygen, % saturation
Sensor Type...........Rapid Pulse - Clark type, polarographic
Range.....................0 to 200 % air saturation
Accuracy................+/- 2 % air saturation
Resolution..............0.1 % air saturation

Dissolved Oxygen, mg/L (Calculated from % air saturation, temperature and salinity)
Sensor Type........... Rapid Pulse - Clark type polarographic
Range.....................0 to 20 mg/L
Accuracy................+/- 0.2 mg/L
Resolution..............0.01 mg/L

Conductivity[*]
Sensor Type...........4 electrode cell
Range.....................0 to 100 mS/cm
Accuracy................+/- 0.5% of reading + 0.001 mS/cm
Resolution..............0.01 mS/cm or 1 uS/cm

Salinity
Sensor Type............Calculated from conductivity and temperature
Range......................0 to 70 ppt
Accuracy.................+/- 1.0% of reading or 0.1 ppt, whichever is greater
Resolution...............0.01 ppt

pH
Sensor Type...........Glass combination electrode
Range.....................2 to 14 units
Accuracy................+/- 0.2 units
Resolution..............0.01 units

pH - Low Ionic Strength
Sensor Type...........Glass combination electrode with open junction and low impedance glass
Range.....................2 to 14 units
Accuracy................+/- 0.2 units
Resolution..............0.01 units

ORP
Sensor type............ Platinum ring
Range......................-999 to 999 mv
Accuracy................+/-20 mv
Resolution...............0.1 mv

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[*] Report outputs of specific conductance (conductivity corrected to 25 C), resistivity, and total dissolved solids are also provided. These values are automatically calculated from conductivity according to algorithms found in *Standard Methods for the Examination of Water and Wastewater* (Ed 1989).

1.4 HOW TO USE THIS MANUAL

This manual provides information for operating and maintaining the Model 600XL Environmental Monitoring System. Sections 1 through 3 provide an overview of setup, calibration, and operational procedures. These first three chapters should provide enough information for you to understand the basic capabilities of the 600XL system and begin sampling. Sections 4 through 9 provide a more detailed explanation of system operations, software, principles of operation, maintenance, and performance troubleshooting. Appendices A-G provide information about health and safety, warranty, accessories, and options.

NOTE: Because of the many features and applications of this versatile product, some sections of this manual may not apply to the specific system you have purchased.

This manual is organized to let you quickly understand and operate the 600XL system. However, it cannot be stressed too strongly that informed and safe operation is more than just knowing which buttons to push. An understanding of the principles of operation, calibration techniques, and system setup is necessary to obtain accurate and meaningful results.

Regular maintenance is required to keep the 600XL functioning properly. Precautions regarding the handling of reagents are also essential for the safety of system operators (see Appendix A for health and safety information).

The early parts of this manual will teach you how to get the 600XL system running. Additional topics are included to help you understand the science it employs, how to use it most effectively and safely, and how to keep it operating correctly.

The 600XL can be purchased with external battery or power supply capability. Additionally, all probes, cables and accessories can be ordered as options or ordered together as a system.

If you have any questions about this product or its application, please contact our customer service department or authorized dealer for assistance. See Appendix C for contact information.

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2. INITIAL SETUP

2.1 UNPACKING

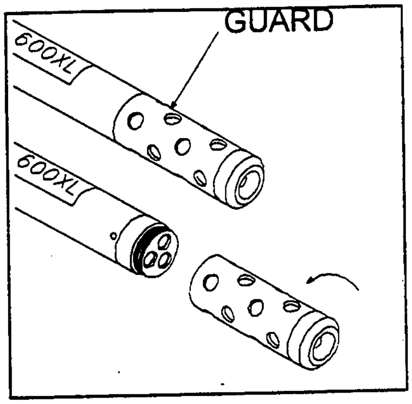
Remove the instrument from the shipping container. Be careful not to discard any parts or supplies. Check off all items on the packing list and inspect all assemblies and components for damage. If any parts are damaged or missing, contact your representative immediately. If you do not know from which dealer your 600XL was purchased, refer to Appendix C for contact information.

NOTE: Reagents for the 600XL are not packaged in the same carton as the instrument. These materials must be ordered separately and will arrive in a separate package.

2.2 SONDE SETUP

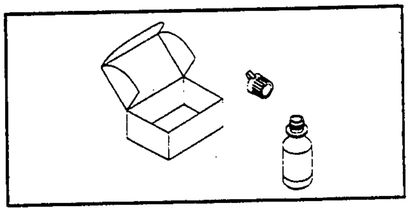
SENSORS

1. Remove the Model 600XL probe guard by hand.

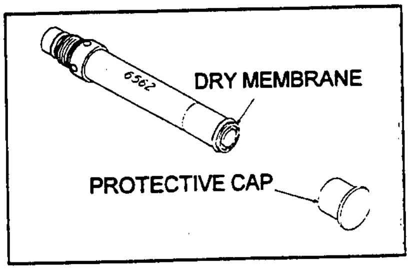


2. NOTE: Step 2 is for the preparation of the 6562 dissolved oxygen probe only. To install other probes, proceed to step 3.

   A. Open the membrane kit and prepare electrolyte. Dissolve the KCl in the dropper bottle by filling it to the neck with distilled water and shaking until the solid is fully dissolved. After dissolution is complete, wait 10-15 minutes unit the solution is free of bubbles.



   B. Remove protective cap and the dry membrane from the 600XL dissolved oxygen probe. NOTE: The dissolved oxygen probe is shipped with a protective dry membrane on the sensor tip. It is very important not to scratch or contaminate the sensor tip. Handle the new probe with care. Avoid touching or hitting of the sensor tip.



C. Hold the probe in a vertical position and apply a few drops of KCl solution to the tip. The fluid should completely fill the small moat around the electrodes and form a meniscus on the tip of the sensor. Be sure no air bubbles are stuck to the face of the sensor. If necessary, shake off the electrolyte and start over.

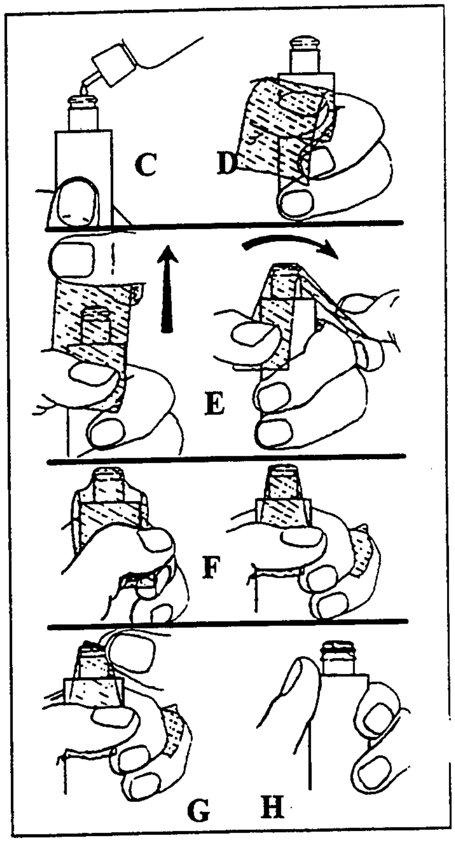
D. Secure a membrane between your left thumb and the probe body. Always handle the membrane with care, touching it at the ends only.

E. With the thumb and forefinger of your right hand, grasp the free end of the membrane. With one continuous motion, gently stretch it up, over, and down the other side of the sensor. The membrane should conform to the face of the sensor.

F. Secure the end of the membrane under the forefinger of your left hand.

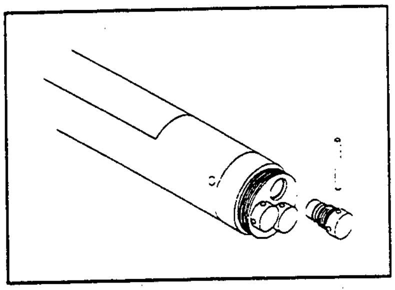
G. Roll the O-ring over the end of the probe, being careful not to touch the membrane surface with your fingers. There should be no wrinkles or trapped air bubbles. Small wrinkles may be removed by lightly tugging on the edges of the membrane. If bubbles are present, remove the membrane and repeat steps C-G.

H. Trim off any excess membrane with a sharp knife or scissors. Make sure the temperature sensor is not covered by excess membrane. Being careful not to get water in the connector, rinse off the excess KCl solution.

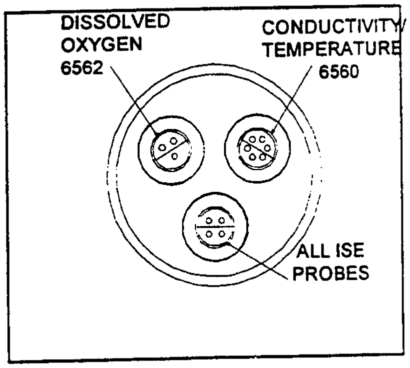


NOTE: Some users find it more convenient to mount the sensor vertically in a vise with rubber jaws while applying the electrolyte and membrane.

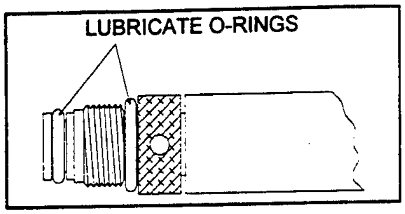
3. Using the probe installation tool supplied in the 6570 maintenance kit, remove the port plugs and locate the port with the connector corresponding to the probe you wish to install.



Note: 6562 Dissolved oxygen probe = 3-pin connector
6560 Conductivity/Temperature = 6-pin connector
6561 pH probe = 4 pin connector
6563 ORP probe = 4 pin connector
6565 Combination pH/ORP probe = 4 pin connector
6564 LIS pH probe = 4 pin connector
6567 combination LIS pH/ORP probe = 4 pin connector

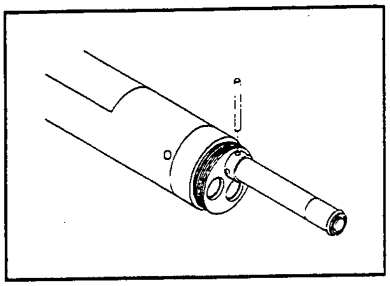


4. Apply a thin coat of O-ring lubricant (supplied in the YSI 6570 maintenance kit) to the O-rings on the connector side of the probe.

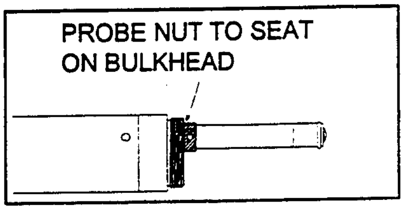


5. NOTE: Before installing probe into sonde, be sure probe port is free of moisture.

Insert the probe into the correct port and gently rotate the probe until the two connectors align.

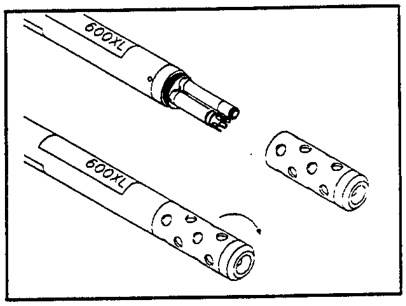


6. With connectors aligned, screw down the probe nut using the probe installation tool. **CAUTION**: Use care not to cross thread the probe nut. Seat nut on face of bulkhead. Do not over tighten.



7..Repeat steps 3-6 for all remaining probes.

8. Replace the 600XL probe guard.



CABLES

Some versions of the Model 600XL have permanently attached cables. If your 600XL has a cable which is non-detachable (no stainless steel connector), parts of this section will not be relevant.

To attach a cable to the 600XL, remove the waterproof cap from the sonde connector and set it aside carefully for later reassembly. Now connect your YSI PC interface cable to the sonde connector. A built-in "key" will ensure proper pin alignment; rotate the cable gently until the "key" engages and then tighten the connectors together by rotating clockwise.

The other end of the cable is a military-style 8-pin connector. This connector plugs directly into the 610 D and 610 DM display/loggers. Most other applications will require the use of an adapter. For example, to connect the 600XL to a computer, use a YSI 6095 MS8 to DB-9 adapter.

POWER

Some type of external power supply is required to power the 600XL sonde. For laboratory setup and calibration with the sonde interfaced to a computer, the YSI 6038 (110 VAC) or 6037 (220 VAC) is ideal. Most adapters include a short pigtail for power that plugs into the power supply. After attaching the three pin connector on the power supply to the pigtail, simply plug the power supply into the appropriate outlet. If you have purchased a 610-series display/logger for use with your 600XL, attachment of the cable to the 610 will allow your sonde to be powered from the batteries in the display/logger or from the 610 power supply if its batteries are not fully charged.

3. BASIC OPERATION

In the previous Section, you learned how to install probes and set up the PC6000 and 600XL sonde software. In this Section, you will learn how to calibrate and run the Model 600XL and how to view your data on a computer display. If you choose to use your 600XL with a 610-series display/logger, refer to the operations manual for the 610 to obtain similar instructions to those provided below.

3.1 CALIBRATION TIPS

WARNING: Reagents used to calibrate and check this instrument may be hazardous to your health. Refer to Appendix A for health and safety information.

Before you begin the calibration procedures outlined below, you may find it helpful to follow some or all of these calibration tips.

1. Remove the sonde stainless steel weight on the bottom of the sonde guard by turning it counterclockwise. This allows the calibration solutions access to the probes with minimal displacement of fluid within the calibration cup. Additionally, carry-over from one solution to the next is reduced.

2. Fill a large bucket with ambient temperature water for rinsing the sonde between calibration solutions.

3. Have several clean, absorbent paper towels or cotton cloths available to dry the sonde between rinses and calibration solutions. It is important to remove as much residual liquid as possible from the sonde after each rinse. Shake the sonde to remove excess rinse water from the inside of the guard. Then dry the outside of the sonde and guard. Drying the sonde and probes in this way reduces carry-over contamination of calibrator solutions and increases the accuracy of the calibration, particularly lower conductivity calibration standards.

4. It is not necessary to remove the probe guard to rinse and dry the probes between calibration solutions. The inaccuracy resulting from simply rinsing the probe compartment and drying the outside of the sonde is minimal.

3.2 CALIBRATION PROCEDURES

WARNING: Calibration reagents may be hazardous to your health. Refer to Appendix A for health and safety information.

A calibration cup is supplied with the Model 600XL. Because the calibration cup fits over the outside of the sonde sensor guard, it is not recommended or necessary to remove the guard to calibrate the sensors. Follow the procedures below to calibrate the sensors. Only *basic* DO percent saturation, Conductivity, pH, and Depth calibration procedures are discussed in this section. Temperature does not require calibration and is, therefore, not included in the Calibrate menu. ORP calibration is required only infrequently and is discussed in Section 4.2. For more detailed calibration procedures, which can be used to enhance the accuracy of some measurements, see Section 4.2.

From the sonde Main menu select **2. Calibrate.** The Calibrate menu will be displayed.

```
---------------------Calibrate-------------------------
1. Conductivity         4. ISE1-pH
2. Dissolved Oxy        5. ISE2-Orp
3. Pressure-Abs

Select option (0 for previous menu):
```

Selection of any of the parameters from the Calibrate menu listing will require the user to input a numerical value and then press Enter. For example, for calibration of specific conductance, the following display will be shown during the calibration sequence.

```
Enter SpCond in mS/cm (10):
```

The number in parentheses is the default value of this parameter and will be used in the calibration if *only* Enter is pressed without typing in another value. Similar prompts will be displayed calibration of all parameters, but for some sensors, such as pH, no default values are provided. In these cases, the user must input a numerical value and then press Enter.

After the calibration value is input and Enteris pressed, a real-time display similar to the following will then appear on the screen. Note that all parameters which have been enabled will appear - not just the one being calibrated at the moment. The user should carefully observe the stabilization of the readings of the parameter which is being calibrated and, when the readings are stable for approximately 30 seconds, press Enter to implement the calibration.

```
==========================================================
 Temp   SpCond   Cond      Sal
  C     mS/cm    mS/cm     ppt
----------------------------------------------------------
To calibrate, press <Enter> when the readings are stable.
 23.00   10.0     10.0      15.7

Select option  (0 for previous menu)
```

NOTE: If an ERROR message appears, begin the calibration procedure again. Be certain that the value you enter for the calibration standard is correct. Also see Section 8, Troubleshooting for more information on error messages.

CAUTION: Be certain to **immerse the entire sonde** in solution standards for calibration of all parameters. Most calibrations require readings not only from the sensor being calibrated but also from the temperature sensor.

Specific start-up calibration procedures for all sensors which commonly require calibration are provided in the following paragraphs of this section. Remember that these are basic protocols designed to get the user up and running with regard to the 600XL. The more-detailed discussion of sensor calibration found in Section 4.2 should be examined prior to use of the instrument in the field.

NOTE: If the particular sensor listed is not installed in your sonde, proceed to the next sensor until the calibration protocol is complete.

CONDUCTIVITY

NOTE: This procedure calibrates not only conductivity, but also specific conductance, salinity, and total dissolved solids.

Place approximately 300 mL of conductivity standard in a clean and dry calibration cup. The conductivity standard you choose should be within the same conductivity range as the water you are preparing to sample. However, we do not recommend using standard less than 1 mS/cm. For example:

- For fresh water choose a 1 mS/cm conductivity standard.
- For brackish water choose a 10 mS/cm conductivity standard.
- For sea water choose a 50 mS/cm conductivity standard.

Caution: Before proceeding insure that the sensor is as dry as possible. Ideally, rinse the conductivity sensor with a small amount of standard that can be discarded. Be certain that you avoid cross contamination of standard solutions with other solutions. Make certain that there are no salt deposits around the oxygen and pH/ORP probes, particularly if you are employing standards of low conductivity.

Without removing the sonde guard, *carefully* immerse the probe end of the sonde into the solution. Gently rotate and/or move the sonde up and down to remove any bubbles from the conductivity cell. The probe must be completely immersed past its vent hole.

Allow at least one minute for temperature equilibration before proceeding.

From the Calibrate menu, select **1. Conductivity** to access the Conductivity calibration procedure and then **1. SpCond.** to access the specific conductance calibration procedure. Enter the calibration value of the standard you are using (mS/cm at 25 C) and press Enter. The current values of all enabled sensors will appear on the screen and will change with time as they stabilize.

Observe the readings under Specific Conductance or Conductivity and when they show no significant change for approximately 30 seconds, press Enter. The screen will indicate that the calibration has been accepted and prompt you to press Enter again to return to the Calibrate menu.

Rinse the sonde in tap or purified water and dry the sonde.

pH 2-POINT

Place approximately 200 mL of pH 7 buffer in a clean calibration cup. *Carefully* immerse the probe end of the sonde into the solution.

Allow at least 1 minute for temperature equilibration before proceeding.

From the **Calibrate** menu, select **4. ISE1 pH** to access the pH calibration choices and then **2. 2-Point**. Press Enter and input the value of the buffer (7 in this case) at the prompt. Press Enter and the current values of all enabled sensors will appear on the screen and will change with time as they stabilize in the solution. Observe the readings under pH and when they show no significant change for approximately 30 seconds, press Enter. The display will indicate that the calibration is accepted.

After the pH 7 calibration is complete, press Enter again, as instructed on the screen, to continue.

Rinse the sonde in water and dry the sonde before proceeding to next step.

Place approximately 200 mL of a second pH buffer solution in a clean calibration cup. The second buffer might be pH 4 if the sample is expected to be acidic or pH 10 if the sample is expected to be basic. *Carefully* immerse the probe end of the sonde into the solution.

Allow at least 1 minute for temperature equilibration before proceeding.

Press Enter and input the value of the second buffer at the prompt. Press Enter and the current values of all enabled sensors will appear on the screen and will change with time as they stabilize in the solution. Observe the readings under pH and when they show no significant change for approximately 30 seconds, press Enter. After the second value calibration is complete, press Enter again, as instructed on the screen, to return to the Calibrate menu.

Rinse the sonde in water and dry the sonde.

Thoroughly rinse and dry the calibration cups for future use.

DISSOLVED OXYGEN

Place approximately 1/8 inch of water or a wet sponge in the bottom of the calibration cup. Place the probe end of the sonde into the calibration cup. Make certain that the DO and the temperature probes are not immersed in the water. Wait approximately 10 minutes for the air in the calibration cup to become water saturated and for the temperatures of the thermistor and the oxygen probe to equilibrate. Make certain that the calibration cup is vented to the atmosphere.

From the **Calibrate** menu, select **2. Dissolved Oxy** to access the DO % calibration procedure.

Enter the current barometric pressure in mm of Hg . *Remember that barometer readings which appear in meteorological reports are generally corrected to sea level and are not useful for you calibration procedure unless they are uncorrected.*

NOTE: Inches of Hg x 25.4 mm/inch = mm Hg

Press Enter and the current values of all enabled sensors will appear on the screen and will change with time as they stabilize. Observe the readings under DO % and when they show no significant change for approximately 30 seconds, press Enter. The screen will indicate that the calibration has been accepted and prompt you to press Enter again to return to the Calibrate menu.

Rinse the sonde in water and dry the sonde.

NOTE: Calibration of dissolved oxygen in the DO % procedure also results in calibration of the DO mg/L mode and vice versa.

NOTE: The above procedure is designed to calibrate your dissolved oxygen sensor for use in sampling applications where the sensor is being pulsed continuously in the Run mode because both "Auto sleep" and "Wait for DO" functions have been disabled as described in Section 2. If your 600XL is to be used in a monitoring application in which data is being captured to a computer or data collection platform, "Auto sleep" and "Wait for DO" will be activated and the calibration displays will be somewhat different. See Section 4 for details.

DEPTH

Following the DO calibration, leave the sonde in water-saturated air. Make certain that the sonde is not submerged in water for the depth calibration.

From the Calibrate menu, select **4. Depth** to access the depth calibration procedure. Input 0.00 or some known sensor offset in feet. Press Enter and monitor the stabilization of the depth readings with time. When no significant change occurs for approximately 30 seconds, press Enter to confirm the calibration and zero the sensor with regard to current barometric pressure.

After depth is zeroed, press Enter again, as instructed on the screen, to return to the Calibrate menu.

The sensors are now calibrated. Press Esc until the sonde Main menu is displayed.

APPENDIX A-7

Procedure for YSI 6000 Sonde

Table of Contents – Appendix A-7

1. INTRODUCTION

1.1 DESCRIPTION

The YSI Model 6000 Environmental Monitoring System is a multiparameter, water quality measurement, and data logging system. It is intended for use in research, assessment, and regulatory compliance applications.

Standard measurement parameters include:

- » Dissolved Oxygen
- » Conductivity
- » Specific Conductance
- » Salinity
- » Total Dissolved Solids
- » Resistivity
- » Temperature

Optional measurement parameters include:

- » pH
- » ORP
- » Depth

The Model 6000 is ideal for profiling and monitoring water conditions in lakes, rivers, wetlands, estuaries, coastal waters, and monitoring wells. It can be left unattended for weeks at a time with measurement parameters sampled at your setup interval and data securely saved in the unit's internal memory. The Model 6000 can be used 500 feet below the water's surface or in as little as a few inches of water. The fast sensor response of the Model 6000 and its built-in data logging make it ideal for vertical profiling. Its small size means it can fit down 4 inch diameter monitoring wells.

A new Rapid Pulse Dissolved Oxygen Sensor (patent pending) exhibits low stirring dependence and, therefore, provides accurate results without an expensive and bulky stirrer. Because no stirring is required, battery life is extended and sensor drift caused by passive fouling is minimized.

If necessary, dissolved oxygen, conductivity, temperature, pH and ORP sensors are quickly replaced in the field.

The Model 6000 communicates with an ASCII terminal or a computer with a terminal emulation program. Every Model 6000 comes with IBM compatible PC based software for simple and convenient setup and data handling. Reports and plots are automatically generated and their presentation easily customized. Data is easily exported to any spreadsheet program for more sophisticated data processing.

The RS-232C and SDI-12 interfaces provide maximum versatility for system networking and real time data collection. Several Model 6000 units are easily installed in a vertical string providing valuable water quality data at a variety of depths. For real time results, the Model 6000 can interface to radio telemetry systems and satellite, telephone, or cellular phone data collection platforms.

The Model 6000 is internally powered by eight C-size alkaline batteries that are easily replaced in the field, without disassembly of the unit. The minimal power requirements of the Rapid Pulse Dissolved Oxygen Sensor combined with state-of-the-art electronic circuits and software provide 30 days of battery life during normal use.

The Model 6000 comes with 256 kilobytes of memory; enough to store 130,000 individual readings.

The Model 6000 comes with a 25 foot cable for interfacing with a computer or terminal. The cable is waterproof at the sonde end and can be used in the lab or field. Optional underwater cables are available in a variety of lengths and are waterproof at both ends.

See Appendix D for a complete list of accessories and calibration reagents.

1.2 GENERAL SPECIFICATIONS

See also Section 1.3 Sensors Specifications.

Operating Environment
Medium: fresh, sea, or polluted water
Temperature: -5 to +45 C
Depth: 0 to 500 feet

Storage Temperature: -40 to +60 C

Material: PVC, Stainless Steel

Dimensions
Diameter: 3.50 inches (8.89 cm)
Length: 19.5 inches (49.53 cm)
Weight: 6.5 pounds (2.95 Kg)

Computer Interface
RS-232C
SDI-12

Software: Works with an IBM PC compatible computer, with 3 1/2 or 5 1/4 inch, high or low density floppy disk drive.
Minimum RAM requirement: 256K
Optional graphic adaptor for plotting

Internal logging memory size: 256 kilobytes 130,000 individual readings

Power: 12 VDC - 8 C-size Alkaline Batteries (included)
External 12 VDC (optional)

1.3 SENSOR SPECIFICATIONS

See also section 1.2 General Specifications. Performance testing of the YSI Model 6000 suggests the following typical performance.

Depth
Sensor Type...........Stainless steel strain gage
Range.................0 to 500 feet
Accuracy.............. +/- 1.5 feet
Resolution............0.1 foot

Temperature
Sensor Type...........Thermistor
Range.................-5 to 45 C
Accuracy.............. +/- 0.4 C
Resolution............0.1 C

Dissolved Oxygen % saturation
Sensor Type...........Rapid Pulse - polarographic
Range.................0 to 200 % air saturation
Accuracy.............. +/- 2 % air saturation
Resolution............0.1 % air saturation

Dissolved Oxygen mg/L
Sensor Type...........Calculated from % air saturation, temperature and salinity.
Range.................0 to 20 mg/L
Accuracy.............. +/- 0.2 mg/L
Resolution............0.01 mg/L

Conductivity*
Sensor Type............4 electrode cell
Range..................0 to 100 mS/cm
Accuracy............... +/-(1% of reading + 0.05 mS/cm)
Resolution.............0.01 mS/cm or 1 uS/cm

*Report outputs of specific conductance (conductivity corrected to 25 C), resistivity and total dissolved solids are also provided. These values are automatically calculated from conductivity according to algorithms found in *Standard Methods for the Examination of Water and Wastewater* (ed 1989).

Salinity

Sensor Type..........Calculated from conductivity and temperature
Range................0 to 70 ppt
Accuracy............ +/-0.2 ppt
Resolution...........0.1 ppt

pH

Sensor Type..........Glass combination electrode
Range................2 to 14 units
Accuracy............ +/- 0.2 units
Resolution...........0.01 units

ORP

Sensor Type...........Platinum electrode
Range................-999 to 999 mV
Accuracy.............. +/-20 mV
Resolution............1 mV

1.4 HOW TO USE THIS MANUAL

This manual provides information for operating and servicing the YSI Model 6000 Environmental Monitoring System. Chapters 1 through 3 provide an overview of setup, calibration, and operational procedures. These first three chapters should provide enough information for you to understand the basic capabilities of the Model 6000 system and begin sampling. Chapters 4 through 9 provide a more detailed explanation of system operations, software, principles of operation, maintenance, and performance troubleshooting. Chapter 10 is written for authorized service technicians and provides necessary information for repairing the Model 6000. Appendix A-E provide information about health and safety, warranty, accessories, and options.

This manual is organized to give you a quick start into understanding and operating the YSI Model 6000 system. However, it cannot be stressed too strongly that informed and safe operation is more than just knowing which buttons to push. An understanding of the principles of operation, calibration techniques, and system setup is necessary to obtain accurate and meaningful results. Regular maintenance is required to keep the YSI Model 6000 functioning effectively. Precautions regarding the handling of reagents are also essential for the safety of system operators (see appendix A for health and safety information).

The early parts of this manual will teach you how to get the Model 6000 system running. Additional topics are included to help you understand the science it employs, how to use it most effectively and safely, and how to keep it operating correctly.

2. INITIAL SETUP

2.1 UNPACKING

Remove the instrument from the shipping container. Be careful not to discard any parts or supplies. Check off all items on the packing list and inspect all assemblies and components for damage. If any parts are damaged or missing, contact your Dealer Representative immediately. If you do not know from which dealer your YSI Model 6000 was ordered, call YSI Customer Service for assistance, see appendix C.

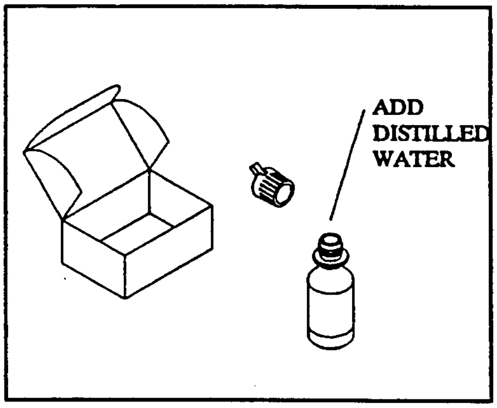
Note: Reagents for the Model 6000 are not packaged in the same carton as the instrument. These materials must be ordered separately and will arrive in a separate package.

2.2 SYSTEM SETUP

YSI 6030 DO/COND/TEMP PROBE

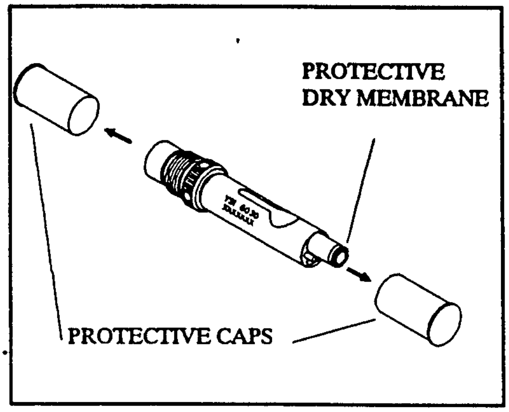
Follow these instructions to prepare your new probe and install it in the Model 6000.

1. Open the membrane kit and prepare the electrolyte. Dissolve the KCl crystals in the dropper bottle by filling it to the neck with distilled water and shaking until the solid is fully dissolved.

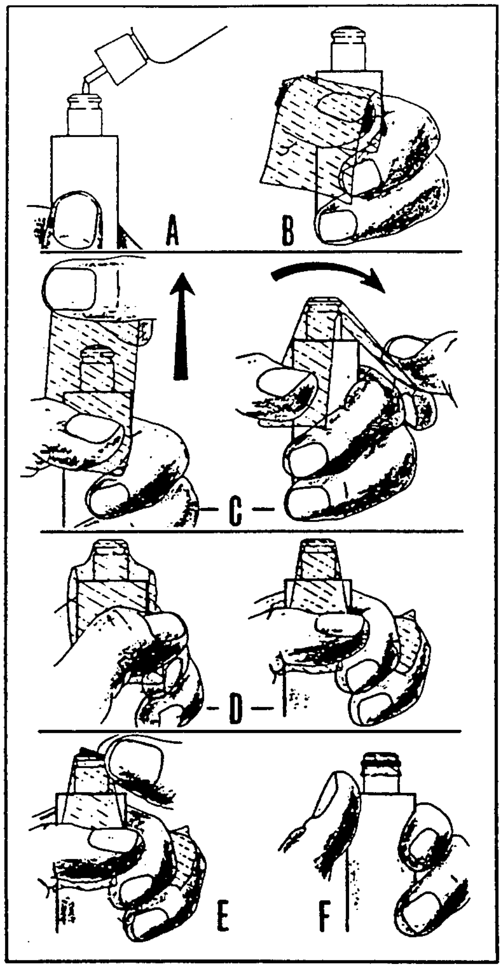


2. Remove the two protective caps and the dry membrane from the 6030 probe.

NOTE: Your probe is shipped with a protective dry membrane on the dissolved oxygen sensor tip. It is very important not to scratch or contaminate the sensor tip. Handle the new probe with care. Avoid touching or accidentally hitting the sensor tip.



3. Follow the procedure described below to install a new membrane on the dissolved oxygen sensor tip.



A. Hold the probe in a vertical position and apply a few drops of KCl solution to the tip. The fluid should completely fill the small moat around the electrodes and form a meniscus on the tip of the sensor. Be sure no air bubbles are stuck to the face of the sensor. If necessary, shake off the electrolyte and start over.

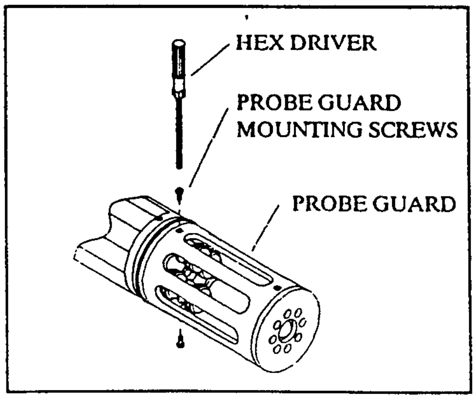
B. Secure a membrane between your left thumb and the probe body. Always handle the membrane with care, touching it at the ends only.

C. With the thumb and forefinger of your right hand, grasp the free end of the membrane. With one continuous motion, gently stretch it up, over, and down the other side of the sensor. The membrane should conform to the face of the sensor.

D. Secure the end of the membrane under the forefinger of your left hand.

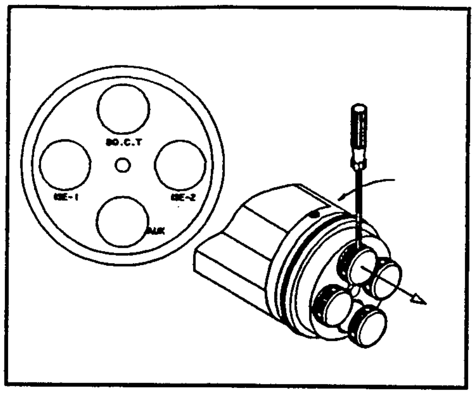
E. Roll the O-ring over the end of the probe, being careful not to touch the membrane surface with your fingers. There should be no wrinkles or trapped air bubbles. Small wrinkles may be removed by lightly tugging on the edges of the membrane.

F. Trim off any excess membrane with a sharp knife or scissors. Make sure the stainless steel temperature sensor is not covered by excess membrane. Being careful not to get water in the connector, rinse off the excess KCl solution.

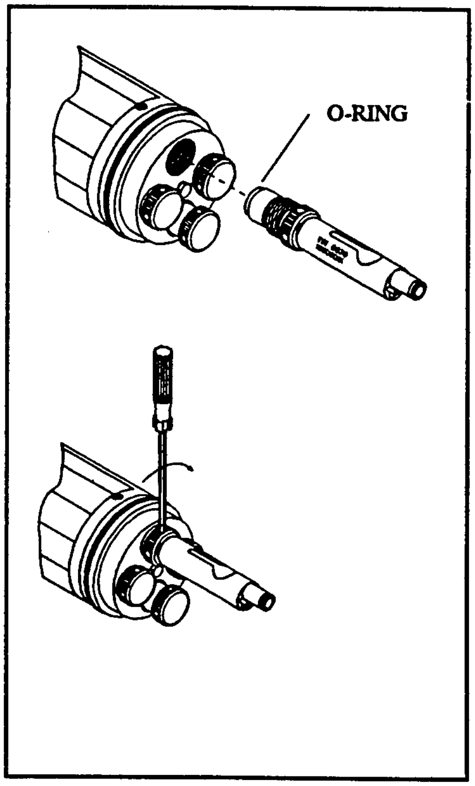


4. Using the hex driver supplied in the 6040 Maintenance Kit, remove the probe guard mounting screws from the sonde. Set the probe guard aside.

NOTE: Do not remove the two allen screws above the guard.



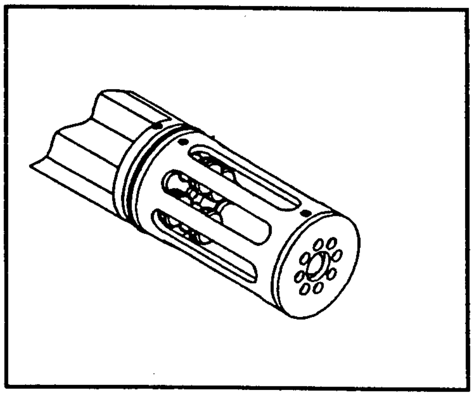
5. Locate the plug which seals the DO.C.T port on the sonde. Using the hex driver to assist you, remove the plug.



6. Install the 6030 probe into the sonde as described below.

A. Apply a very thin coat of O-ring lubricant (supplied in the 6040 Maintenance Kit) to the O-ring on the connector side of the probe. Insert the probe in the port marked DO.C.T.

B. With the connectors aligned, screw down the probe nut. Use the hex driver to assist you in tightening the nut. **DO NOT OVER TIGHTEN.**



7. Replace the probe guard and tighten the probe guard mounting screws.

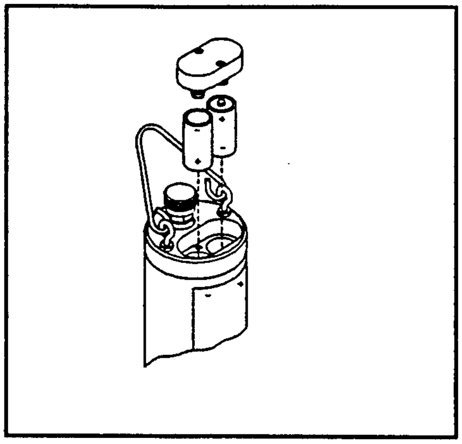
OPTIONAL PROBES

If you have purchased any optional YSI probes, follow the instruction sheet provided with your probe(s) for preparation and installation into the Model 6000. The Model 6000 comes standard with a 6030 Dissolved Oxygen / Conductivity / Temperature probe. 6031 pH, or 6032 ORP probes can be ordered as options and do not require factory installation (see section 4.7 for software setup). A depth sensor is a factory installed option.

INSTALL BATTERIES

Your first set of batteries is supplied with the instrument. Install 8 C-size alkaline batteries as follows:

Use the hex driver, supplied in the 6040 Maintenance Kit, to loosen the battery lid screws.



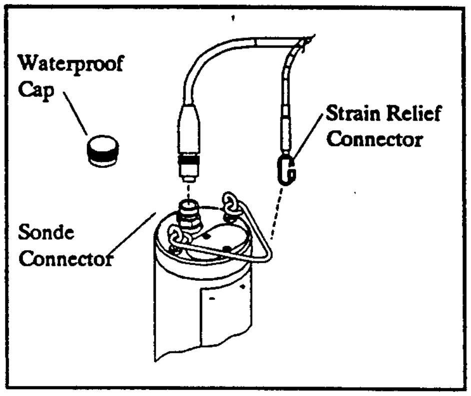
NOTE: The battery lid screws are "captive", it is not necessary to remove them from the lid completely.

Remove the battery lid and install the batteries, as shown.

NOTE: Observe the correct polarity before installing the batteries into the battery chamber.

Be sure the O-ring is installed in the groove of the lid. Check the O-ring and sealing surfaces for any contaminates which could interfere with the O-ring seal of the battery chamber. Remove any contaminates present. Apply a very thin coat of O-ring lubricant (supplied in the 6040 Maintenance Kit) to the O-ring inside the battery chamber lid. Return the battery lid and tighten the screws. DO NOT OVER TIGHTEN.

ATTACH CABLE



Remove the water proof cap from the sonde connector and set aside for later reassembly. Connect the PC interface cable to the sonde connector. A built-in "key" will ensure proper pin alignment. Connect the DB-25 end of the cable to an RS-232 serial port in your computer. Attach the strain relief connector to the sonde handle. Rotate the strain relief connector nut to close the connector's opening.

2.3 SOFTWARE INSTALLATION

PC6000 software is provided with every Model 6000 and is in the back of this instruction manual. Use this software if you have an IBM compatible PC with at least 256KB of RAM and DOS 3.0 or later. If your system is not IBM compatible, use any terminal emulation program with your computer.

Insert the disk into your floppy disk drive. At the C:\ prompt, type the letter of the drive in which the program disk was inserted followed by a colon, then press **Enter**.

Example: A: **Enter.**

To install PC6000 software execute the following command from the DOS prompt:

```
INSTALL     <destination>
```

where "destination" is the drive and directory in which you want the PC6000 files to be installed. For example, the command:

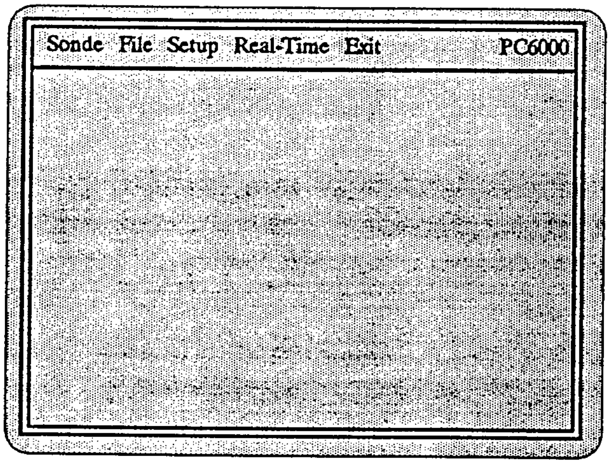
```
INSTALL C:\PC6000
```

will install the PC6000 software to the C: drive and \PC6000 directory.

If you are using a two floppy disk drive system, follow the instructions in section 5.2. After installing the software, remove the disk from the floppy drive and keep the original disk in a safe place.

2.4 SOFTWARE SETUP

To start the software, make your current drive C:\PC6000. Type **PC6000** and press **Enter**. The PC6000 software will load. The following menu will appear at the top of the computer screen:



Use the arrow keys to move the cursor and highlight menu options. Press **Enter** to select a highlighted option. Press **Esc** to cancel an entry. To start, highlight **Setup** then press **Enter.** Check the default setup values.

Comm port:	1	Printer port:	1
Baud rate:	9600	Printer type:	Epson dot-matrix
Date format:	MDY	Menu Colors:	Scheme 1 (mono/LCD)
Date separator:	/	Plot colors:	Scheme 1 (mono)
Time separator:	:	Graphics mode:	Automatic selection hi-res
Radix mark:	.		
CDF delimiter:	,	Export file format:	PRN
		Default scaling technique:	A
Real-Time Setup		Default parameters per plot:	2
Advanced Setup			

Comm port:

Select this option to enter the number of the Comm port (1 or 2) to which the Model 6000 is connected. Be sure to press **Enter** to confirm a new entry. If the default setting is correct there is no need to change it.

Baud rate:

Select this option to check the baud rate. If the baud rate is not set to 9600, set it to 9600. To set the baud rate, highlight it and press **Enter.** A list of possible baud rates will appear. Select 9600 from the list and press **Enter.**

Printer port:

Select this option to specify the parallel port (LPT1,LPT2 or LPT3) to which your printer is connected. If no printer is connected select LPT1.

Printer type:

Select this option to select the type of printer connected to your computer system. From the list, choose the printer that best describes your printer. If your printer is not on the list, refer to your printer's instruction manual to determine what type of printer your printer emulates.

Menu colors:

Select this option to choose a color scheme for the PC6000 menus. As you move the cursor between color schemes, the screen changes to display your selection. Press **Enter** to confirm a selection.

Plot colors:

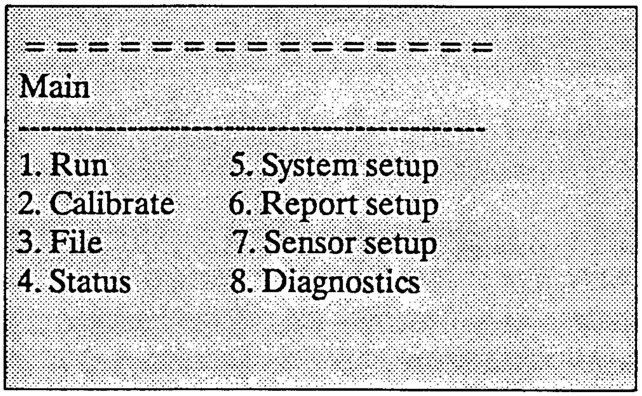
Select this option to choose a color scheme for the PC6000 plots. As you move the cursor between color schemes, the screen changes to display your selection. Press **Enter** to confirm a selection.

The other setup functions are described in section 5.5, but usually are not necessary for a quick start into Model 6000 operations. Press **Esc** to exit the setup menu. The cursor will return to the top line menu.

2.5 SONDE SETUP

There are two sets of software at work within the Model 6000 system. One is resident in your PC and was provided on floppy disk with this instruction manual. The other software is resident in the sonde itself. When you select **Sonde** from the PC6000 top-line menu, you are exiting the PC based software, and are entering the sonde based software.

Highlight and select **Sonde** from the top-line menu. The sonde Main menu will be displayed.



```
================
Main
------------------------------------------
1. Run          5. System setup
2. Calibrate    6. Report setup
3. File         7. Sensor setup
4. Status       8. Diagnostics
```

NOTE: If no menu is displayed and a # appears at the top of the computer screen, type **MENU** and press **Enter.** If a menu other than the Main menu is displayed, press ESC until the Main menu appears. If you can not get to the Main menu, check to be sure the cable is properly connected, the batteries are properly installed and the Comm port and other software parameters are setup as described in Section 2.4, or see Chapter 8, Troubleshooting.

NOTE: The sonde software is menu driven. Select a function by typing its corresponding number. It is not necessary to press **Enter** after a number selection. Use the **0** or **Esc** key to return to a previous menu.

At the Main menu, select **5. System setup**. The System setup menu will be displayed.

```
==============
System-setup
-----------------------------
1. Date & time
2. Radix
3. Communication
4. Instrument ID
```

1. Date & time

Select this option to choose the date format and set the correct date and time. Select the format you desire; month/day/year, day/month/year or year/month/day. Next enter the date and time as prompted on the screen.

Note: For convenience any non-numeric key (such as **space**) in addition to / and : will separate the fields in the date and time entries.

4. Instrument ID

Select this option to identify your instrument with its serial number (located on the back of the sonde) or any other name or number you wish, up to 15 characters. Then press **Enter.**

Press Esc to backup to the Main menu. See Chapter 4 for a more detailed description of the sonde menus. The sonde is now setup and ready to calibrate and run. Proceed to Chapter 3.

3. BASIC OPERATION

In the previous chapter, you learned how to install probes and setup the PC6000 and sonde software. In this chapter, you will learn how to calibrate and run the Model 6000 and upload and process data.

3.1 CALIBRATION TIPS

WARNING: Reagents used to calibrate and check this instrument may be hazardous to your health. Refer to appendix A for health and safety information.

Before you begin the calibration procedures outlined below, you may find it helpful to follow some or all of these calibration tips.

1. Remove and retain the two allen screws at the very bottom of the sonde guard. Remove the bottom plate of the sonde guard (not the entire guard). This allows the calibration solutions access to the probes with minimal displacement of fluid within the calibration cup. Additionally, "carry-over" from one solution to the next is reduced.

2. Fill a 2 1/2 gallon bucket with cool tap water for rinsing the sonde between calibration solutions.

3. Have several clean, absorbent paper towels or cotton cloths available to dry the sonde between rinses and calibration solutions. It is important to remove as much residual liquid as possible from the sonde after each rinse. Drying the sonde and probe in this way reduces carry-over contamination of calibrator solutions and increases the accuracy of the calibration.

4. Fill each of the three calibration cups (supplied) as follows:

The first with a conductivity standard which is in the range of the water to be sampled; see the conductivity section below.

The second with a pH 7 buffer solution.

The third with a pH buffer which is in the range of the water to be sampled; see the pH section below.

3.2 CALIBRATION PROCEDURES

WARNING: Calibration reagents may be hazardous to your health. Refer to appendix A for health and safety information.

From the sonde Main menu select **2. Calibrate.** The Calibrate menu will be displayed.

```
===============
Calibrate
------------------------------
1. Conductivity    4. pH 2 point
2. DO %            5. pH 3 point
3. DO mg/L         6 Depth
```

Three calibration cups are supplied with the Model 6000. Because the calibration cups fit over the outside of the sonde probe guard, it is not necessary to remove the guard to calibrate the sensors. Follow the procedures below to calibrate the sensors. There are two procedures for calibrating the dissolved oxygen and pH sensors. In this chapter, we only discuss **DO %** and **pH 2 point** calibration procedures. Temperature and ORP probes do not require calibration and are, therefore, not included in the Calibrate menu. For other calibration procedures, see section 4.2.

CONDUCTIVITY -

Note: This procedure calibrates not only conductivity, but also specific conductance, salinity, and total dissolved solids.

Place 500 ml (approximately 1 pint) of conductivity standard in a clean calibration cup. The conductivity standard you choose should be within the same conductivity range as the water you are preparing to sample. For example:

- For sea water choose a 50mS/cm conductivity standard.
- For fresh water choose a 1mS/cm conductivity standard.
- For brackish water choose a 10mS/cm conductivity standard.

Without removing the sonde guard, **slowly and carefully** immerse the probe end of the sonde into the solution. Gently rotate and/or move the sonde up and down to remove any bubbles from the conductivity cell.

Allow at least 1 minute for temperature equilibration before proceeding.

Select 1. **Conductivity** to access the Conductivity calibration procedure.

```
Enter SpCond in mS/cm (10.000):
```

Enter the calibration value of the standard you are using (mS/cm at 25 C) and press **Enter.**

NOTE: If an ERROR message appears, begin the conductivity calibration procedure again. Be certain that the value you enter for the calibration standard is correct. Also see Chapter 8, Troubleshooting.

After the calibration procedure is complete, press any key, as instructed on the screen, to return to the Calibrate menu.

Rinse the sonde in cool tap water and gently dry the sonde guard and probes. Be very careful not to touch the membrane on the DO probe.

pH 2 POINT -

Place 500 ml (approximately 1 pint) of pH 7 buffer in a clean calibration cup. **Slowly and carefully** immerse the probe end of the sonde into the solution.

Allow at least 1 minute for temperature equilibration before proceeding.

Select **4. pH 2 point** to access the pH 2 point calibration procedure. Press any key to calibrate to pH 7.

After the pH 7 calibration is complete, press any key, as instructed on the screen, to continue.

Rinse the sonde in cool tap water and gently dry the sonde guard and probes. Be very careful not to touch the membrane on the DO probe.

Place 500 ml (approximately 1 pint) of a second pH buffer solution in a clean calibration cup. The second buffer might be pH 4 if the sample is expected to be acidic; or pH 10 if the sample is expected to be basic. **Slowly and carefully** immerse the probe end of the sonde into the solution.

Allow at least 1 minute for temperature equilibration before proceeding.

Press any key to continue. When prompted on the screen, enter the value of the pH buffer (in this case, 4 or 10), and press **Enter.**

After the second value calibration is complete, press any key, as instructed on the screen, to return to the Calibrate menu.

Rinse the sonde in cool tap water and gently dry the sonde guard and probes. Be very careful not to touch the membrane on the DO probe.

Thoroughly rinse and dry the calibration cups for future use.

DISSOLVED OXYGEN -

Replace the sonde guard bottom plate, which may have been removed to simplify conductivity or pH calibration. Place a wet sponge inside a clean, empty calibration cup. Place the probe end of the sonde into the calibration cup. The oxygen probe will now be in water-saturated air.

Press ESC to return to the sonde Main menu. Select **1. Run** from the sonde Main menu to view the Run menu. Select **1. Discrete sample** from the Run menu to view the Run Discrete-sample menu. Select **3. Sample interval** from the Run Discrete-sample menu. Set the interval to 4 seconds and press **Enter.** Select **1. Start discrete sample** from the Run Discrete-sample menu. After 4 seconds have passed data will begin to display on the screen. Wait 10 - 15 minutes. During these 10 - 15 minutes the calibration chamber will reach 100% water-saturated air, and the D.O. sensor will warm-up and stabilize. After 10 - 15 minutes use the ESC key to return to the sonde Main menu.

Select **2. Calibrate** from the sonde Main menu to view the Calibrate menu. Select **2. DO %**, from the Calibrate menu, to access the DO % calibration procedure. Enter the current barometric pressure in atmospheres, e.g., 745 mm Hg is equivalent to 745/760 or 0.98 atm. Press **Enter** and the computer will indicate that the calibration procedure is in process.

After approximately 1 minute, the calibration will be complete. Press any key, as instructed on the screen, to return to the Calibrate menu.

Note: Calibration of dissolved oxygen in the DO % procedure also results in calibration of the DO mg/L mode and vice versa.

DEPTH -

Keep the sonde in the air -- do not submerge it.

Select **6. Depth** to access the depth calibration procedure. Press any key to zero the depth sensor to local barometric pressure.

After depth is zeroed, press any key, as instructed on the screen, to return to the Calibrate menu.

The sensors are now calibrated. Press Esc until the sonde Main menu is displayed.

3.3 DISCRETE SAMPLING

Use the Discrete sampling mode to turn on or turn off the continuous sampling function. Data will be displayed to the screen and optionally to internal flash disk memory.

Select **1. Run** from the sonde Main menu. The Run menu will be displayed.

```
================
Run
------------------------------
1. Discrete sample
2. Conditional sample
3. Unattended sample
```

Select **1. Discrete sample** from the Run menu. The Run Discrete- sample menu will be displayed.

```
=================
Run Discrete-sample
------------------------------
1. Start discrete sample
2. Site description
3. Sample interval
4. Close file
5. Set Auto-stop timer
```

Select the different functions in this menu to become familiar with the Discrete sampling mode. Remember to use **Esc** to cancel functions or to backup to the previous display.

```
1. Start discrete sample
```

Select this option to start discrete sampling. After the initial sampling time interval has passed, data will be displayed on the screen. A single line of data can be logged to flash disk memory by pressing **1.** (screen prompt reads '1-LOG last sample'). A set of data can be logged to flash disk by pressing **2.** (screen prompt reads '2-LOG ON/OFF'). A message will be displayed that LOG is on, turn it off by selecting **2**. Select Esc or **0** to exit discrete sampling.

2. Site description

Select this option to name the site of the sample if you intend to log data while discrete sampling. Up to 31 characters can be entered. The site description you enter will be displayed with the file name to help you identify the file later.

3. Sample interval

Select this option to set the interval which will pass between samples. Enter the desired interval in seconds. The default sample interval is 4 seconds.

4. Close file

Select this option to close any discrete sample file. A file is opened automatically whenever data is logged to flash disk memory. When you exit discrete sample mode, you can close the file using this function or you can leave it open and log to it later. If you try to setup an unattended sample study or open a new discrete sample file you will be asked if you wish to close the discrete sample file. In order to open a new file, any other open file must first be closed.

5. Set Auto-stop timer

Select this option to setup a timer to automatically turn off discrete sampling. If you begin a discrete sample and forget to turn it off later battery life will be greatly reduced. For this reason, the **Auto-stop timer** can be set up to automatically turn off any active discrete sample if it has been left on for a specified period of time. Simply type in what you feel is an appropriate amount of minutes, and the program will turn off discrete sampling after that many minutes have passed without activity. Setting the timer to **0** disables the Auto-stop timer, thus discrete sampling will never be stopped by the Auto-stop timer.

Press Esc until the sonde Main menu is displayed.

3.4 UNATTENDED SAMPLING

The unattended sampling mode is designed to log accurate readings of all user-defined parameters at intervals you specify. The Model 6000 can be deployed for weeks at a time using this feature. Throughout the deployment, data is stored to internal flash disk memory. This data can be uploaded to the PC6000 software upon completion of the study, or at anytime during the study.

Select 1. **Run** from the sonde Main menu. The Run menu will be displayed.

```
================
Run
------------------------------------------
1. Discrete sample
2. Conditional sample
3. Unattended sample
```

Select 3. **Unattended sample** from the Run menu. The current time and date, all active sensors, battery voltage, and free flash disk space will be displayed.

NOTE: If the current time and date are not correct, your unattended sampling study will not begin or end when you desire. To correct the time and date; see section 2.5.

You will be asked to enter the following information concerning the unattended sampling study you wish to setup.

```
Enter starting date (XX/XX/XX):
Enter starting time (XX:XX:XX):
Enter duration in days (XX):
Enter interval in minutes (1):
Enter site description ():
```

To gain experience, setup an unattended sampling study with the following responses to the above questions:

Enter starting date: *today's date*
Enter starting time: *current time*
Enter duration in days: *1*
Enter interval in minutes: *2*
Enter site description: *First-test*

You will be asked if all start-up information is correct. Check the information **closely**, if you want to change something press **N** and **Enter**. If all information is correct press **Y** and **Enter**. The following message will be displayed briefly:

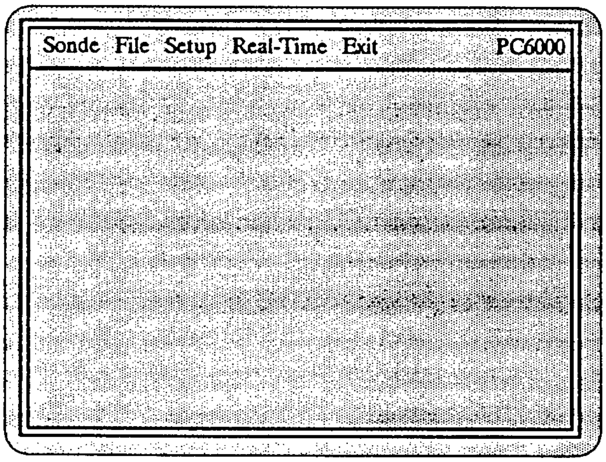
```
********************************************
*INSTRUMENT IS IN UNATTENDED MODE *
********************************************
```

To complete the "first-test" you have just started, disconnect the cable from the sonde and screw on the waterproof connector cap. The unit is now ready for deployment. If the calibration cup is installed, remove it and place the Model 6000 in at least 6 inches of water. Leave it there for an hour. Proceed to the next section.

3.5 RECOVERING DATA FROM THE MODEL 6000

Attach the PC cable to the Model 6000. Connect the other end of the cable to the serial port of your computer.

Boot the PC6000 software and select **Sonde** from the top-line menu.



NOTE: If access to the sonde is denied, first check all cable connections and the comm port information under **Setup** of the PC6000 top-line menu. If all cable connectors are properly attached and the comm port setting is correct, it is likely that the sonde batteries have been depleted. To replace the sonde batteries see section 2.2.

From the sonde Main menu select **1. Run.**

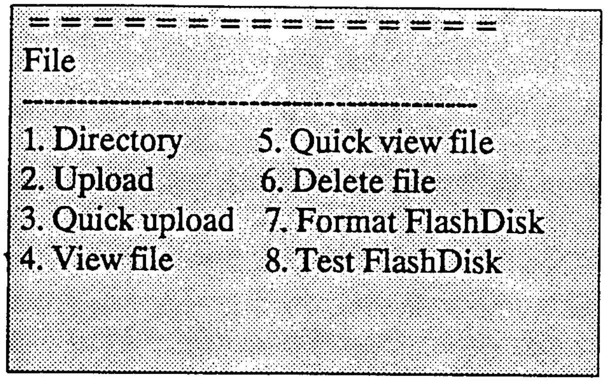
NOTE: If the instrument is busy taking a sample it will display the following message: **Instrument is busy. Please wait.**

Wait until the Run menu appears and select **3. unattended sample.** The screen will display a prompt asking if you wish to cancel the unattended sampling study. Press Y and **Enter.**

NOTE: Data can be uploaded while an unattended sampling study is in progress.

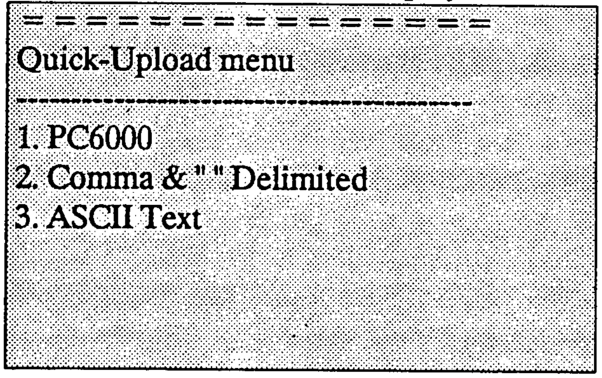
NOTE: If unattended sampling has already stopped automatically because the "duration" you entered earlier has expired, press ESC twice to return to the Main menu.

Return to the sonde Main menu using the **Esc** key. Select **3. File** to display the File menu.



```
================
File
------------------------------------------
1. Directory      5. Quick view file
2. Upload         6. Delete file
3. Quick upload   7. Format FlashDisk
4. View file      8. Test FlashDisk
```

Select **3. Quick upload** to display the Quick-Upload menu.



```
================
Quick-Upload menu
------------------------------------------
1. PC6000
2. Comma & " " Delimited
3. ASCII Text
```

Select **1. PC6000** to upload to the PC6000 software. A secondary menu will appear to show the progress of the upload.

NOTE: Comma & " " Delimited and ASCII Text formats are available for uploading data to a spreadsheet or database program. See section 4.3 for more details.

When the upload is complete, press **Esc** until the sonde Main menu returns. Press **F10** to exit the sonde menu and return to the PC6000 top-line menu.

3.6 LOADING A FILE

Select **File** from the top-line menu.

Sonde File Setup Real-Time Exit YSI PC6000

A box will appear on the screen asking you to enter a filename.

Filename: *.dat

You may type a file name or press **Enter** for a directory. If the directory contains more than one data file, a list of files will be displayed. Use the arrow keys to highlight and the **Enter** key to select the file of choice.

NOTE: If only one data file is resident in the directory, it will be automatically loaded.

After the selected file has been retrieved, the following menu will be displayed:

MODIFY	VIEW	SYSTEM
Parameters	Header	Print Report
Control Info	Report	Print Plot
Scaling Info	Plot	Definitions
	Statistics	Export

Any changes made from this menu will effect only the current file. To change default values for all files, see section 5.5.

Header

Select this option to view general information about the file. Information displayed on the header screen includes the file name, site description, a list of active sensors, and other details about this file. Press **Esc** to return to the File menu.

Report

Select this option to view a time-stamped report of the data collected during the study. Press **Esc** to return to the File menu.

Plot

Select this option to view a plot of the data collected during the study. Press **Esc** until the top-line menu of the PC6000 program appears.

All plots and reports can be customized to your specific requirements, see section 5.4 for details.

6.3 OXIDATION REDUCTION POTENTIAL (ORP)

The Model 6000 determines the Oxidation-Reduction Potential (ORP) of the media by measuring the difference in potential between an electrode which is relatively chemically inert and a reference electrode. The Model 6000 utilizes a field-replaceable combination probe with a platinum tip and an Ag/AgCl reference electrode which utilizes gelled electrolyte. ORP values are presented in millivolts and are not compensated for temperature.

CALIBRATION

No calibration as such is required for the ORP readings of the Model 6000. However, to determine if the probe is functioning properly, the sensor can be immersed in a solution of known ORP. A solution consisting of a mixture of potassium ferricyanide and potassium ferrocyanide in 0.1 M KCl (ZoBell solution) is available from YSI for this accuracy check. The ORP output should be in the 221 to 241 mV range for the ZoBell solution if the ORP probe is within the manufacturer's specifications.

MEASUREMENT PRECAUTIONS

(1) Clean and store the probe according to the manufacturer's instructions.

APPENDIX A-8

Procedure for Temperature: Method 170.1

Temperature - Method 170.1

1.0 Procedure

Perform temperature measurements in accordance with "Temperature" Method 170.1 (Thermometric) as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, and notes.

3.0 Quality Control Samples

None

TEMPERATURE

Method 170.1 (Thermometric)

STORET NO. 00010

1. Scope and Application
    1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
2. Summary of Method
    2.1 Temperature measurements may be made with any good grade of mercury-filled or dial type centigrade thermometer, or a thermistor.
3. Comments
    3.1 Measurement device should be routinely checked against a precision thermometer certified by the National Bureau of Standards.
4. Precision and Accuracy
    4.1 Precision and accuracy for this method have not been determined.
5. Reference
    5.1 The procedure to be used for this determination is found in:
    Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 125, Method 212 (1975).

Approved for NPDES
Issued 1971

APPENDIX A-9

Procedure for pH: Method 150.1 or Method 150.2

Table of Contents – Appendix A-9

pH - Method 150.1 or Method 150.2

1.0 Procedure

Perform pH measurements by either Method 150.1 or 150.2 (attached) as appropriate.

2.0 Recordkeeping

Retain all machine printouts, worksheets, and notes.

3.0 Quality Control Samples

Periodically, reanalyze calibration buffers.

pH

Method 150.1 (Electrometric)

STORET NO.
Determined on site 00400
Laboratory 00403

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes and acid rain (atmospheric deposition).
2. Summary of Method
   2.1 The pH of a sample is determined electrometrically using either a glass electrode in combination with a reference potential or a combination electrode.
3. Sample Handling and Preservation
   3.1 Samples should be analyzed as soon as possible preferably in the field at the time of sampling.
   3.2 High-purity waters and waters not at equilibrium with the atmosphere are subject to changes when exposed to the atmosphere, therefore the sample containers should be filled completely and kept sealed prior to analysis.
4. Interferences
   4.1 The glass electrode, in general, is not subject to solution interferences from color, turbidity, colloidal matter, oxidants, reductants or high salinity.
   4.2 Sodium error at pH levels greater than 10 can be reduced or eliminated by using a "low sodium error" electrode.
   4.3 Coatings of oily material or particulate matter can impair electrode response. These coatings can usually be removed by gentle wiping or detergent washing, followed by distilled water rinsing. An additional treatment with hydrochloric acid (1 + 9) may be necessary to remove any remaining film.
   4.4 Temperature effects on the electrometric measurement of pH arise from two sources. The first is caused by the change in electrode output at various temperatures. This interference can be controlled with instruments having temperature compensation or by calibrating the electrode-instrument system at the temperature of the samples. The second source is the change of pH inherent in the sample at various temperatures. This error is sample dependent and cannot be controlled, it should therefore be noted by reporting both the pH and temperature at the time of analysis.
5. Apparatus
   5.1 pH Meter-laboratory or field model. A wide variety of instruments are commercially available with various specifications and optional equipment.

Approved for NPDES
Issued 1971
Editorial revision 1978 and 1982

5.2 Glass electrode.

5.3 Reference electrode–a calomel, silver-silver chloride or other reference electrode of constant potential may be used.

NOTE 1: Combination electrodes incorporating both measuring and reference functions are convenient to use and are available with solid, gel type filling materials that require minimal maintenance.

5.4 Magnetic stirrer and Teflon-coated stirring bar.

5.5 Thermometer or temperature sensor for automatic compensation.

6. Reagents

6.1 Primary standard buffer salts are available from the National Bureau of Standards and should be used in situations where extreme accuracy is necessary.

6.1.1 Preparation of reference solutions from these salts require some special precautions and handling(1) such as low conductivity dilution water, drying ovens, and carbon dioxide free purge gas. These solutions should be replaced at least once each month.

6.2 Secondary standard buffers may be prepared from NBS salts or purchased as a solution from commercial vendors. Use of these commercially available solutions, that have been validated by comparison to NBS standards, are recommended for routine use.

7. Calibration

7.1 Because of the wide variety of pH meters and accessories, detailed operating procedures cannot be incorporated into this method. Each analyst must be acquainted with the operation of each system and familiar with all instrument functions. Special attention to care of the electrodes is recommended.

7.2 Each instrument/electrode system must be calibrated at a minimum of two points that bracket the expected pH of the samples and are approximately three pH units or more apart.

7.2.1 Various instrument designs may involve use of a "balance" or "standardize" dial and/or a slope adjustment as outlined in the manufacturer's instructions. Repeat adjustments on successive portions of the two buffer solutions as outlined in procedure 8.2 until readings are within 0.05 pH units of the buffer solution value.

8. Procedure

8.1 Standardize the meter and electrode system as outlined in Section 7.

8.2 Place the sample or buffer solution in a clean glass beaker using a sufficient volume to cover the sensing elements of the electrodes and to give adequate clearance for the magnetic stirring bar.

8.2.1 If field measurements are being made the electrodes may be immersed directly in the sample stream to an adequate depth and moved in a manner to insure sufficient sample movement across the electrode sensing element as indicated by drift free (< 0.1 pH) readings.

8.3 If the sample temperature differs by more than 2°C from the buffer solution the measured pH values must be corrected. Instruments are equipped with automatic or manual

---

(1) National Bureau of Standards Special Publication 260.

compensators that electronically adjust for temperature differences. Refer to manufacturer's instructions.

8.4 After rinsing and gently wiping the electrodes, if necessary, immerse them into the sample beaker or sample stream and stir at a constant rate to provide homogeneity and suspension of solids. Rate of stirring should minimize the air transfer rate at the air water interface of the sample. Note and record sample pH and temperature. Repeat measurement on successive volumes of sample until values differ by less than 0.1 pH units. Two or three volume changes are usually sufficient.

8.5 For acid rain samples it is most important that the magnetic stirrer is not used. Instead, swirl the sample gently for a few seconds after the introduction of the electrode(s). Allow the electrode(s) to equilibrate. The air-water interface should not be disturbed while measurement is being made. If the sample is not in equilibrium with the atmosphere, pH values will change as the dissolved gases are either absorbed or desorbed. Record sample pH and temperature.

9. Calculation

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

10. Precision and Accuracy

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

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

(FWPCA Method Study 1, Mineral and Physical Analyses)

10.2 In a single laboratory (EMSL), using surface water samples at an average pH of 7.7, the standard deviation was ±0.1.

Bibliography

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 460, (1975).
2. Annual Book of ASTM Standards, Part 31, "Water", Standard D1293–65, p 178 (1976).
3 Peden, M. E. and Skowron, L. M., Ionic Stability of Precipitation Samples, Atmospheric Environment, Vol. 12, pp. 2343-2349, 1978.

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

pH, Continuous Monitoring (Electrometric)—Method 150.2

1. Scope and Application

1.1 This method is applicable to the continuous pH measurement of drinking, surface, and saline waters, domestic and industrial waste waters.

2. Summary of Method

2.1 The pH of a sample is determined electrometrically using a glass electrode with a reference electrode or a single combination electrode.

3. Sample Handling and Preservation

3.1 The composition of the water or waste contacting the measuring electrode system must be representative of the total flow from the water body.

4. Interferences

4.1 The glass electrode, in general, is not subject to solution interferences from color, turbidity, colloidal matter, oxidants, reductants or high salinity

4.2 Sodium error at pH levels greater than 10 can be reduced or eliminated by using a "low sodium error" electrode.

4.3 Manually inspect the conditions of the electrodes every 30 days for coating by oily materials or buildup of lime. If oil and grease and/or scale buildup are not present, this time interval may be extended.

4.3.1 Coatings of oil, grease and very fine solids can impair electrode response. These can usually be removed by gentle wiping and detergent washing. The use of flow-through electrode housings which provide higher flow velocity helps to prevent the coating action.

4.3.2 Heavy particulate matter such as lime accumulation can be removed by careful scrubbing or immersion in dilute (1+9) hydrochloric acid. Continuous monitoring under these conditions benefits from ultrasonic or other in-line continuous cleaning methods.

4.4 Temperature effects on the electrometric measurement of pH arise from two sources. The first is caused by the change in electrode output at various temperatures. This interference can be controlled with instruments having temperature compensation or by calibrating the electrode-instrument system at the temperature of the samples. For best results, meters having automatic temperature compensation should be calibrated with solutions within 5°C of the temperature of the stream to be measured. The second source is the change of pH inherent in the sample at various temperatures. This error is sample dependent and cannot be controlled, it should therefore be noted by reporting both the pH and temperature at the time of analysis.

5. Apparatus

5.1 pH Monitor - A wide variety of instruments are commercially available with various specifications and optional equipment. For unattended use, the monitor should be equipped with automatic or fixed temperature compensation and with a recorder or alarm function.

5.2 Glass electrode - with shielded cable between electrode and monitor unless preamplification is used.

[illegible] Reference electrode - a [illegible]ence electrode with a constant potential and with either a visible electrolyte or viscous gel fill.
NOTE 1: Combination electrodes incorporating both measuring and reference functions are convenient to use and are available with solid, gel-type filling materials that require minimal maintenance.

5.4 Temperature sensor - for automatic compensator covering general ambient temperature range.

5.5 Electrode mounting - to hold electrodes; may be flow through (for small flows), pipe mounted or immersion.

6. Reagents

6.1 Primary standard buffer salts are available from the National Bureau of Standards and should be used in situations where extreme accuracy is required.

6.1.1 Preparation of reference solutions from these salts require some special precautions and [illegible]dling[1] such as low conductivity [illegible]on water, drying ovens, and [illegible]on dioxide free purge gas. These solutions should be replaced at least once each month.

6.2 Secondary buffers may be prepared from NBS salts or purchased as a solution from commercial vendors. Use of these commercially available solutions, which have been validated by comparison to NBS standards, is recommended for routine operation. These buffers may be retained for at least six months if kept stoppered.

7. Calibration

7.1 Immersion type electrodes - easily removed from mounting.

7.1.1 The electrode should be calibrated at a minimum of two points that bracket the expected pH of the water/waste and are approximately three pH units or more apart.

7.1.2 Repeat calibration adjustments on successive portions of the two buffer solutions until readings are within ±0.05 pH units of the buffer value. If calibration problems occur, see 4.3.

7.1.3 Because of the wide variety of instruments available, no detailed operating instructions are provided. Instead, the analyst should refer to the particular manufacturer's instructions.

7.1.4 Calibration against two buffers should be carried out at least daily. If the pH of the fluid being measured fluctuates considerably, the calibration should be carried out more often. Calibration frequencies may be relaxed if historical data supports a longer period between calibration.

7.2 Immersion type electrodes - not easily removed from mounting.

7.2.1 Collect a grab sample of the flowing material from a point as close to the electrode as possible. Measure the pH of this grab sample as quickly as possible with a laboratory - type pH meter. Adjust the calibration control of the continuous monitor to the reading obtained.

7.2.2 The temperature and condition of the grab sample must remain constant until its pH has been measured by the laboratory pH meter. The temperature of the sample should be measured and the temperature compensator of the laboratory pH meter adjusted.

7.2.3 The laboratory - type pH meter should be calibrated prior to use against two buffers as outlined in 7.1.

7.2.4 The continuous pH monitoring system should be initially calibrated against two buffers as outlined in 7.1 before being placed into service. Recalibration (every 30 days) at two points is recommended if at all possible to ensure the measuring electrode is in working order. If this is not possible, the use of electrode testing features for a broken or malfunctioning electrode should be considered when purchasing the equipment.

7.2.5 The indirect calibration should be carried out at least once a day. If the pH of the fluid being measured fluctuates considerably, the calibration should be carried out more often. Calibration frequencies may be relaxed if historical data support a longer period between calibration.

7.2.6 If the electrode can be removed from the system, but with difficulty, it should be directly calibrated as in 7.1 at least once a month.

7.3 Flow-through type electrode - easily removed from its mounting.

7.3.1 Calibrate using buffers as in 7.1. The buffers to be used may be the process stream itself as one buffer, and as a second buffer after adjustment of pH by addition of an acid or base. This will provide the larger volumes necessary to calibrate this type electrode.

7.3.2 Since the velocity of sample flow-through a flow through electrode can produce an offset error in pH reading, the user must have data on hand to show that the offset is known and compensation has been accomplished.

7.4 Flow-through type electrode - not easily removed from its mounting.

7.4.1 Calibrate as in 7.2.

7.4.2 Quality control data must be on hand to show the user is aware of possible sample flow velocity effects.

8. Procedure

8.1 Calibrate the monitor and electrode system as outlined in Section 7.

8.2 Follow the manufacturer's recommendation for operation and installation of the system.

8.3 In wastewaters, the electrode may require periodic cleaning. After manual cleaning, the electrode should be calibrated as in 7.1 or 7.2 before returning to service.

8.4 The electrode must be placed so that the water or waste flowing past the electrode is representative of the system.

9. Calculations

9.1 pH meters read directly in pH units. Reports pH to the nearest 0.1 unit and temperature to the nearest °C.

10. Precision and Accuracy

10.1 Because of the wide variability of equipment and conditions and the changeable character of the pH of many process waters and wastes, the precision of this method is probably less than that of Method 150.1; however, a precision of 0.1 pH unit

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[1] [illegible]tional Bureau of Standards Special Publication

should be attainable in the range of pH 6.0 to 8.0. Accuracy data for continuous monitoring equipment are not available at this time.

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APPENDIX A-10

Procedure for Oxidation Reduction Potential: Method 2580

2580 OXIDATION-REDUCTION POTENTIAL (ORP) (PROPOSED)*

2580 A. Introduction

1. Significance

Oxidation and reduction (redox) reactions mediate the behavior of many chemical constituents in drinking, process, and wastewaters as well as most aquatic compartments of the environment.[1-5] The reactivities and mobilities of important elements in biological systems (e.g., Fe, S, N, and C), as well as those of a number of other metallic elements, depend strongly on redox conditions. Reactions involving both electrons and protons are pH- and Eh-dependent; therefore, chemical reactions in aqueous media often can be characterized by pH and Eh together with the activity of dissolved chemical species. Like pH, Eh represents an intensity factor. It does not characterize the capacity (i.e., poise) of the system for oxidation or reduction.

The potential difference measured in a solution between an inert indicator electrode and the standard hydrogen electrode should not be equated to Eh, a thermodynamic property, of the solution. The assumption of a reversible chemical equilibrium, fast electrode kinetics, and the lack of interfering reactions at the electrode surface are essential for such an interpretation. These conditions rarely, if ever, are met in natural water.

Thus, although measurement of Eh in water is relatively straightforward, many factors limit the interpretation of these values. These factors include irreversible reactions, electrode poisoning, the presence of multiple redox couples, very small exchange currents, and inert redox couples. Eh values measured in the field correlate poorly with Eh values calculated from the redox couples present. Nevertheless, measurement of redox potential, when properly performed and interpreted, is useful in developing a more complete understanding of water chemistry.

2. Sampling and Storage

Do not store samples; analyze on collection. Minimize both atmospheric contact and delay in analysis.

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* Approved by Standard Methods Committee, 1990.

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2580 B. Oxidation-Reduction Potential Measurement in Clean Water

1. General Discussion

a. Principle: Electrometric measurements are made by potentiometric determination of electron activity (or intensity) with an inert indicator electrode and a suitable reference electrode. Ideally, the indicator electrode will serve as either an electron donor or acceptor with respect to electroactive oxidized or reduced chemical species in solution. At redox equilibrium, the potential difference between the ideal indicator electrode and the reference electrode equals the redox potential of the system. However, inert indicator electrodes that behave ideally in all aqueous systems, particularly in natural waters, do not exist. Electrodes made of platinum are most commonly used for Eh measurements. They have limitations,[1] as do alternative materials such as gold and graphite.

The standard hydrogen reference electrode is fragile and impractical for routine laboratory and field use. Therefore, silver:silver-chloride or calomel reference electrodes are used commonly. The redox potential measurement is corrected for the difference between the potential of the reference electrode and that of the standard hydrogen electrode. See Section 4500-H^+, pH Value.

It is not possible to calibrate Eh electrodes over a range of redox potentials (as is done with pH electrodes). Instead, standard solutions that exhibit both chemical stability and known redox potentials for specific indicator electrodes are used to check electrode response at the temperature of measurement.

The potential of the platinum (Pt) Eh electrode versus the Ag/AgCl reference electrode with KCl electrolyte in ZoBell's solution ($3 \times 10^{-3}M$ potassium ferrocyanide and $3 \times 10^{-3}M$ potassium ferricyanide in $0.1M$ KCl)[2] has been measured as a function of temperature.[3] Good agreement was obtained between Eh values measured with this electrode pair in ZoBell's solution and those calculated from the stability constants at 8 to 85°C. The potential of the ZoBell's solution with this electrode configuration as a function of temperature can be calculated:[4]

$$Eh, V = 0.428 - 0.0022\,(T - 25)$$

where T = solution temperature, °C. Alternatively, select the value from Table 2580:I.

To determine the Eh of a sample relative to the standard hydrogen electrode, measure Eh of both sample and standard solution at the same temperature (i.e., ±0.1°C). Then calculate Eh value of the sample:

$$Eh_{system} = E_{observed} + Eh_{ZoBell\ reference} - Eh_{ZoBell\ observed}$$

where:

$E_{observed}$ = sample potential relative to reference electrode,

$Eh_{ZoBell\ reference}$ = theoretical Eh of reference electrode and Zobell's solution, relative to the standard hydrogen electrode (see Table 2580:I), and

$Eh_{ZoBell\ observed}$ = observed potential of ZoBell's solution, relative to the reference electrode.

The measurements described above can be applied analogously to other indicator electrode/reference electrode pairs and standard solutions.

b. Interferences: Specific interferences may be due to operation of either indicator or reference electrode, redox capacity or poise of the sample, sample preservation and handling, and temperature equilibration.

1) Sorption and poisoning effects on electrodes—Contamination of the electrode surface, salt bridge, or internal electrolyte in the case of reference electrodes, can lead to excessive drift, poor electrode response, and artifact potentials. Organic matter, sulfide, and bromide may cause these problems, particularly in long-term electrode use.[1,5-7] If excessive drift occurs or erratic performance of paired electrodes is observed in redox standard solutions after appropriate cleaning, refilling, or regeneration procedures, discard the faulty electrode and use a new one.

2) pH variations—Redox potential is sensitive to pH if hydrogen ion or hydroxide ion is involved in the redox half-cells. Cell potentials tend to increase as proton concentration increases (i.e., pH decreases) and Eh values drop as hydroxide concentrations increase (i.e., pH increase).

3) Sample handling and preservation—The sample poise will govern the resistance of the sample to change in redox potential; this phenomenon is analogous to the resistance to pH change afforded by buffer capacity. Except in concentrated process streams, sludges, leachates, and highly reducing or treated waters, the concentrations of oxidized or reduced species may be fairly low (i.e., $<10^{-4}M$). Under these conditions, handle reduced

TABLE 2580:I. POTENTIAL OF ZOBELL'S SOLUTION AS FUNCTION OF TEMPERATURE

T °C	E *V*	T °C	E *V*
1	0.481	16	0.448
2	0.479	17	0.446
3	0.476	18	0.443
4	0.474	19	0.441
5	0.472	20	0.439
6	0.470	21	0.437
7	0.468	22	0.435
8	0.465	23	0.432
9	0.463	24	0.430
10	0.461	25	0.428
11	0.459	26	0.426
12	0.457	27	0.424
13	0.454	28	0.421
14	0.452	29	0.419
15	0.450	30	0.417

samples very carefully to avoid exposure to atmospheric oxygen. A closed cell sampling configuration may be used.[4,8] Samples cannot be stored or preserved; analyze at sampling.

4) Temperature equilibration—Obtain Eh standard solution reading for the electrode pair at a temperature as close as possible to that of the sample. Temperature determines the Eh reference potential for a particular solution and electrode pair. It also may affect the reversibility of the redox reaction, the magnitude of the exchange current, and the stability of the apparent redox potential reading, particularly in poorly poised solutions. Hold temperature constant for all measurements and report it with Eh results.

2. Apparatus

a. pH or millivolt meter: Use a pH meter or other type of high-impedance potentiometer capable of reading either pH or millivolts (mV). For most applications, a meter scale readable to ± 1400 mV is sufficient.

b. Reference electrode consisting of a half-cell providing a constant electrode potential. See Section 4500-H$^+$.B.2*b*.

c. Oxidation-reduction indicator electrode: The platinum electrode is used most commonly. A noble metal or graphite electrode may be useful for specific applications.

1) Noble metal electrode—Noble metal (i.e., gold or platinum) foil, wire, or billet types of electrode are inert and resistant to chemical reaction. Clean and polish electrode surface to insure reliable performance. Platinum electrodes may be cleaned by strong acid soaking,[9,10] hydrogen peroxide and detergent washing,[11] and anodic activation.[10] Abrasive polishing with crocus cloth, jeweler's rouge, or 400 to 600 grit wet/dry carborundum paper may be best.[5]

2) Graphite electrode—A wax-impregnated graphite (WIG) electrode may be used, especially in aqueous suspensions or soils.[12,13] The WIG electrode is more resistant to electrode poisoning than electrodes made of platinum wire.

d. Beakers: Preferably use polyethylene, TFE, or glass beakers.

e. Stirrer: Use a magnetic TFE-coated stirring-bar-type mixer.

f. Flow cell: Use for continuous flow measurements and for poorly buffered solutions.

3. Reagents

a. Standard redox solutions: Standardize the electrode system against redox solutions that provide stable known Eh values over a range of temperatures. Although standard solutions are available, they do not cover the anticipated range of Eh values. Commercially prepared solutions may be used, particularly in field testing. The composition and Eh values of standard solutions are shown in Table 2580:II. With reasonable care, these solutions are stable for several months.

b. Eh electrode cleaners: Use either:

1) Aqua regia—Mix 1 volume conc nitric acid with 3 volumes conc hydrochloric acid. Prepare fresh and discard after use.

2) Chromic acid—Dissolve 5 g potassium dichromate, $K_2Cr_2O_7$, in 500 mL conc sulfuric acid.

4. Procedure

a. Instrument calibration: Follow manufacturer's instructions for using pH/millivolt meter and in preparing electrodes for use. Use a shorting lead to verify the zero point on the meter's millivolt scale. Equilibrate the standard solution to the temperature of the sample. Immerse electrodes in the gently stirred, standard solution in a beaker (or flow cell). Turn on meter, placing the function switch in the millivolt mode.

After several minutes for electrode equilibration, record reading to nearest millivolt. If the reading is more than ± 10 mV from the theoretical redox standard value at that temperature, replace reference electrode fluid and repeat the measurement. If that procedure fails to bring the reading to within ± 10 mV of the theoretical value, polish the sensing element of the indicator electrode with carborundum paper, crocus cloth, or jeweler's rouge. Rinse electrode thoroughly and recheck reading with a fresh portion of the standard solution. If the reading is

TABLE 2580:II. PREPARATION OF REDOX STANDARD SOLUTIONS

Standard Solution	Potentials of Pt Electrode vs. Selected Reference Electrodes at 25°C in Standard Solution					Weight of Chemicals Needed 1000 mL Aqueous Solution at 25°C
	Calomel	Silver:Silver Chloride Ag/AgCl			Standard Hydrogen	
	Hg/Hg_2Cl_2 saturated KCl	KCl 1.00*M*	KCl 4.00*M*	KCl saturated		
Light's solution[14]	+430	+439	+475	+476	+675	39.21 g ferrous ammonium sulfate, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ 48.22 g ferric ammonium sulfate, $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$ 56.2 mL sulfuric acid, H_2SO_4, sp gr 1.84
ZoBell's solution*[2]	+183	+192	+228	+229	+428	1.4080 g potassium ferrocyanide, $K_4Fe(CN)_6 \cdot 3H_2O$ 1.0975 g potassium ferricyanide, $K_3Fe(CN)_6$ 7.4555 g potassium chloride, KCl

* Store in dark plastic bottle in a refrigerator.

within ± 10 mV of the theoretical value, record it and the temperature. If the reading is not within ± 10 mV, repeat the cleaning procedure above or try another electrode. Then rinse the electrode with distilled water and proceed with the sample measurement.

Useful treatments for noble metal electrodes in restoring performance after long periods of use include immersion in warm (70°C) aqua regia for 1 to 2 min or 5 min in 6*N* HNO_3 after bringing to a boil. Alternatively treat with chromic acid solution followed by 6*N* HCl and rinse with water.

b. Sample analysis: Check system for performance with the standard solution, rinse electrodes thoroughly with sample water, then immerse them in the gently stirred sample. Let equilibrate, record Eh value to the nearest millivolt, and temperature to ±0.1°C. Repeat with a second sample portion to confirm successive readings within ±10 mV. Equilibration times vary and may take many minutes in poorly poised solutions. Successive readings that vary less than ±10 mV over 10 min are adequate for most purposes.

Make continuous flow or pumped sample measurements, particularly of poorly poised solutions, in a closed flow cell after external calibration of the electrode system. Recalibrate daily and more frequently if turbid, organic-rich, or high-dissolved-solids solutions are being measured.

See Table 2580:III for recommended combinations of electrodes, standards, and sample handling.

5. Trouble Shooting

a. Meter: Use a shorting lead to establish meter reading at zero millivolts whenever possible. If the meter cannot be zeroed, follow the manufacturer's instructions for service.

b. Electrodes: If the potentiometer is in good working order, the fault may be in the electrodes. Frequently, renewal of the filling solution for the salt bridge for the reference electrode is sufficient to restore electrode performance. Another useful check is to oppose the emf of a questionable reference electrode with that of the same type known to be in good order. Using an adapter, plug the good reference electrode into the indicator electrode jack of the potentiometer. Then plug the questionable electrode into the reference electrode jack. With the meter in the millivolt position, immerse electrodes in an electrolyte (e.g., KCl) solution and then into a redox standard solution. The two millivolt readings should be 0 ± 5 mV for both solutions. If different electrodes are used (e.g., silver:silver chloride versus calomel or vice versa), the reading should be 44 ± 5 mV for a good reference electrode.

TABLE 2580:III. RECOMMENDED COMBINATIONS FOR SELECTED SAMPLE TYPES

Sample Type	Indicator Electrode(s)	Reference Electrode	Type of Sample Cell
Process stream (low Br^-) (S^{2-})	Pt or Au	Calomel or silver: silver chloride	Closed continuous flow (dual indicator electrode)
(high Br^-)	Pt or Au	Calomel or silver: silver chloride with salt bridge (double junction reference electrode)	
Natural waters			
Surface waters	Pt or Au	Calomel or silver: silver chloride	Closed continuous flow (dual indicator electrode) or beaker
Groundwater	Pt or Au	Calomel or silver: silver chloride	Closed continuous flow (dual indicator electrode)
Soils, sludges	WIG, Pt wire	Calomel or silver: silver chloride	Beaker or soil core

Unless an indicator electrode has been poisoned, physically damaged, or shorted out, it usually is possible to restore function by proper cleaning.

6. Calculation

$$Eh_{\text{system}} = E_{\text{observed}} + E_{\text{reference standard}} - E_{\text{reference observed}}$$

Report temperature at which readings were made.

7. Precision and Bias

Standard solution measurements made at stable temperatures with a properly functioning electrode system should be accurate to within ±10 mV. Calibration precision as reflected by the agreement of dual platinum electrodes versus an Ag:AgCl reference electrode for over a 2-year period has been estimated at ±15 mV (i.e., one standard deviation) in ZoBell's solution (N = 78) at approximately 12°C. Precision on groundwater samples (N = 234) over the same period has been estimated at ±22 mV (i.e., ± one standard deviation) in a closed flow cell.[15]

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APPENDIX A-11

Procedure for Non-Purgeable Organic Carbon: Method 415.1

Total Organic Carbon - Method 415.1 with Dohrmann DC-190

1.0 Procedure

Perform Total Organic Carbon analysis in accordance with "Organic Carbon, Total", Method 415.1 (Combustion or Oxidation) and in accordance with chapters 6 and 10 of the operating manual for the Dohrmann 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.

3.0 Quality Control Samples

For each batch of samples, perform a method blank, reagent 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 machine. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.

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, %	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 $^{\circ}$C

Furnace Temperature = Furnace set point (Furnace light is green.) For most applications, the temperature should be 680°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.

DAILY OPERATION

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. $\text{NPOC} \xrightarrow[\text{Furnace}]{\text{TC}} CO_2$
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. $\text{IC} \xrightarrow[\text{Chamber}]{\text{IC}} CO_2$
TC	Any water sample.	Sample injected into TC port. $\text{TC} \xrightarrow[\text{Furnace}]{\text{TC}} CO_2$
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. $\text{POC Gas} \rightarrow \text{IC Scrubber} \rightarrow \text{POC} \xrightarrow[\text{Furnace}]{\text{TC}} CO_2$
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. $\text{Sample} \xrightarrow{800^{\circ}C} CO_2$

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.

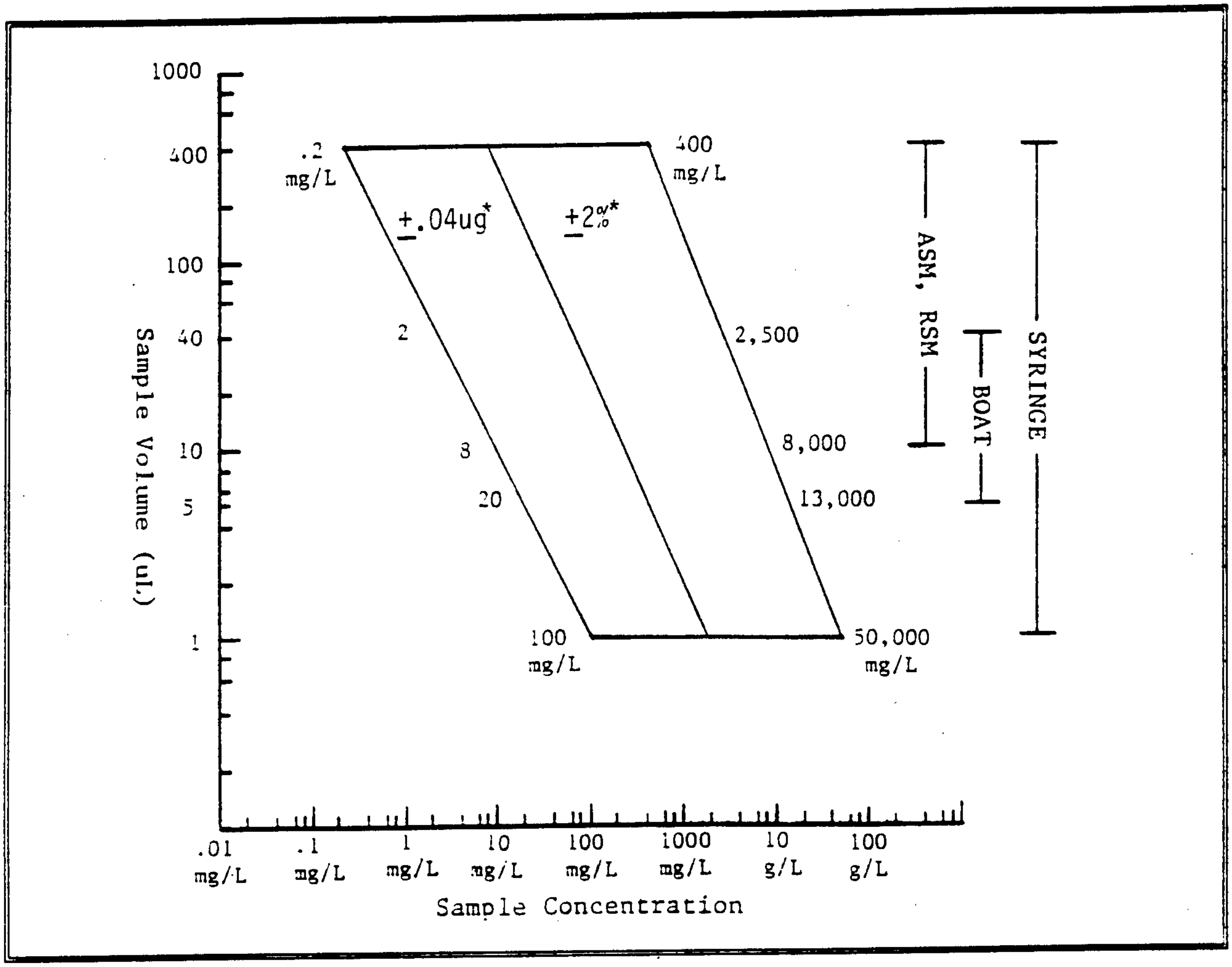
Table 6.2
INLET MODE SELECTION

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

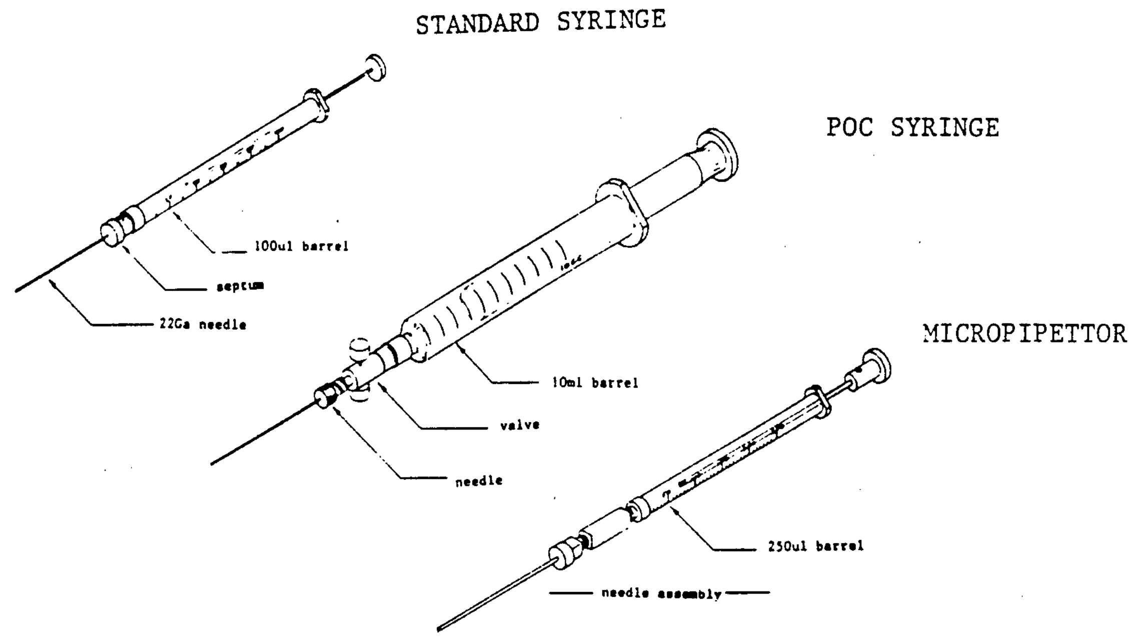


FIGURE 6.2. SYRINGE ASSEMBLIES

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.

TC or IC

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.

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

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 <u>for all volumes</u>.

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.

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.

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.

POC

⚠WARNING

Acid hazard.
Check installation
before sparging
or priming. See
manual sect. 5.

Priming and sparging steps involve acid pumping into the appropriate vessels. Make sure the plumbing is properly connected to avoid acid injury to persons or property.

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 F_n and b_n are recalculated each time either the Calibration Factor or the System Blank is updated. It should be noted that if the old value b_o is already 0, the new value b_n 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 [illegible]eration 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 stablize 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:

$$\textbf{g Compound} = \frac{\textbf{mw} \times \textbf{\%C}}{\textbf{N} \times \textbf{12.01}}$$

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):

$$\frac{342.29 \times 1\%}{12 \times 12.01} = 2.375 \text{ g.}$$

TC and IC WORKING STANDARDS

<u>Use:</u>

Calibration or system check-out.
Choose the standard concentration to match the working range of your samples.

<u>Requirements:</u>

Reagent water.
Clean volumetric flasks and volumetric pipets.

<u>Preparation:</u>

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°C. **Minimize exposure to atmosphere.**

Bottle volume: Between 100 - 200 mL, depending upon the concentration.

Replace weekly.

<u>System Performance Check:</u> (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 A-12

Procedure for Chemical Oxygen Demand: Method 410.4

Chemical Oxygen Demand
EPA Method 410.4
with Hach DR/2000

1.0 Procedure

Perform analysis for Chemical Oxygen Demand on the Hach DR/2000 in accordance with manufacturer's instructions (Method 8000 attached).

2.0 Recordkeeping

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

3.0 Quality Control Samples

For each batch of samples, perform a method blank and one quality control sample made from a sucrose solution. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.

4.0 References

"Chemical Oxygen Demand Method 410.4 (Colorimetric, Automated; Manual)", *Methods for Chemical Analysis of Water and Wastes - Revised March 1983*, (U. S.) Enviromental Protection Agencey, Cincinnati, OH, P84-128677

"5220 Chemical Oxygen Demand (COD)", "5220 D. Closed Reflux, Colorimetric Method", *Standard Methods for the Analysis of Water and Wastewater*, 19th ed., [American Public Health Association]

CHEMICAL OXYGEN DEMAND

Method 410.4 (Colorimetric, Automated; Manual)

STORET NO. 00340

1. Scope and Application
   1.1 This method covers the determination of COD in surface waters, domestic and industrial wastes.
   1.2 The applicable range of the automated method is 3–900 mg/1 and the range of the manual method is 20 to 900 mg/1.
2. Summary of Method
   2.1 Sample, blanks and standards in sealed tubes are heated in an oven or block digestor in the presence of dichromate at 150°C. After two hours, the tubes are removed from the oven or digestor, cooled and measured spectrophotometrically at 600 nm.
3. Sample Handling and Preservation
   3.1 Collect the samples in glass bottles if possible. Use of plastic containers is permissible if it is known that no organic contaminants are present in the containers.
   3.2 Samples should be preserved with sulfuric acid to a $pH < 2$ and maintained at 4°C until analysis.
4. Interferences
   4.1 Chlorides are quantitatively oxidized by dichromate and represent a positive interference. Mercuric sulfate is added to the digestion tubes to complex the chlorides.
5. Apparatus
   5.1 Drying oven or block digestor, 150°C
   5.2 Corning culture tubes, 16 x 100 mm or 25 x 150 mm with Teflon lined screw cap
   5.3 Spectrophotometer or Technicon AutoAnalyzer
   5.4 Muffle furnace, 500°C.
6. Reagents
   6.1 Digestion solution: Add 10.2 g $K_2Cr_2O_7$, 167 ml conc. H_2SO_4 and 33.3 g $HgSO_4$ to 500 ml of distilled water, cool and dilute to 1 liter.
   6.2 Catalyst solution: Add 22 g Ag_2SO_4 to a 4.09kg bottle of conc. H_2SO_4. Stir until dissolved.
   6.3 Sampler wash solution: Add 500 ml of conc H_2SO_4 to 500 ml of distilled water.
   6.4 Stock potassium acid phthalate: Dissolve 0.850 g in 800 ml of distilled water and dilute to 1 liter. 1 ml = 1 mg COD
      6.4.1 Prepare a series of standard solutions that cover the expected sample concentrations by diluting appropriate volumes of the stock standard.
7. Procedure
   7.1 Wash all culture tubes and screw caps with 20% H_2SO_4 before their first use to prevent contamination. Trace contamination may be removed from the tubes by igniting them in a muffle oven at 500°C for 1 hour.

Pending approval for Section 304(h), CWA
Issued 1978

7.2 Automated
  7.2.1 Add 2.5 ml of sample to the 16 x 100 mm tubes.
  7.2.2 Add 1.5 ml of digestion solution (6.1) and mix.
  7.2.3 Add 3.5 ml of catalyst solution (6.2) carefully down the side of the culture tube.
  7.2.4 Cap tightly and shake to mix layers.
  7.2.5 Process standards and blanks exactly as the samples.
  7.2.6 Place in oven or block digestor at 150°C for two hours.
  7.2.7 Cool, and place standards in sampler in order of decreasing concentration. Complete filling sampler tray with unknown samples.
  7.2.8 Measure color intensity on AutoAnalyzer at 600 nm.

7.3 Manual
  7.3.1 The following procedure may be used if a larger sample is desired or a spectrophotometer is used in place of an AutoAnalyzer.
  7.3.2 Add 10 ml of sample to 25 x 150 mm culture tube.
  7.3.3 Add 6 ml of digestion solution (6.1) and mix.
  7.3.4 Add 14 ml of catalyst solution (6.2) down the side of culture tube.
  7.3.5 Cap tightly and shake to mix layers.
  7.3.6 Place in oven or block digestor at 150°C for 2 hours.
  7.3.7 Cool, allow any precipitate to settle and measure intensity in spectrophotometer at 600 nm. Use only optically matched culture tubes or a single cell for spectrophotometric measurement.

8. Calculation
  8.1 Prepare a standard curve by plotting peak height or percent transmittance against known concentrations of standards.
  8.2 Compute concentration of samples by comparing sample response to standard curve.

9. Precision and Accuracy
  9.1 Precision and accuracy data are not available at this time.

Bibliography

1. Jirka, A. M., and M. J. Carter, "Micro-Semi-Automated Analysis of Surface and Wastewaters for Chemical Oxygen Demand." Anal. Chem. 47:1397, (1975).

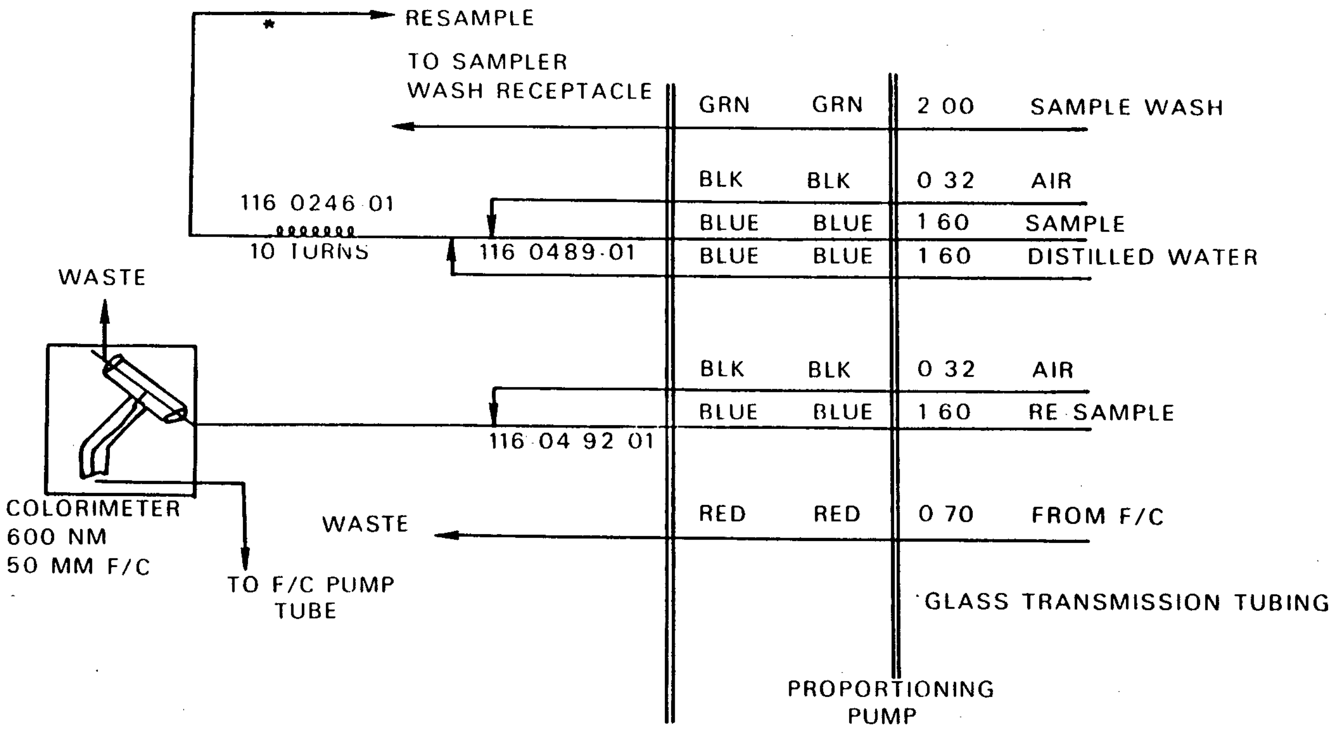


FIGURE 1 C O D MANIFOLD AA1 OR AA 11

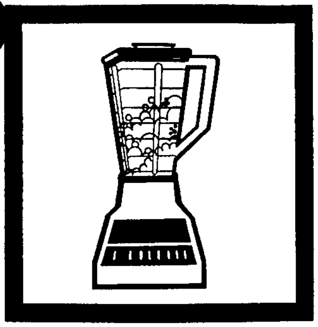
Method 8000

OXYGEN DEMAND, CHEMICAL

For water, wastewater and seawater

Reactor Digestion Method*; USEPA approved for reporting**

DIGESTION



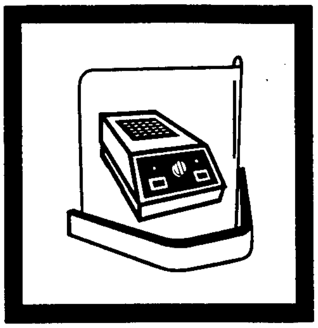
1. Homogenize 500 mL of sample for 2 minutes in a blender.

0 to 15,000 mg/L Note: *Homogenize 100 mL of sample. Pour the homogenized sample into a 250–mL beaker and stir with a magnetic stirrer.*

Note: *Blending ensures distribution of solids and improves accuracy and reproducibility.*

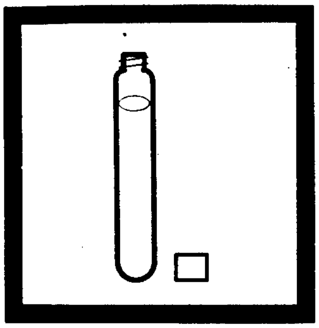
Note: *If samples cannot be analyzed immediately, see Sampling and Storage following these procedures.*

Caution: *Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if inappropriately handled or accidently misused. Please read all warnings and the safety section of this manual. Appropriate eye protection and clothing should be used for adequate user protection. If contact occurs, flush the affected area with running water. Follow instructions carefully.*



2. Turn on the COD Reactor. Preheat to 150 °C. Place the plastic shield in front of the reactor.

Caution: *Ensure safety devices are in place to protect analyst from splattering should reagent leaking occur.*

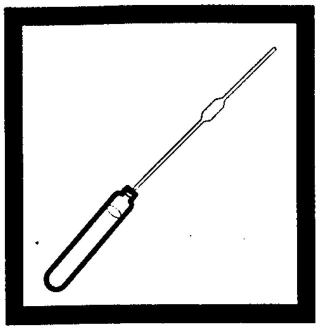


3. Remove the cap of a COD Digestion Reagent Vial for the appropriate range:

Sample Concentration Range (mg/L)	COD Digestion Reagent Vial Type
0 to 150	Low Range
0 to 1,500	High Range
0 to 15,000	High Range Plus

Use the cap tool provided to loosen the High Range Plus vials caps.

Note: *The reagent mixture is light–sensitive. Keep unused vials in the opaque shipping container, in a refrigerator if possible. The amount of light striking the vials during the test will not affect results.*



4. Hold the vial at a 45–degree angle. Pipet 2.00 mL (0.2 mL for the 0 to 15,000 mg/L range) of sample into the vial.

0 to 15,000 mg/L Note: *Pipet only 0.20 mL of sample, not 2.00 mL, using a TenSette Pipet. For greater accuracy a minimum of three replicates should be analyzed and the results averaged.*

Note: *Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Do not run tests with vials which have been spilled. If contact occurs, wash with running water.*

Note: *For proof of accuracy, use COD standard solutions (preparation given in the Accuracy Check) in place of the sample.*

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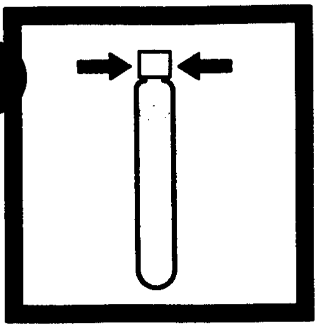
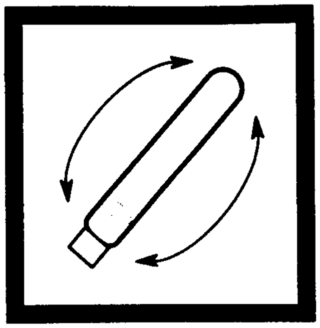
*Jirka, A.M.; Carter, M.J. *Analytical Chemistry*, 1975, 47(8), 1397

***Federal Register*, April 21, 1980, 45(78), 26811–26812

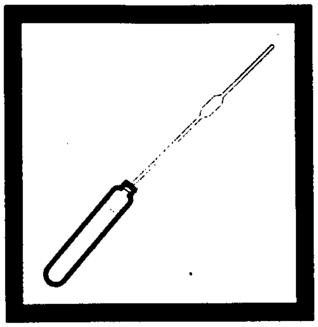


5. Replace the vial cap tightly. Use the cap tool provided, if necessary. Rinse the COD vial with deionized water and wipe the vial clean with a paper towel.



6. Hold the vial by the cap and over a sink. Invert gently several times to mix the contents. Place the vial in the preheated COD Reactor.

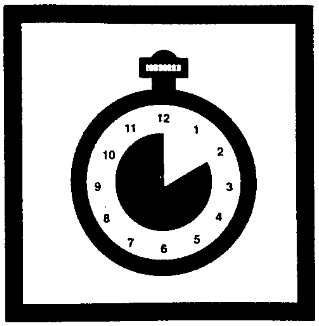
Note: The vial will become very hot during mixing.



7. Prepare a blank by repeating Steps 3 to 6, substituting 2.00 mL (0.2 mL for the 0 to 15,000 mg/L range) deionized water for the sample.

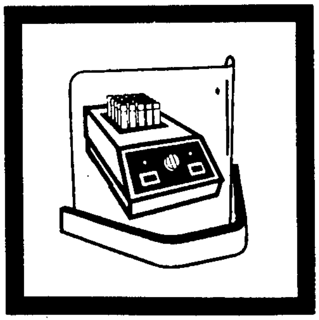
Note: Be sure the pipet is well rinsed, or use a clean pipet.

Note: One blank must be run with each set of samples. All tests (samples and blank) should be run with the same lot of vials. The lot number appears on the container label.

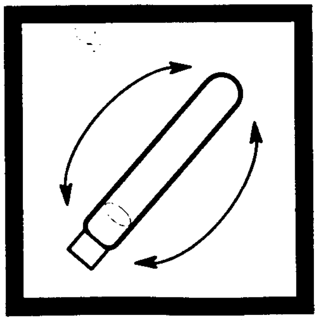


8. Heat the vials for 2 hours.

Note: Many wastewater samples containing easily oxidized materials are digested completely in less than two hours. If desired, measure the concentration (while still hot) at 15 minute intervals until it remains unchanged. At this point, the sample is completely digested. Cool the vials to room temperature for final measurement.



9. Turn the reactor off. Wait about 20 minutes for the vials to cool to 120 °C or less.



10. Invert each vial several times while still warm. Place the vials into a rack. Wait until the vials have cooled to room temperature.

Note: If a pure green color appears in the reacted sample, the reagent capacity may have been exceeded. Measure the COD and, if necessary, repeat the test with a diluted sample.

Colorimetric
or
Titrimetric

11. Use one of the following analytical techniques to determine the sample concentration:

Colorimetric determination,
0 to 150 mg/L COD
Colorimetric determination,
0 to 1,500 mg/L COD
Colorimetric determination,
0 to 15,000 mg/L COD
Buret titration

COD
GH, etc. 0-150

COLORIMETRIC DETERMINATION, 0 to 150 mg/L COD

%T 4 PROG 3 0

READ ENTER

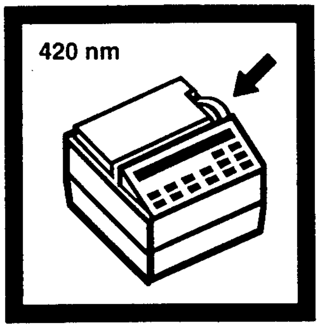
1. Enter the stored program number for chemical oxygen demand (COD), low range.

Press: **4 3 0 READ/ENTER**

The display will show:
DIAL nm TO 420

Note: DR/2000s with software versions 3.0 and greater will display "P" and the program number.

Note: Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.



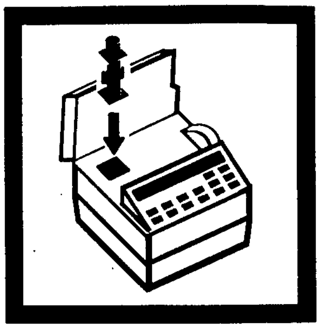
2. Rotate the wavelength dial until the small display shows:
420 nm

Note: Approach the wavelength setting from the higher to lower values.

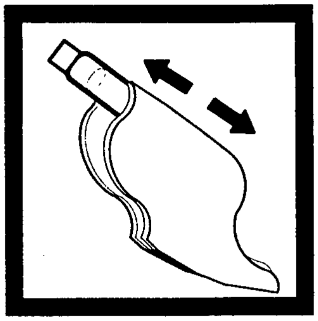
READ ENTER

3. Press: **READ/ENTER**

The display will show:
mg/l COD L

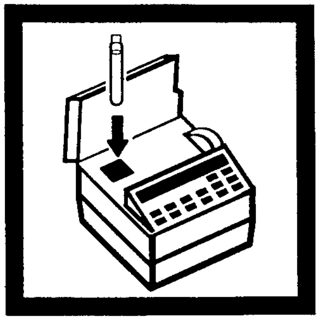


4. Place the COD Vial Adapter into the cell holder with the marker to the right.



5. Clean the outside of the blank with a towel.

Note: Wiping with a damp towel, followed by a dry one will remove fingerprints or other marks.



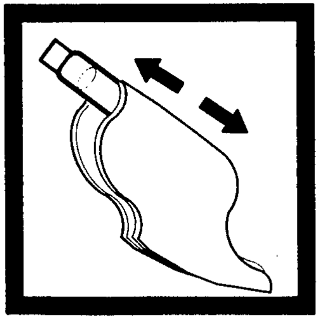
6. Place the blank into the adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.

Note: The blank is stable when stored in the dark; see Blanks for Colorimetric Determination following these procedures.

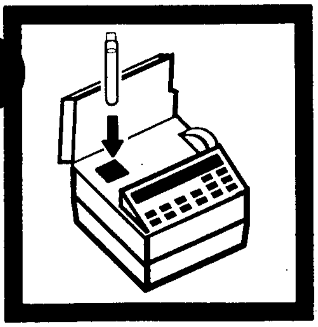
CLEAR ZERO

7. Press: **ZERO**

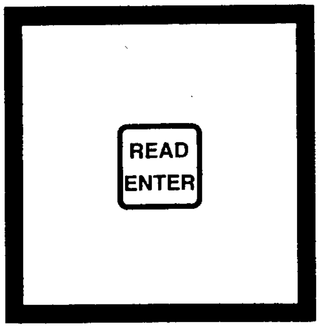
The display will show:
WAIT
then:
0. mg/l COD L



8. Clean the outside of the sample vial with a towel.



9. Place the sample vial into the adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.



10. Press: **READ/ENTER**

The display will show:
WAIT
then the result in mg/L COD will be displayed.

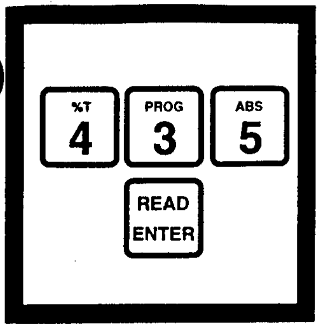
Note: In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

Note: For most accurate results with samples near 150 mg/L COD, repeat the analysis with a diluted sample.

COD
CH ETC.

0-150

COLORIMETRIC DETERMINATION, 0 to 1,500 and 0 to 15,000 mg/L COD



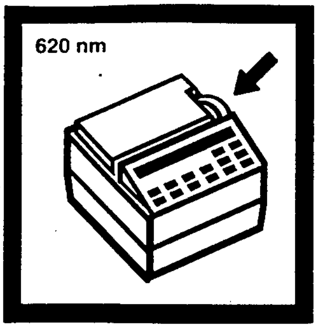
1. Enter the stored program number for chemical oxygen demand, high range.

Press: **4 3 5 READ/ENTER**

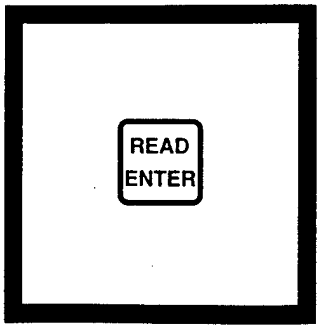
The display will show:
DIAL nm TO 620

Note: DR/2000s with software versions 3.0 and greater will display "P" and the program number.

Note: Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

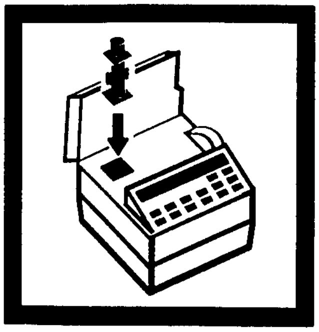


2. Rotate the wavelength dial until the small display shows:
620 nm

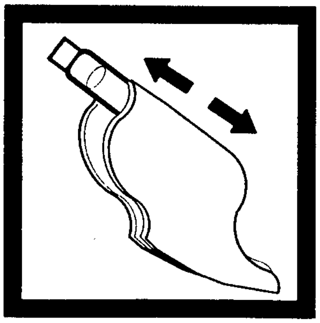


3. Press: **READ/ENTER**

The display will show:
mg/l COD H

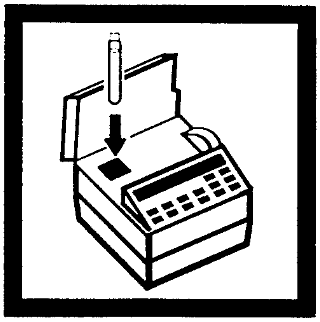


4. Place the COD Vial Adapter into the cell holder with the marker to the right.



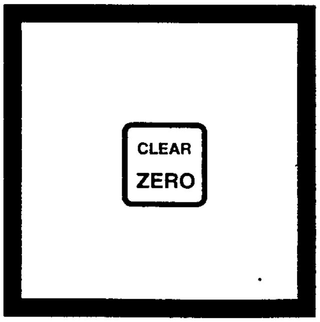
5. Clean the outside of the blank with a towel.

Note: Wiping with a damp towel followed by a dry one will remove fingerprints or other marks.



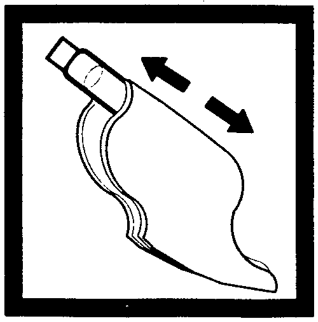
6. Place the blank into the adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.

Note: The blank is stable when stored in the dark. See Blanks for Colorimetric Determination following these procedures.

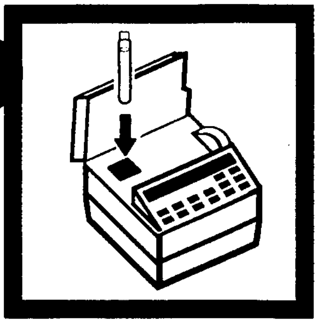


7. Press: **ZERO**

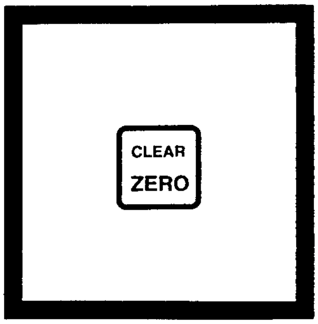
The display will show:
WAIT
then:
0. mg/l COD H



8. Clean the outside of the sample vial with a towel.



9. Place the sample vial in the adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.



10. Press: **READ/ENTER**

The display will show:
WAIT
then the result in mg/L COD will be displayed.

0 to 15,000 mg/L Note:
When High Range Plus COD Digestion Reagent Vials are used, multiply the displayed value by ten.

Note: *In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.*

Note: *For most accurate results with samples near 1,500 or 15,000 mg/L COD, repeat the analysis with a diluted sample.*

APPENDIX A-13

Procedure for Ammonia Nitrogen: Method 350.1

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

6.3.5 Syringe filters - Titan nylon 25-mm syringe filters - 0.45 micron. SRI Catalog number 44525-NN or equivalent.

6.3.6 Syringes - 10 cc syringe with Luer Lok, B-D Part 309604 or equivalent. (Smaller volumes are acceptable)

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

7.1.1 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 9.2.

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 Pour samples and standards into vials. If samples have particulate matter, filter them into the sample vial with a syringe and nylon syringe filter. 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

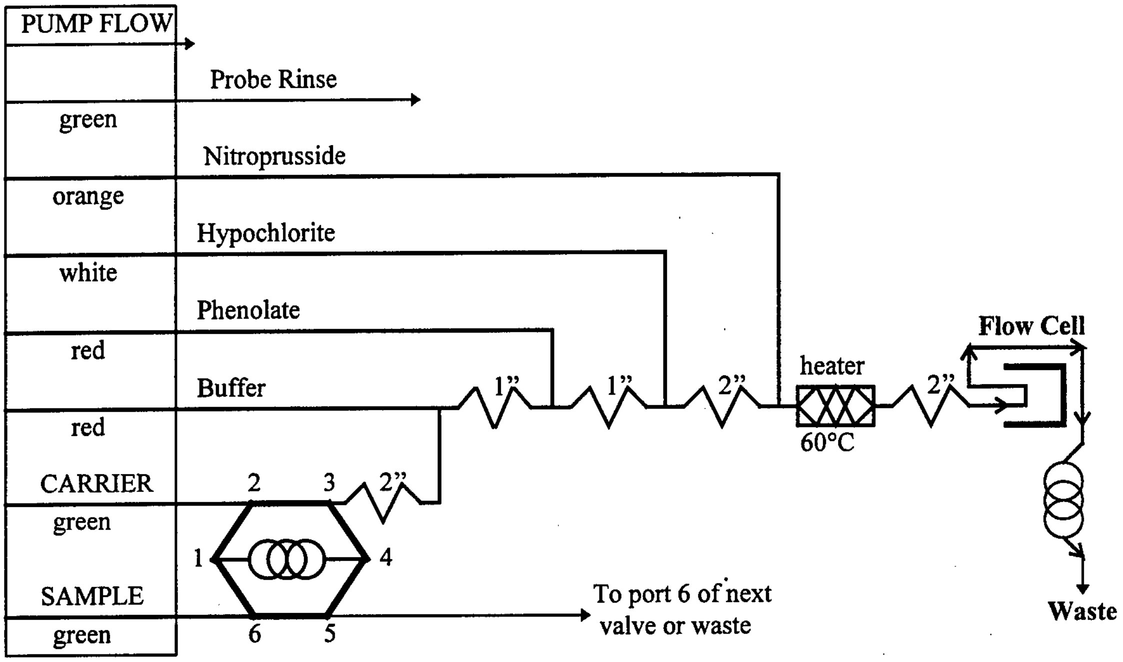
Load Time: 0.0

Load period 25.0

Inject Period: 45.0

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

9.2 Ammonia Manifold Diagram



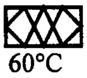
Sample Loop = 13 cm x 0.5 mm i.d.

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 

includes 650 cm of tubing wrapped around the heater block at the specified temperature.

10.0 ATTACHMENTS AND APPENDICES

None

End of Procedure

NITROGEN, AMMONIA

Method 350.1 (Colorimetric, Automated Phenate)

STORET NO. Total 00610
Dissolved 00608

1. Scope and Application
   1.1 This method covers the determination of ammonia in drinking, surface, and saline waters, domestic and industrial wastes in the range of 0.01 to 2.0 mg/1 NH_3 as N. This range is for photometric measurements made at 630–660 nm in a 15 mm or 50 mm tubular flow cell. Higher concentrations can be determined by sample dilution. Approximately 20 to 60 samples per hour can be analyzed.
2. Summary of Method
   2.1 Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.
3. Sample Handling and Preservation
   3.1 Preservation by addition of 2 ml conc. H_2SO_4 per liter and refrigeration at 4°C.
4. Interferences
   4.1 Calcium and magnesium ions may be present in concentration sufficient to cause precipitation problems during analysis. A 5% EDTA solution is used to prevent the precipitation of calcium and magnesium ions from river water and industrial waste. For sea water a sodium potassium tartrate solution is used.
   4.2 Sample turbidity and color may interfere with this method. Turbidity must be removed by filtration prior to analysis. Sample color that absorbs in the photometric range used will also interfere.
5. Apparatus
   5.1 Technicon AutoAnalyzer Unit (AAI or AAII) consisting of:
      5.1.1 Sampler.
      5.1.2 Manifold (AAI) or Analytical Cartridge (AAII).
      5.1.3 Proportioning pump.
      5.1.4 Heating bath with double delay coil (AAI).
      5.1.5 Colorimeter equipped with 15 mm tubular flow cell and 630–660 nm filters.
      5.1.6 Recorder.
      5.1.7 Digital printer for AAII (optional).

Approved for NPDES following preliminary distillation, Method 350.2.
Issued 1974
Editorial revision 1978

6. Reagents

6.1 Distilled water: Special precaution must be taken to insure that distilled water is free of ammonia. Such water is prepared by passage of distilled water through an ion exchange column comprised of a mixture of both strongly acidic cation and strongly basic anion exchange resins. The regeneration of the ion exchange column should be carried out according to the instruction of the manufacturer.

NOTE 1: All solutions must be made using ammonia-free water.

6.2 Sulfuric acid 5N: Air scrubber solution. Carefully add 139 ml of conc. sulfuric acid to approximately 500 ml of ammonia-free distilled water. Cool to room temperature and dilute to 1 liter with ammonia-free distilled water.

6.3 Sodium phenolate: Using a 1 liter Erlenmeyer flask, dissolve 83 g phenol in 500 ml of distilled water. In small increments, cautiously add with agitation, 32 g of NaOH. Periodically cool flask under water faucet. When cool, dilute to 1 liter with distilled water.

6.4 Sodium hypochlorite solution: Dilute 250 ml of a bleach solution containing 5.25% NaOCl (such as "Clorox") to 500 ml with distilled water. Available chlorine level should approximate 2 to 3%. Since "Clorox" is a proprietary product, its formulation is subject to change. The analyst must remain alert to detecting any variation in this product significant to its use in this procedure. Due to the instability of this product, storage over an extended period should be avoided.

6.5 Disodium ethylenediamine-tetraacetate (EDTA) (5%): Dissolve 50 g of EDTA (disodium salt) and approximately six pellets of NaOH in 1 liter of distilled water.

NOTE 2: On salt water samples where EDTA solution does not prevent precipitation of cations, sodium potassium tartrate solution may be used to advantage. It is prepared as follows:

6.5.1 Sodium potassium tartrate solution: 10% $NaKC_4H_4O_6 \cdot 4H_2O$. To 900 ml of distilled water add 100 g sodium potassium tartrate. Add 2 pellets of NaOH and a few boiling chips, boil gently for 45 minutes. Cover, cool, and dilute to 1 liter with ammonia-free distilled water. Adjust pH to 5.2 ±.05 with H_2SO_4. After allowing to settle overnight in a cool place, filter to remove precipitate. Then add 1/2 ml Brij-35(4) (available from Technicon Corporation) solution and store in stoppered bottle.

6.6 Sodium nitroprusside (0.05%): Dissolve 0.5 g of sodium nitroprusside in 1 liter of distilled water.

6.7 Stock solution: Dissolve 3.819 g of anhydrous ammonium chloride, NH_4Cl, dried at 105°C, in distilled water, and dilute to 1000 ml. 1.0 ml = 1.0 mg NH_3–N.

6.8 Standard Solution A: Dilute 10.0 ml of stock solution (6.7) to 1000 ml with distilled water. 1.0 ml = 0.01 mg NH_3–N.

6.9 Standard solution B: Dilute 10.0 ml of standard solution A (6.8) to 100.0 ml with distilled water. 1.0 ml = 0.001 mg NH_3–N.

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6.10 Using standard solutions A and B, prepare the following standards in 100 ml volumetric flasks (prepare fresh daily):

NH_3–N, mg/l	ml Standard Solution/100 ml
	Solution B
0.01	1.0
0.02	2.0
0.05	5.0
0.10	10.0
	Solution A
0.20	2.0
0.50	5.0
0.80	8.0
1.00	10.0
1.50	15.0
2.00	20.0

NOTE 3: When saline water samples are analyzed, Substitute Ocean Water (SOW) should be used for preparing the above standards used for the calibration curve; otherwise, distilled water is used. If SOW is used, subtract its blank background response from the standards before preparing the standard curve.

Substitute Ocean Water (SOW)

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

7. Procedure

7.1 Since the intensity of the color used to quantify the concentration is pH dependent, the acid concentration of the wash water and the standard ammonia solutions should approximate that of the samples. For example, if the samples have been preserved with 2 ml conc. H_2SO_4/liter, the wash water and standards should also contain 2 ml conc. H_2SO_4/liter.

7.2 For a working range of 0.01 to 2.00 mg NH_3–N/1 (AAI), set up the manifold as shown in Figure 1. For a working range of .01 to 1.0 mg NH_3–N/1 (AAII), set up the manifold as shown in Figure 2. Higher concentrations may be accommodated by sample dilution.

7.3 Allow both colorimeter and recorder to warm up for 30 minutes. Obtain a stable baseline with all reagents, feeding distilled water through sample line.

7.4 For the AAI system, sample at rate of 20/hr, 1:1. For the AAII use a 60/hr 6:1 cam with a common wash.

7.5 Arrange ammonia standards in sampler in order of decreasing concentration of nitrogen. Complete loading of sampler tray with unknown samples.

7.6 Switch sample line from distilled water to sampler and begin analysis.

8. Calculations

8.1 Prepare appropriate standard curve derived from processing ammonia standards through manifold. Compute concentration of samples by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 In a single laboratory (EMSL), using surface water samples at concentrations of 1.41, 0.77, 0.59 and 0.43 mg NH_3–N/1, the standard deviation was ±0.005.

9.2 In a single laboratory (EMSL), using surface water samples at concentrations of 0.16 and 1.44 mg NH_3–N/1, recoveries were 107% and 99%, respectively.

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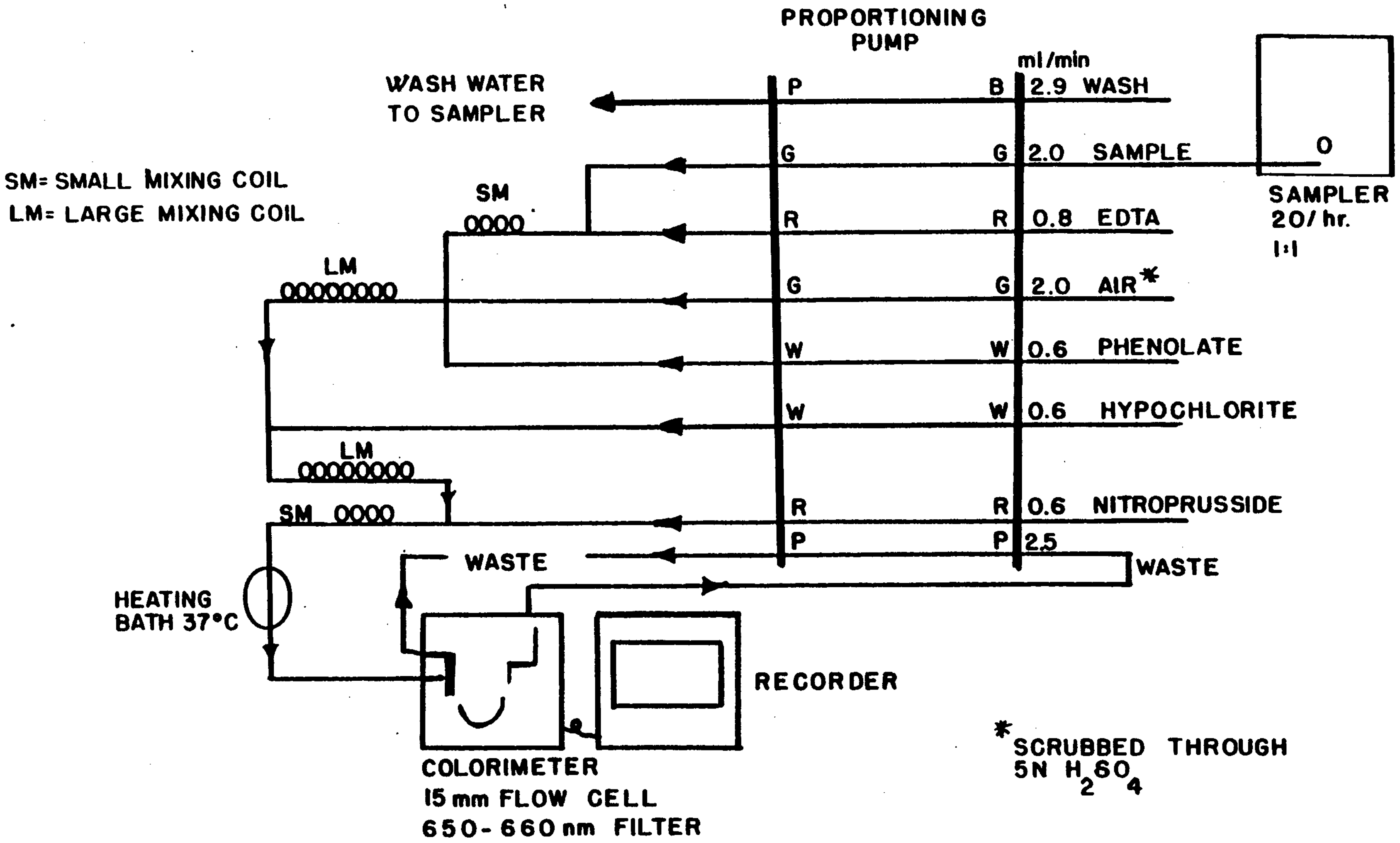

FIGURE 1 AMMONIA MANIFOLD AA I

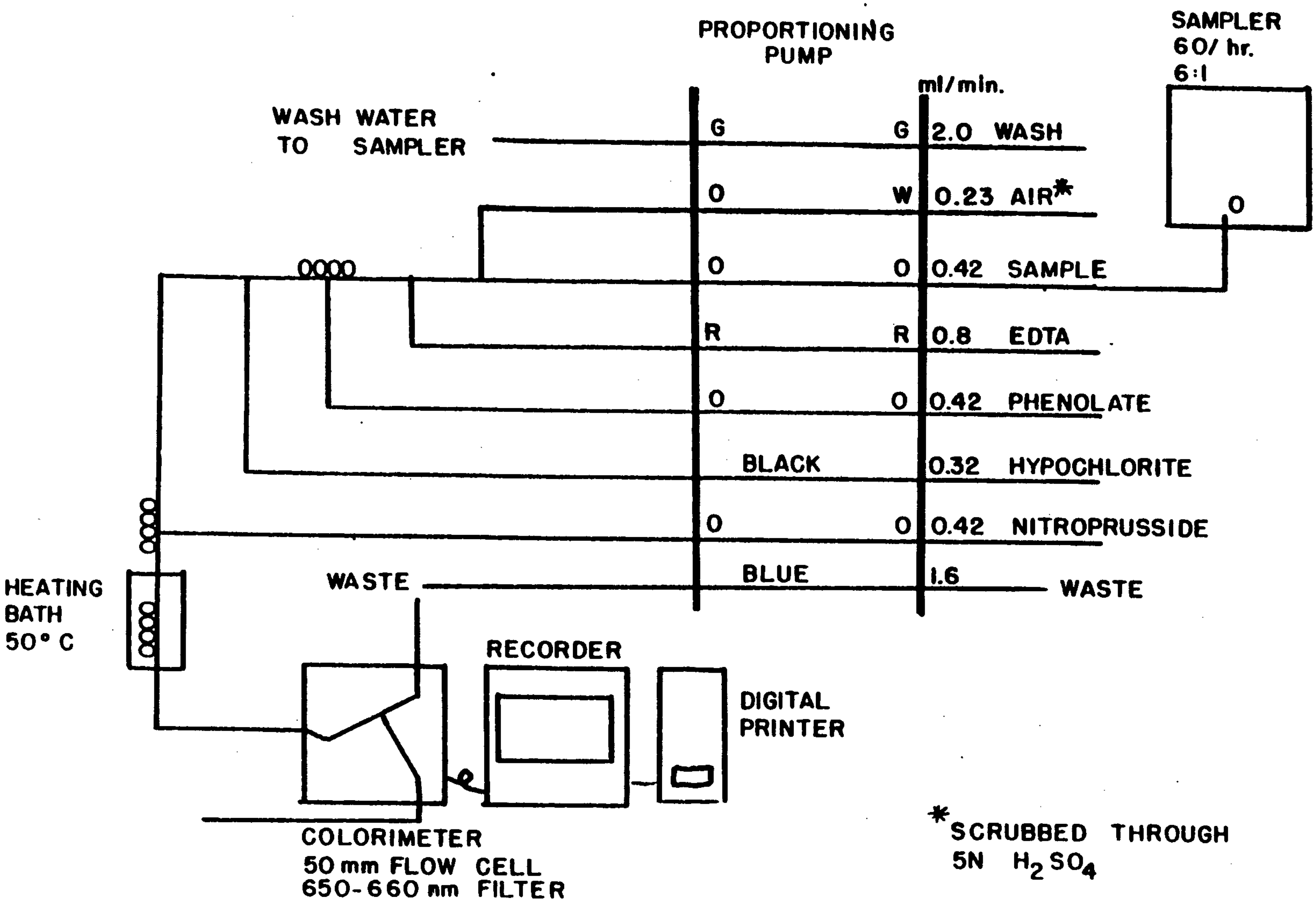

FIGURE 2. AMMONIA MANIFOLD AA II

APPENDIX A-14

Procedure for Total Kjeldahl Nitrogen: Method 351 Series

Table of Contents – Appendix A-14

Nitrogen, Total Kjehldahl - Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAII)

1.0 Procedure

Perform analysis for Total Kjehldahl Nitrogen (Method 351.2) in accordance with procedures for the Technicon II AutoAnalyzer, or for the Lachat Quick Chem 8000 flow injection analyzer as attached.

2.0 Recordkeeping

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

3.0 Quality Control Samples

For each batch of samples, perform a method blank, reagent 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 machine. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.

NITROGEN, KJELDAHL, TOTAL

Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAII)

STORET NO. 00625

1. Scope and Application
   1.1 This method covers the determination of total Kjeldahl nitrogen in drinking and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogeneous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines. The applicable range of this method is 0.1 to 20 mg/1 TKN. The range may be extended with sample dilution.
2. Summary of Method
   2.1 The sample is heated in the presence of sulfuric acid, K_2SO_4 and $HgSO_4$ for two and one half hours. The residue is cooled, diluted to 25 ml and placed on the AutoAnalyzer for ammonia determination. This digested sample may also be used for phosphorus determination.
3. Definitions
   3.1 Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described below.
   3.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free-ammonia value (Method 350.2, Nitrogen, Ammonia, this manual) from the total Kjeldahl nitrogen value.
4. Sample Handling and Preservation
   4.1 Samples may be preserved by addition of 2 ml of conc H_2SO_4 per liter and stored at 4°C. Even when preserved in this manner, conversion of organic nitrogen to ammonia may occur. Therefore, samples should be analyzed as soon as possible.
5. Apparatus
   5.1 Block Digestor–40
   5.2 Technicon Manifold for Ammonia (Figure 1)
   5.3 Chemware TFE (Teflon boiling stones), Markson Science, Inc., Box 767, Delmar, CA 92014)
6. Reagents
   6.1 Mercuric Sulfate: Dissolve 8 g red mercuric oxide (HgO) in 50 ml of 1:4 sulfuric acid (10 ml conc H_2SO_4: 40 ml distilled water) and dilute to 100 ml with distilled water.
   6.2 Digestion Solution: (Sulfuric acid-mercuric sulfate-potassium sulfate solution): Dissolve 133 g of K_2SO_4 in 700 ml of distilled water and 200 ml of conc H_2SO_4. Add 25 ml of mercuric sulfate solution and dilute to 1 liter.

Pending approval for NPDES
Issued 1978

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6.3 Sulfuric Acid Solution (4%): Add 40 ml of conc. sulfuric acid to 800 ml of ammonia free distilled water, cool and dilute to 1 liter.

6.4 Stock Sodium Hydroxide (20%): Dissolve 200 g of sodium hydroxide in 900 ml of ammonia-free distilled water and dilute to 1 liter.

6.5 Stock Sodium Potassium Tartrate Solution (20%): Dissolve 200 g sodium potassium tartrate in about 800 ml of ammonia-free distilled water and dilute to 1 liter.

6.6 Stock Buffer Solution: Dissolve 134.0 g of sodium phosphate, dibasic (Na_2HPO_4) in about 800 ml of ammonia free water. Add 20 g of sodium hydroxide and dilute to 1 liter.

6.7 Working Buffer Solution: Combine the reagents in the stated order; add 250 ml of stock sodium potassium tartrate solution (6.5) to 200 ml of stock buffer solution (6.6) and mix. Add xx ml sodium hydroxide solution (6.4) and dilute to 1 liter. See concentration ranges, Table I, for composition of working buffer.

6.8 Sodium Salicylate/Sodium Nitroprusside Solution: Dissolve 150 g of sodium salicylate and 0.3 g of sodium nitroprusside in about 600 ml of ammonia free water and dilute to 1 liter.

6.9 Sodium Hypochlorite Solution: Dilute 6.0 ml sodium hypochlorite solution (clorox) to 100 ml with ammonia free distilled water.

6.10 Ammonium chloride, stock solution: Dissolve 3.819 g NH_4Cl in distilled water and bring to volume in a 1 liter volumetric flask. 1 ml = 1.0 mg NH_3-N.

7. Procedure

Digestion

7.1 To 20 or 25 ml of sample, add 5 ml of digestion solution (6.2) and mix (use a vortex mixer).

7.2 Add (4–8) Teflon boiling stones (5.3). Too many boiling chips will cause the sample to boil over.

7.3 With Block Digestor in manual mode set low and high temperature at 160°C and preheat unit to 160°C. Place tubes in digestor and switch to automatic mode. Set low temperature timer for 1 hour. Reset high temperature to 380°C and set timer for 2 1/2 hours.

7.4 Cool sample and dilute to 25 ml with ammonia free water.

Colorimetric Analysis

7.5 Check the level of all reagent containers to ensure an adequate supply.

7.6 Excluding the salicylate line, place all reagent lines in their respective containers, connect the sample probe to the Sampler IV and start the proportioning pump.

7.7 Flush the Sampler IV wash receptacle with about 25 ml of 4.0% sulfuric acid (6.3).

7.8 When reagents have been pumping for at least five minutes, place the salicylate line in its respective container and allow the system to equilibrate. If a precipitate forms after the addition of salicylate, the pH is too low. Immediately stop the proportioning pump and flush the coils with water using a syringe. Before restarting the system, check the concentration of the sulfuric acid solutions and/or the working buffer solution.

TABLE 1

CONCENTRATION RANGES
(NITROGEN)

	Dilution loops						
	Initial sample		Resample				
No.	Sample line	Diluent line	Resample line	Diluent line	Approx. std. cal. setting	Range PPM N (± 10%)	ml stock NaOH per liter working buffer solution
1	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0–0.5	250
2	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0–1.5	250
3	.16 (ORN/YEL)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0–1	120
4	.16 (ORN/YEL)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0–5	120
5	.16 (ORN/YEL)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	700	0–2	80
6	.16 (ORN/YEL)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	100	0–10	80

7.9 To prevent precipitation of sodium salicylate in the waste tray, which can clog the tray outlet, keep the nitrogen flowcell pump tube and the nitrogen Colorimeter "To Waste" tube separate from all other lines or keep tap water flowing in the waste tray.

7.10 After a stable baseline has been obtained start the Sampler.

8. Calculations

8.1 Prepare standard curve by plotting peak heights of processed standards against concentration values. Compute concentrations by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 In a single laboratory (EMSL), using sewage samples of concentrations of 1.2, 2.6, and 1.7 mg N/1, the precision was ±0.07, ±0.03 and ±0.15, respectively.

9.2 In a single laboratory (EMSL), using sewage samples of concentrations of 4.7 and 8.74 mg N/1, the recoveries were 99 and 99%, respectively.

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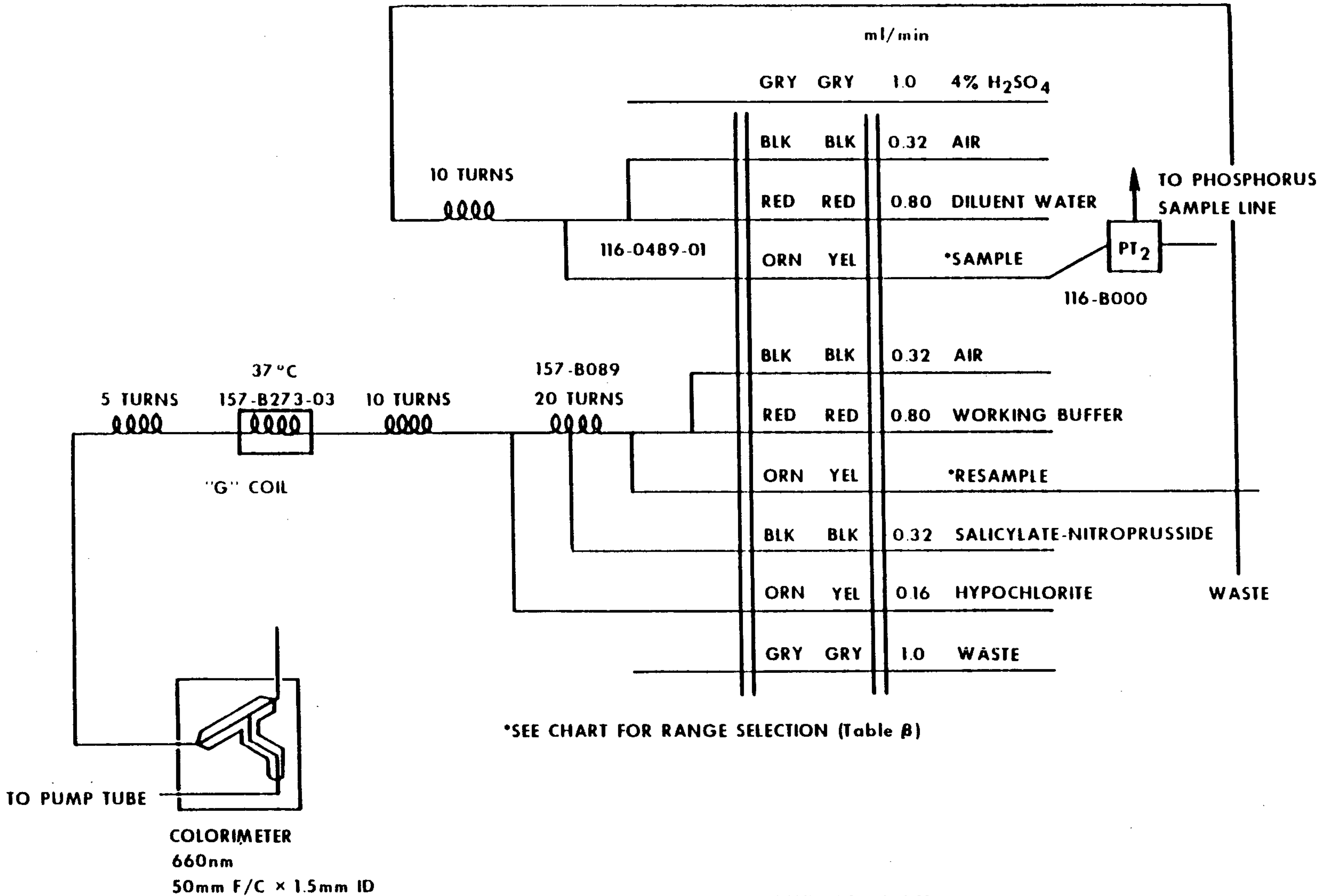


*SEE CHART FOR RANGE SELECTION (Table *B*)

FIGURE 1. AMMONIA MANIFOLD AAII

QuikChem METHOD 10-107-06-2-D

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY

(BLOCK DIGESTOR METHOD)

Written by David H. Diamond

Applications Group

Revision Date:

18 October 1994

LACHAT INSTRUMENTS
6645 WEST MILL ROAD
MILWAUKEE, WI 53218, USA

LACHAT
INSTRUMENTS

QuikChem Method 10-107-06-2-D

Total Kjeldahl Nitrogen in Waters

0.2 to 20 .0 mg N/L

-- Principle --

This method covers the determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids. proteins and peptides to ammonia but may not the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

-- Interferences --

1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.4% (v/v) H_2SO_4 in the diluted digestion sample with no change in signal intensity.
2. High nitrate cocncentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

-- Special Apparatus --

1. Heating Unit
2. Block Digestor/75 mL tubes (Lachat Part. No. 1800-000)
3. 5 mL and 20 mL Repipet Dispensers

Revised by Karin Wendt 18 October 1994 written by D. Diamond and copyrighted on 24 June 92 by**Lachat Instruments. 6645 West Mill Road. Milwaukee. WI 53218.** USA. Phone: (414) 358-4200 FAX: 1-414-358-4206.

Contents

QUIKCHEM METHOD 10-107-06-2-D

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY (BLOCK DIGESTOR METHOD)

1. SCOPE AND APPLICATION

1.1. The method covers the determination of total Kjeldahl nitrogen in water and wastewater.

1.2. 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.

1.3. The applicable range is 0.2 to 20 mg N/L. The method detection limit is 0.02 mg N/L. 90 samples per hour can be analyzed.

1.4. Samples containing particulates should be filtered or homogenized.

2. SUMMARY OF METHOD

2.1. 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 an analyzed for ammonia. This digested sample may also be used for phosphorus determination.

2.2. 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.

2.3. Organic nitrogenous the difference obtained by subtracting the free-ammonia concentration from the total Kjeldahl nitrogen concentration.

2.4. Approximately 0.1 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.

2.5. 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 EDTA in the buffer prevents precipitation of calcium and magnesium.

3. DEFINITIONS

3.1. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

3.2. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3. INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4. LABORATORY SPIKED BLANK (LSB) -- an aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LSB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5. LABORATORY SPIKED SAMPLE MATRIX (LSM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LSM is analyzed exactly like sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LSM corrected for background concentrations.

3.6. LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that is digested exactly as a sample including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7. LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.

3.8. MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9. METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10. QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to spike an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11. STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4. INTERFERENCES

4.1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.5% (v/v) H_2SO_4 in the diluted digestion sample with no change in signal intensity.

4.2. 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.

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

5. SAFETY

5.1. 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. Cautions are included for known extremely hazardous materials.

5.2. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3. The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1. Mercury (Reagents 1 and 2)

5.3.2. Sulfuric Acid (Reagents 1, 2 and 6)

5.3.3. Sodium Nitroprusside (Reagent 4)

6. EQUIPMENT AND SUPPLIES

6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

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

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

  6.3.1. Sampler

  6.3.2. Multichannel proportioning pump

  6.3.3. Reaction unit or manifold

  6.3.4. Colorimetric detector

  6.3.5. Data system

6.4. Special apparatus

  6.4.1. Heating Unit

  6.4.2. Block Digestor/75 mL (Lachat Part. No. 1800-000)

  6.4.3. 5 mL and 20 mL repipet dispensers

  6.4.4. Vortex mixer

7. REAGENTS AND STANDARDS

7.1. PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

Degassing with Helium

To prevent bubble formation. the water carrier is degassed with helium. Use He at 20 lb/in^2 through a helium degassing wand. Bubble He vigorously through the solution for one minute. If air spikes continue to be a problem, the buffer can also be degasssed.

Reagent 1. Mercuric Sulfate Solution

To a 100 mL volumetric flask add approximately 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.

Reagent 2. Digestion Solution

In 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. Prepare fresh monthly.

Reagent 3. Buffer

By Volume: In a **1 L** volumetric flask containing **900 mL water** completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ($Na_2HPO_4 \cdot 7H_2O$). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** (NaOH), dilute to the mark and invert to mix. Degas weekly and prepare fresh monthly.

By Weight: To a tared **1 L** container add **958 g water** and completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ($Na_2HPO_4 \cdot 7H_2O$). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** (NaOH). Stir or shake until dissolved. Degas weekly and prepare fresh monthly.

Reagent 4. 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 invert to mix. Store in a dark bottle and prepare fresh monthly.

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

Reagent 5. 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. Prepare fresh daily.

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 DI water**. Shake to mix. Prepare fresh daily.

Reagent 6. Diluent 5.0% (V/V) Sulfuric Acid

NOTE: Diluent is prepared to dilute off scale samples. This reagent is not used on-line.

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared **1 L** container, add **760 g water** and **250 mL Reagent 2 (Digestion Solution)**. Invert to mix.

7.2. PREPARATION OF STANDARDS

Prepare standards in DI water daily or preserve them with 2 mL/L sulfuric acid. Once preserved, standards may be stored for 28 days. Standards in digest matrix may be stored for up to 28 days. If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.

Digested Standards

NOTE: Working standards prepared in DI water are digested per the procedure in section 8.

Standard 1: Stock Standard 1000 mg N/L

In a **1 L** volumetric flask dissolve **3.819 g ammonium chloride** (NH_4Cl) that has been dried for two hours at 110°C in about **800 mL DI water**. Dilute to the mark and invert to mix. As an alternative, primary standard grade ammonium sulfate is available from Fisher Scientific, cat. no. A938-500.

Standard 2. Working Stock Standard 20.0 mg N/L

By Volume: In a **250 mL** volumetric flask, dilute **5.0 mL Stock Standard 1** to the mark with **DI water**. Invert to mix.

By Weight: To a tared **1 L** container add about **20 g Stock Standard 1**. Divide the exact weight of the standard solution by **0.02** and dilute up to this resulting total weight with **DI water**. Shake to mix.

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	20.00	10.00	5.00	2.00	1.00	0.50	0.00

By Volume

Volume (mL) of **Standard 2** diluted to 100 mL with DI water	100	50	25	10	5	2.5	0

By Weight

Weight (g) of **Standard 2** diluted to final weight (~250 g) divide by **factor** below with DI water.	250.0	125	62.5	25	12.5	6.25	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.50	0.25	0.10	0.05	0.025	0

Non-Digested Standards

Standard 3. Blank in Digestion Matrix (0.00 mg N/L)

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared **1 L** container, add **760 g water** and **250 mL Reagent 2 (Digestion Solution)**. Invert to mix.

Standard 4. High Standard in Digestion Matrix (20.0 mg N/L)

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Add **20 mL** of Standard 1 (1000 mg N/L). Allow the solution to cool and dilute to the mark with DI water. Invert to mix. Prepare fresh monthly.

By Weight: To a tared **1 L** container, add **740 g water** and **250 mL Reagent 2 (Digestion Solution)**. Add **20 g** of Standard 1 (1000 mg N/L) and shake to mix.

Note: Non-Digested standards will need to be labeled to reflect the changing concentration or dilution which occurs during the digestion procedure. The following formula can be used to calculate the adjustment. For example, using a final volume of 21 mL for the digestate and an initial sample volume of 20 mL results in a labeled concentration of a 5.25 mg P/L for a 5.00 mg P/L non-digested standard.

$$\text{Labeled non-digested standard concentration} = \frac{\text{final digestate volume}}{\text{initial sample volume}} \times \begin{array}{c}\text{standard}\\ \text{concentration}\end{array}$$

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	20.00	10.00	5.00	2.00	1.00	0.500	0.00

By Volume

Volume (mL) of **Standard 2** diluted to 100 mL with Reagent 6	100	50	25	10	5	2.5	0

By Weight

Weight (g) of **Standard 2** diluted to final weight (~250 g) divide by **factor** below with Reagent 6.	250.0	125	62	25	12.5	6.25	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.50	0.25	0.10	0.05	0.025	0

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with dilute hydrochloric acid (0.5 M) and then rinsed with reagent water. The volume collected should be sufficient to insure a representative sample, allow for replicate analysis and minimize waste disposal.

8.2. Samples should be preserved to pH < 2 and cooled to $4^{o}C$ at the time of collection.

8.3. Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at $4^{o}C$ and may be held for up to 28 days.

9. QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards, the lowest concentration being $>$ 10X MDL. If any determined concentration exceeds the known values by +/-10%, linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3. Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within +/-10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) spiked at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the spiked reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates, t= 2.528 for twenty one replicates].

S = standard deviation of the replicate analyses.

MDLs should be determined every 6 months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3. ASSESSING LABORATORY PERFORMANCE

9.3.1. Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2. Laboratory Spiked Blank (LSB) -- The laboratory must analyze at least one LSB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3. The laboratory must used LSB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (X) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \overline{X} + 3S$$

$$\text{LOWER CONTROL LIMIT} = \overline{X} - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LSB. These data must be kept on file and be available for review.

9.3.4. Instruments Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/-10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/-10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

9.4. ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1. Laboratory Spiked Sample Matrix (LSM) -- The laboratory must add a known amount of analyte to a minimum of 10% of routine samples. In each case the LSM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory spiked blank.

9.4.2. Calculate the percent recovery for each analyte, corrected for concentrations measured in the unspiked sample, and compare these values to the designated LSM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_s - C}{s} \text{ X100}$$

Where,

R = percent recovery

C_s = spiked sample concentration.

C = sample background concentration.

s = concentration equivalent of analyte added to sample.

9.4.3. If the recovery of any analyte falls outside the designated LSM recovery range and the laboratory performance for that analyte is shown to be in control the recovery problem encountered with the LSM is judged to be either matrix or solution related, not system related.

9.4.4. Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10. CALIBRATION AND STANDARDIZATION

10.1. Prepare a series of 7 standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (suggested range in section 7.2).

10.4. Calibrate the instrument as description in section 11.

10.2. Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

10.3. After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed +/-10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

[illegible]. PROCEDURE

11.1. DIGESTION PROCEDURE

NOTE: Some laboratories prepare standards in DI water and process them through the digestion as outlined below. Other laboratories calibrate using standards in the digest matrix, i.e., **NOT** digested. Instructions for preparing standards in the digest matrix are given in section 7 of this method, following the instructions for preparing standards in DI water. At a minimum, two blanks and one standard should be prepared in DI water and digested.

11.1.1. Both standards and samples should be carried through this procedure. If samples have been preserved with sulfuric acid, standards should be preserved in the same manner.

11.1.2. To 20.0 mL of sample or standard add 5 mL digestion solution and mix. This is efficiently accomplished using an acid resistant 5 mL repipet device (EM Science, 108033-1, available through major scientific supply companies.)

11.1.3. Add 2 - 4 Hengar granules or 10 - 12 teflon stones to each tube. Hengar (Alundum) granules and teflon stones are effective for smooth boiling. Hengar granules are available from Fisher Scientific, cat. no. S145-500. Teflon stones are available from Markson Science. cat. no. 248-808, (800) 528-5114.

11.1.4. Ensure that the digestion tubes are dry on the outside and that all tubes contain boiling stones. 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 sample should have boiled off before increasing the temperature in step 5.

11.1.5. Continue to digest for 1.5 additional hours with the controller set to 380°C. This time includes the ramp time for the block temperature to come up to 380°C. The typical ramp time is 50 - 60 minutes. 380°C must be maintained for 30 minutes.

11.1.6. Before removing samples, gather the necessary supplies to dilute the samples with water. Remove the samples from the block and allow exactly 5 minutes to cool. Add water to the samples rapidly so that all samples are diluted within 10 minutes of removal from the block.

11.1.7. Add 19.0 mL DI water to each tube and vortex to mix. The total final volume should be 20 mL. The longer the samples have been allowed to cool, the longer the samples should be vortexed. For samples diluted at 5 minutes, 10 seconds of vortexing is sufficient. For samples which have cooled for greater than 10 minutes. up to 30 seconds of vortexing may be necessary.

11.1.8. If samples are not run immediately they should be diluted, vortexed and covered with lab film or capped tightly.

11.2. SYSTEM START-UP PROCEDURE

11.2.1. Prepare reagent and standards as described in section 7.

11.2.2. Set up manifold as shown in section 17.1.

11.2.3. Input peak timing and integration window parameters as specified in section 17.

11.2.4. Pump DI 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.

11.2.5. Place standards in the autosampler. and fill the sample tray. Input the information required by data system. such as concentration, replicates and QC scheme.

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

11.2.7. After a stable baseline has been obtained. start the sampler and perform analysis (please refer to system notes).

11.4. SYSTEM NOTES

11.4.1. Allow at least 15 minutes for the heating unit to warm up to 60°C.

11.4.2. If sample concentrations are greater than the high standard the digested sample should be diluted with **Reagent 6**. When the digital diluter is used, **Reagent 6** should be used as diluent. Do not dilute digested samples or standards with **DI water**.

11.4.3 If the salicylate reagent is merged with a sample containing sulfuric acid in the absence of the buffer solution, the salicylate reagent will precipitate. If this occurs all teflon manifold tubing should be replaced. To prevent this, prime the system by first placing the buffer transmission line in the buffer. Pump until the air bubble introduced during the transfer reaches the "T" fitting on the manifold. Then place all other transmission lines in the proper containers.

11.4.4. In normal operation nitroprusside gives a yellow background color which combines with the blue indosalicylate to give an emerald green color. This is the normal color of the solution in the waste container.

11.4.5. In normal operation the digest blank will result in a peak of about 1/5 the area of the 0.5 mg N/L standard. This peak is due to the acid in the digest and is present in every injection. Since this blank is constant for all samples and standards it will not effect data quality.

11.4.6. If phosphorus is also determined with the Lachat System. a second helium degassing tube should be purchased and the tubes should be dedicated to the individual chemistries.

11.4.7. If baseline drifts. peaks are too wide. or other problems with precision arise, clean the manifold by the following procedure:

A. Place transmission lines in water and pump to clear reagents (2-5 minutes).

B. Place reagent lines in **1 M hydrochloric acid** (1 volume of HCl added to 11 volumes of water) and pump for several minutes.

C. Place all transmission lines in water and pump for several minutes.

D. Resume pumping reagents.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply the answer by the appropriate dilution factor.

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

12.3. Report results in mg N/L.

13. METHOD PERFORMANCE

13.1. The method performance data are presented as method support data in section 19.2. This data was generated according to Lachat Standard Operating Procedure J001, Lachat FIA Support Data Generation.

14. POLLUTION PREVENTION

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

14.2. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Society's Department of Government Regulations and Science Policy," 115 16Th Street N. W., Washington D. C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

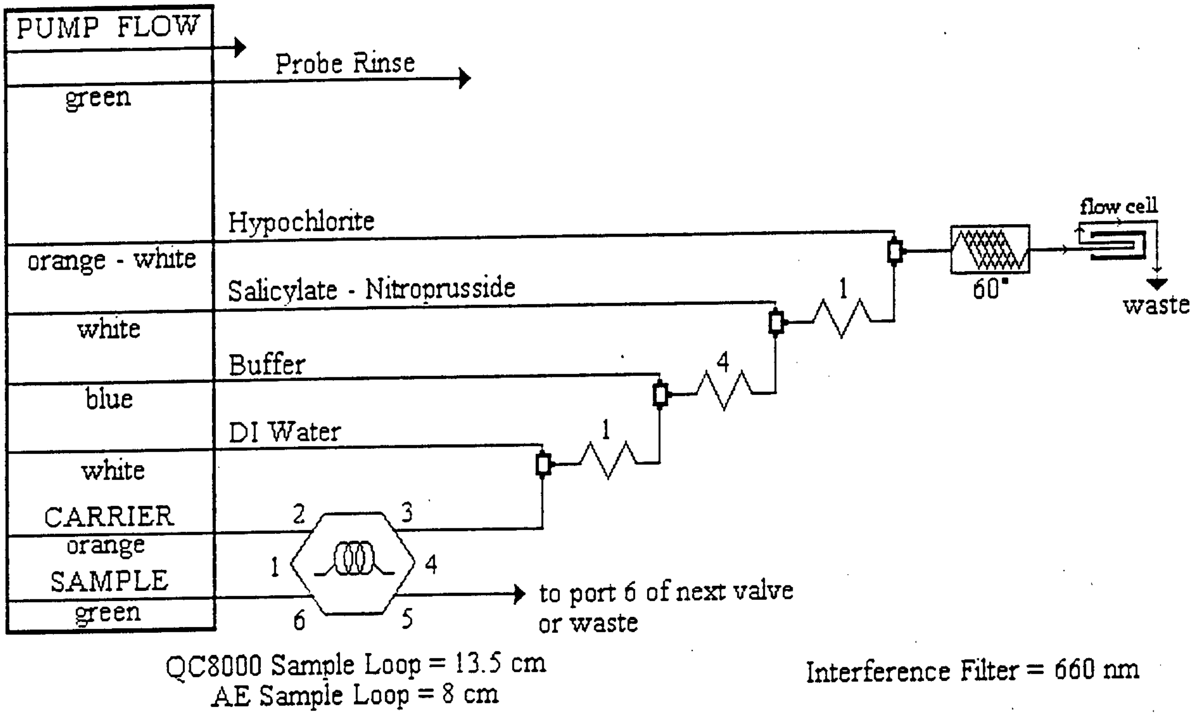
15.1. The Environmental Protection Agency (USEPA) requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waster discharge permit and regulations, and by complying with all solid and hazardous waste regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Sect. 14.3.

16. REFERENCES

1. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 351.2
2. ASTM, Water(I), Volume 11.01, Method D3590-89. Test Methods for Kjeldahl Nitrogen in Water, p. 447
3. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 350.1
4. Code of Federal Regulations 40, Chapter 1, Part 136, Appendix B.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

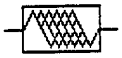
17.1. TOTAL KJELDAHL NITROGEN MANIFOLD DIAGRAM:



CARRIER is helium degassed water.

1 is **70 cm** of tubing on a **1 inch** coil support

4 is **255 cm** of tubing on a **4 inch** coil support

Apparatus: Standard valve. flow cell, and detector head modules are used. The  shows **650 cm of heated tubing**. All manifold tubing is **0.8 mm (0.032 in) i.d**. This is **5.2 uL/cm**.

MANIFOLD DIAGRAM REVISION DATE: 15 July 1992 by D. Diamond - 26Jul94 lc

17.2 DATA SYSTEM PARAMETERS FOR THE QUIKCHEM AE

Sample throughput: 90 samples/hour; 60 s/sample
Pump speed: 35
Cycle Period: 45 s

Inject to start of peak period: 38 s

Presentation, Data Window

Top Scale Response: 0.32 abs

Bottom Scale Response: 0.00 abs

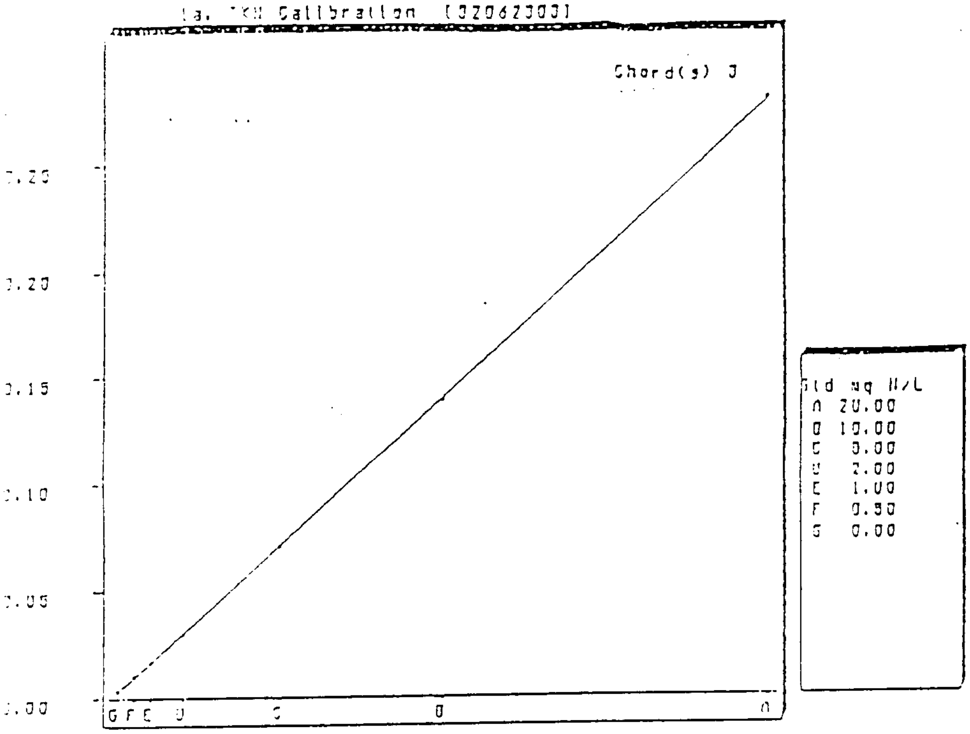
Segment/Boundaries: A: 20.00 mg N/L

E: 1 mg N/L

F: 0.00 mg N/L

Series 4000/System IV Settings: Gain = 420 x 1

17.3 QUIKCHEM AE SUPPORT DATA



```
                      Calibration Statistics Report
Cal Ref: 92062303                                      06/24/92 03:48 pm
 Method: TKN-F

                              Channel: TKN

                        Correlation Coefficients

Seg Stds     Full      Chord 1   Chord 2   Chord 3   Chord 4   Chord 5
 1  A-E    0.9999    0.9992    1.0000    1.0000    0.9999    0.4217 ?
 2  E-G    0.9977    0.7302 ?  1.0000    1.0000    0.9994    0.5000 ?

                  Percent Standard Deviation in Slope

 1  A-E      0.7       1.6       0.2       0.1       0.6       67.
 2  E-G      1.6      51.5 ?     0.0       0.0       4.3       72.
```

```
******************************************************************************
      QuikChem AE Calibration Report for Calibration 92062303
                          Method: TKN
             This calibration was done on 06/23/92 at  07:51 pm
             This report prepared on 06/24/92 at  03:44 pm
******************************************************************************

                                        --- Average Concentrations ---   Baseline Corrected
            Analyte          Units       Known   Determined  % Residual   Average Absorbance
Standard A, TKN              mg N/L      20.000    20.042      -0.21          0.2795

                                        --- Average Concentrations ---   Baseline Corrected
            Analyte          Units       Known   Determined  % Residual   Average Absorbance
Standard B, TKN              mg N/L      10.000     9.963       0.37          0.1394

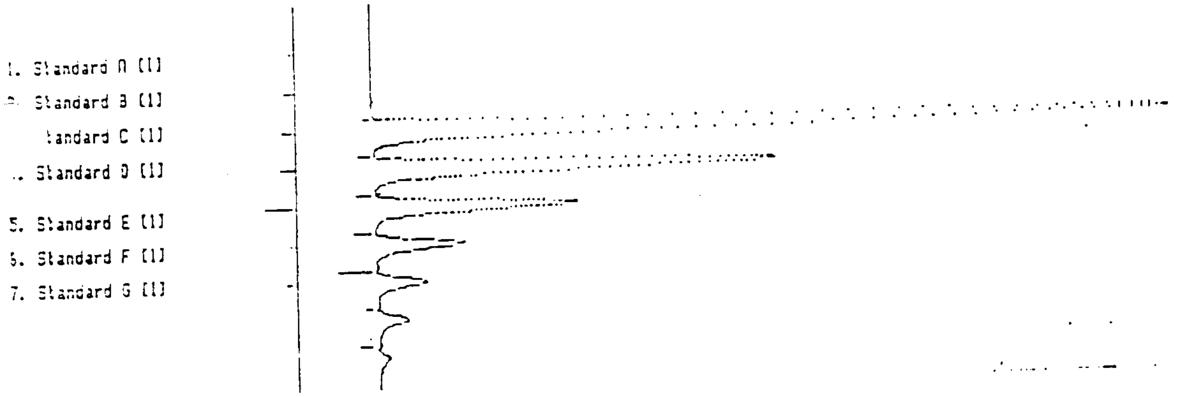
                                        --- Average Concentrations ---   Baseline Corrected
            Analyte          Units       Known   Determined  % Residual   Average Absorbance
Standard C, TKN              mg N/L       5.000     4.994       0.12          0.0714

                                        --- Average Concentrations ---   Baseline Corrected
            Analyte          Units       Known   Determined  % Residual   Average Absorbance
Standard D, TKN              mg N/L       2.000     2.007      -0.34          0.0301

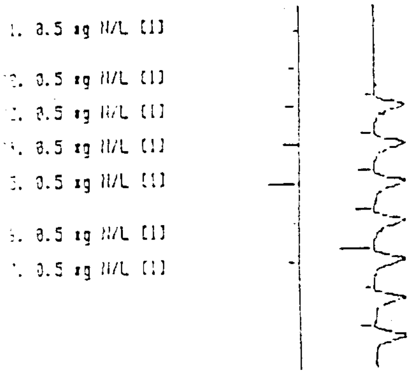
                                        --- Average Concentrations ---   Baseline Corrected
            Analyte          Units       Known   Determined  % Residual   Average Absorbance
Standard E, TKN              mg N/L       1.000     1.000       0.00          0.0168

                                        --- Average Concentrations ---   Baseline Corrected
            Analyte          Units       Known   Determined  % Residual   Average Absorbance
Standard F, TKN              mg N/L       0.500     0.500      -0.01          0.0101

                                        --- Average Concentrations ---   Baseline Corrected
            Analyte          Units       Known   Determined  % Residual   Average Absorbance
Standard G, TKN              mg N/L       0.000    -0.008      ***.**         0.0033
```



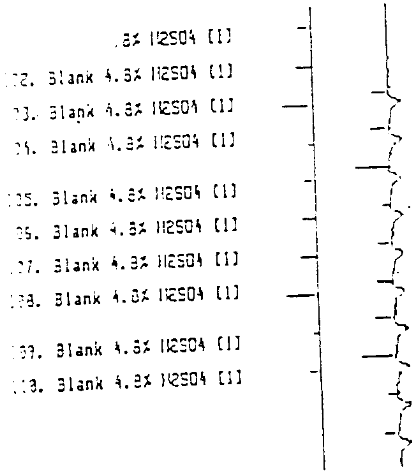
ack 1 (Ref: 92062300) 06/23/1992, 08:08 pm



Cup#	Sample ID	1 TKN mg N/L
101	0.5 mg N/L	0.492<
102	0.5 mg N/L	0.496<
103	0.5 mg N/L	0.506
104	0.5 mg N/L	0.492<
105	0.5 mg N/L	0.479<
106	0.5 mg N/L	0.487<
107	0.5 mg N/L	0.503

MDL
----
mean = 0.494
s = 0.0092
(3.14) s = 0.029 mg N/L

ack 1 (Ref: 92062306) 06/23/1992, 07:51 pm

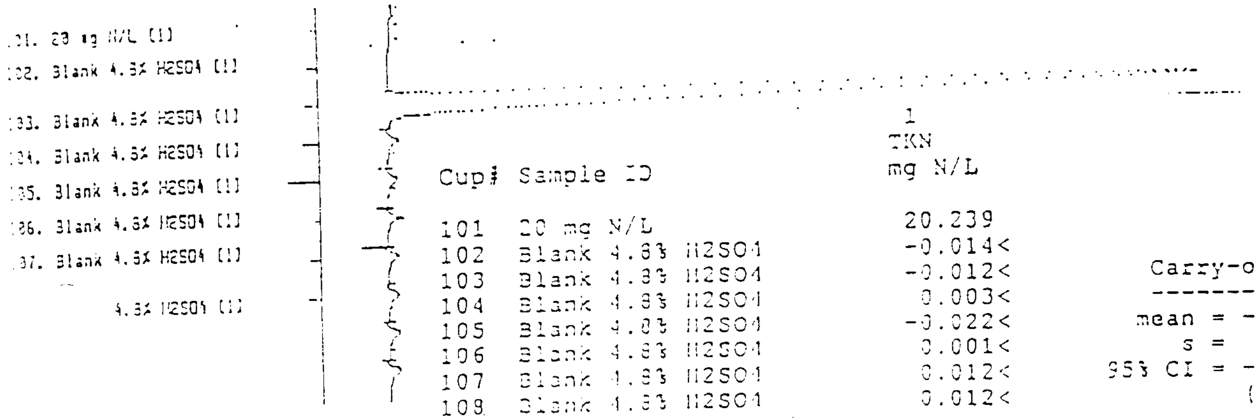


Cup#	Sample ID	1 TKN mg N/L
101	Blank 4.8% H2SO4	0.010<
102	Blank 4.8% H2SO4	-0.004<
103	Blank 4.8% H2SO4	0.007<
104	Blank 4.8% H2SO4	0.012<
105	Blank 4.8% H2SO4	-0.003<
106	Blank 4.8% H2SO4	-0.000<
107	Blank 4.8% H2SO4	0.021<
108	Blank 4.8% H2SO4	0.008<
109	Blank 4.8% H2SO4	0.015<
110	Blank 4.8% H2SO4	-0.007<

EMDL DATA

Mean = 0.0059
s = 0.0091
(4.65) s = 0.04 mg N/L

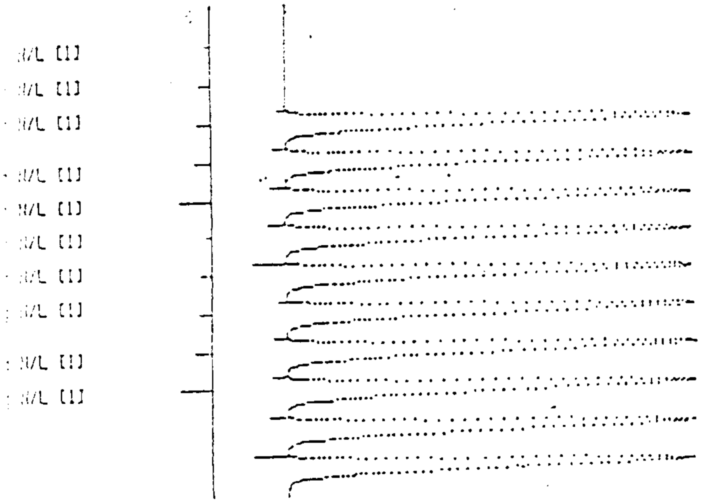
Rack 1 (Ref: 92062310) 06/23/1992, 08:29 pm



Cup#	Sample ID	1 TKN mg N/L
101	20 mg N/L	20.239
102	Blank 4.8% H2SO4	-0.014<
103	Blank 4.8% H2SO4	-0.012<
104	Blank 4.8% H2SO4	0.003<
105	Blank 4.8% H2SO4	-0.022<
106	Blank 4.8% H2SO4	0.001<
107	Blank 4.8% H2SO4	0.012<
108	Blank 4.8% H2SO4	0.012<

Carry-over
----
mean = -0.0029
s = 0.0133
95% CI = -0.015 to 0.009
(passes)

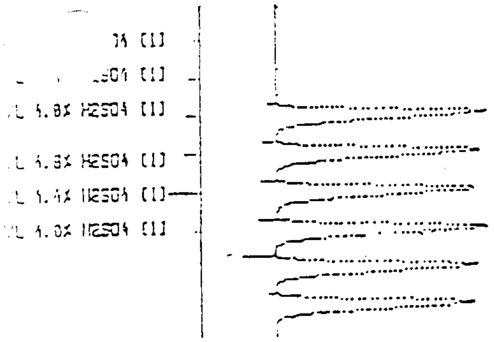
```
Ref: 92062309)   06/23/1992, 08:15 pm
```



```
                                           1
                                          TKN
Cup# Sample ID                          mg N/L

101  10.0 mg N/L                        10.017
102  10.0 mg N/L                        10.022
103  10.0 mg N/L                         9.971        Precision
104  10.0 mg N/L                         9.989        ---------
105  10.0 mg N/L                        10.001      mean = 10.06
106  10.0 mg N/L                        10.096         s = 0.065
107  10.0 mg N/L                        10.125      %RSD = 0.64
108  10.0 mg N/L                        10.102
109  10.0 mg N/L                        10.068
110  10.0 mg N/L                        10.065
```

```
Ref: 92062307)   06/23/1992, 08:00 pm
```



```
                                     1
                                    TKN
Cup# Sample ID                    mg N/L

101  5.0 mg N/L 4.8% H2SO4         5.039               Acid Effect
102  5.0 mg N/L 4.4% H2SO4         4.893               -----------
103  5.0 mg N/L 4.0% H2SO4         4.753      mean (4.8%) = 5.025 mg N/L
104  5.0 mg N/L 4.8% H2SO4         5.011      mean (4.4%) = 4.859 mg N/L
105  5.0 mg N/L 4.4% H2SO4         4.825      mean (4.0%) = 4.775 mg N/L
106  5.0 mg N/L 4.0% H2SO4         4.757
```

```
Ref: 92062311)   06/23/1992, 08:38 pm
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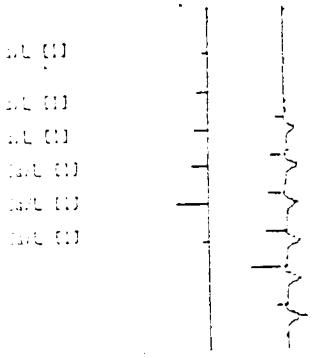


```
                                   1
                                  TKN
Cup# Sample ID                  mg N/L

101  20 mg Ca/L                 -0.002<      Calcium Interference
102  20 mg Ca/L                  0.001<      --------------------
103  20 mg Ca/L                  0.021<    <0.10 mg N/L at 100 mg Ca/L
104  100 mg Ca/L                 0.044<
105  100 mg Ca/L                 0.051<
106  100 mg Ca/L                 0.080<
```

17.4 DATA PARAMETERS FOR THE QUIK CHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput:	90 samples/hour; 60 s/sample
Pump speed:	35
Cycle Period:	45 s

Analyte data:

Peak Base Width:	39 s
% Width Tolerance:	100
Threshold:	11537
Inject to Peak Start:	42 s
Chemistry:	Direct

Calibration Data:

Levels	1	2	3	4	5	6	7
Concentrations mg P/L	20.00	10.00	5.00	2.00	1.00	0.50	0.00

Calibration Fit Type: 1st Order Polynomial

Weighting Method: None

Sampler Timing:

Min. Probe in Wash Period: 14 s

Probe in Sample Period: 20 s

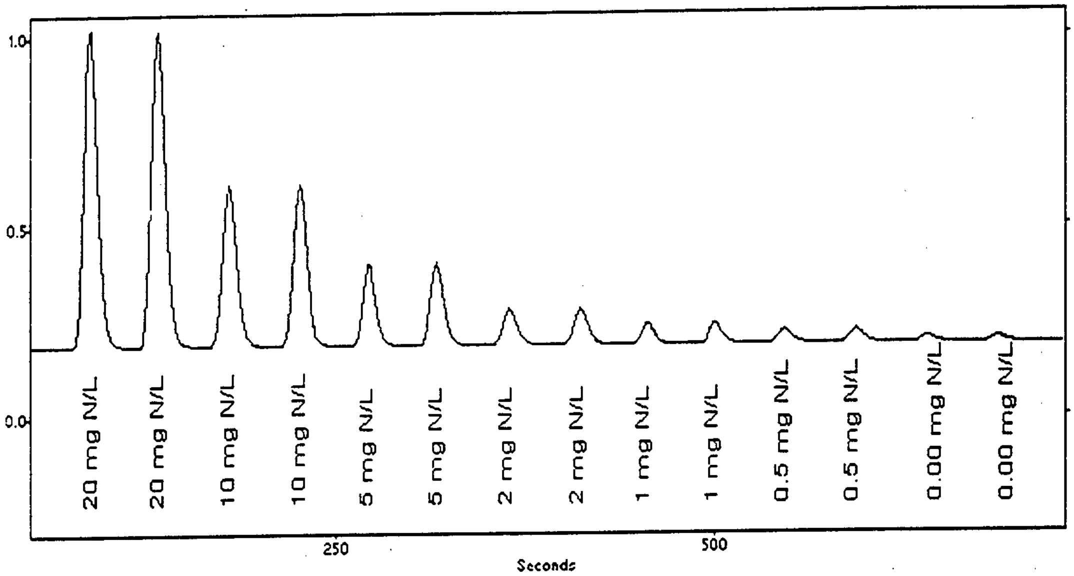
Valve Timing:

Load Period: 20 s

Inject Period: 25 s

Load Time: 0.0 s

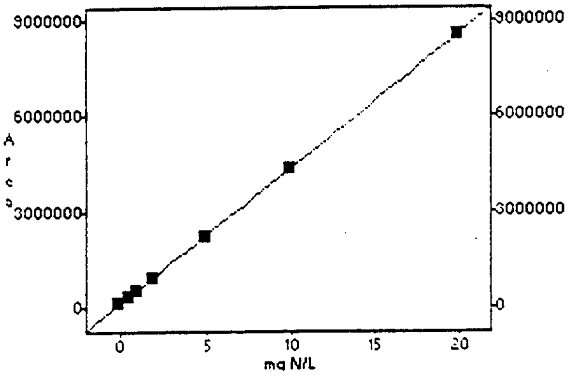
17.5 QUIKCHEM 8000 SUPPORT DATA



ACQ. TIME: Aug 22, 1994 13:25:57
DATA FILENAME: C:\OMNION\DATA\1010762E\082294C2.FDT
METHOD FILENAME: C:\OMNION\METHODS\1010762D\1010762D.MET

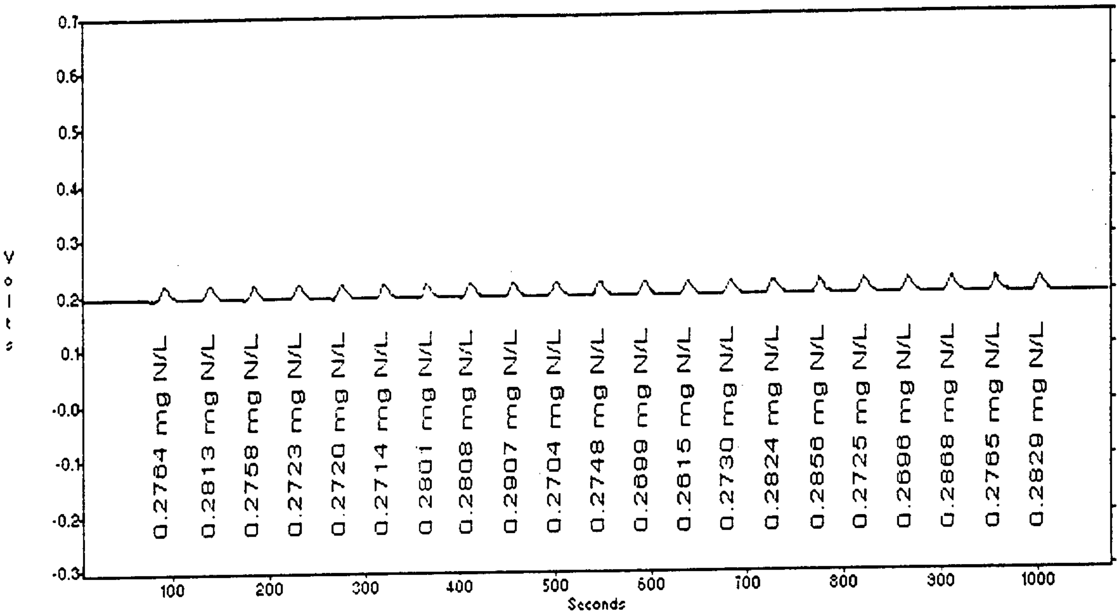
Calibration Graph and Statistics

Level	Area	mg CN⁻/L	Determined	Rep 1	Rep 2	Replic STD	Replic RSD	% residual
1	8596849	20	20.000	8596849	8634613	26703.2	0.3	-0.0
2	4383597	10	10.020	438597	4373046	7460.7	0.2	-0.2
3	2248447	5	4.960	2248447	2246723	1218.7	0.1	0.8
4	990856	2	1.991	990856	978804	8522.1	0.9	0.9
5	574638	1	0.997	574638	566821	5527.9	1.0	0.3
6	366814	0.5	0.504	366814	364718	1481.9	0.4	-0.9
7	167977	0	0	167977	165993	1403.2	0.8	---



Scaling: None
Weighting: None
1st Order Poly
Conc = 2.369e-006Area-3.646e-001
$R^2 = 1.000$

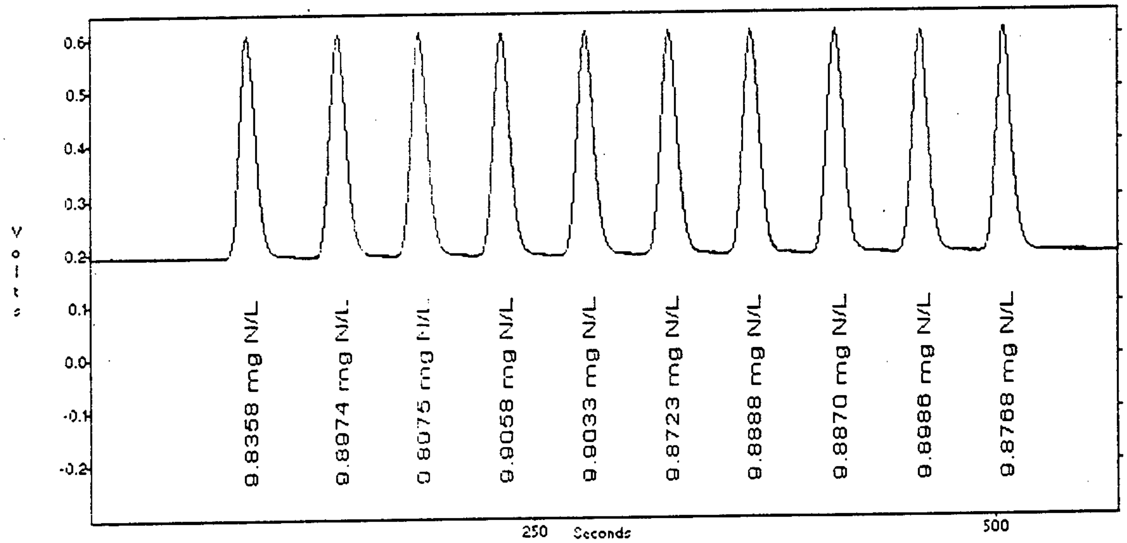
Figure 2. Method Detection Limit



MDL = 0.020 mg N/L

ACQ. TIME:	Aug 22, 1994 13:54:02
DATA FILENAME:	C:\OMNION\DATA\1010762E\082294M1.FDT
METHOD FILENAME:	C:\OMNION\METHODS\1010762D\1010762D.MET

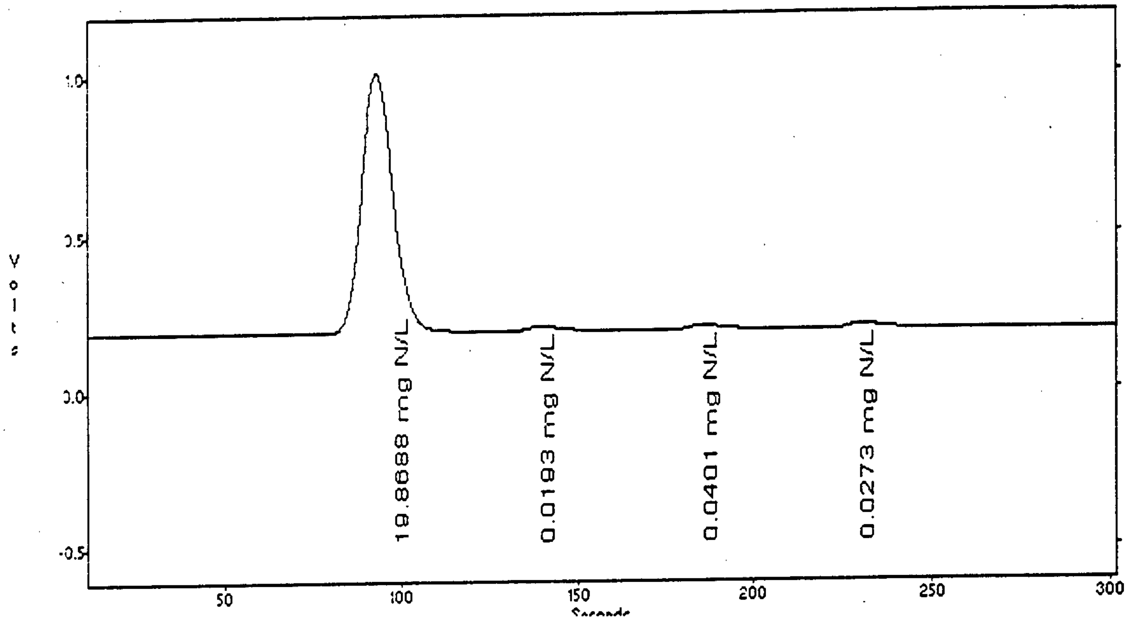
Figure 3. Precision



Precision = 0.211 % RSD

ACQ. TIME:	Aug 22, 1994 14:21:28
DATA FILENAME:	C:\OMNION\DATA\1010762E\082294P1.FDT
METHOD FILENAME:	C:\OMNION\METHODS\1010762D\1010762D.MET

Figure 4. Carryover



ACQ. TIME: Aug 22, 1994 14:32:42
DATA FILENAME: C:\OMNION\DATA\1010762E\082294R1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1010762D\1010762D.MET

QuikChem METHOD 10-107-06-2-E

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY

(BLOCK DIGESTOR METHOD)

Written by David H. Diamond

Applications Group

Revision Date:

18 October 1994

LACHAT INSTRUMENTS
6645 WEST MILL ROAD
MILWAUKEE, WI 53218, USA

QuikChem Method 10-107-06-2-E

Total Kjeldahl Nitrogen in Waters

0.1 to 5.0 mg N/L

-- Principle --

This method covers the determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia but may not the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

-- Interferences --

1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.4% (v/v) H_2SO_4 in the diluted digestion sample with no change in signal intensity.
2. High nitrate cocncentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

-- Special Apparatus --

1. Heating Unit
2. Block Digestor/75 mL tubes (Lachat Part. No. 1800-000)
3. 5 mL and 20 mL Repipet Dispensers

Revised by Karin Wendt 18 October 1994 written by D. Diamond and copyrighted on 24 June 92 by**Lachat Instruments, 6645 West Mill Road, Milwaukee, WI 53218,** USA. Phone: (414) 358-4200 FAX: 1-414-358-4206.

Contents

QUIKCHEM METHOD 10-107-06-2-E

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY (BLOCK DIGESTOR METHOD)

1. SCOPE AND APPLICATION

1.1. The method covers the determination of total Kjeldahl nitrogen in water and wastewater.

1.2. 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.

1.3. The applicable range is 0.1 to 5 mg N/L. The method detection limit is 0.02 mg N/L. 90 samples per hour can be analyzed.

1.4. Samples containing particulates should be filtered or homogenized.

2. SUMMARY OF METHOD

2.1. 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 an analyzed for ammonia. This digested sample may also be used for phosphorus determination.

2.2. 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.

2.3. Organic nitrogenous the difference obtained by subtracting the free-ammonia concentration from the total Kjeldahl nitrogen concentration.

2.4. Approximately 0.1 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.

2.5. 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 EDTA in the buffer prevents precipitation of calcium and magnesium.

3. DEFINITIONS

3.1. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards. but without the analyte.

3.2. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3. INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4. LABORATORY SPIKED BLANK (LSB) -- an aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LSB is analyzed exactly like a sample. and its purpose is to determine whether the methodology is in control. and whether the laboratory is capable of making accurate and precise measurements.

3.5. LABORATORY SPIKED SAMPLE MATRIX (LSM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LSM is analyzed exactly like sample. and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LSM corrected for background concentrations.

3.6. LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that is digested exactly as a sample including exposure to all glassware. equipment. and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents. or the apparatus.

3.7. LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.

3.8. MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity. health hazards, physical properties, fire, and reactivity data including storage. spill. and handling precautions.

3.9. METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified. measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10. QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to spike an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11. STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4. INTERFERENCES

4.1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.5% (v/v) H_2SO_4 in the diluted digestion sample with no change in signal intensity.

4.2. 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.

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

5. SAFETY

5.1. 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. Cautions are included for known extremely hazardous materials.

5.2. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3. The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1. Mercury (Reagents 1 and 2)

5.3.2. Sulfuric Acid (Reagents 1, 2 and 6)

5.3.3. Sodium Nitroprusside (Reagent 4)

6. EQUIPMENT AND SUPPLIES

6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

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

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1. Sampler

6.3.2. Multichannel proportioning pump

6.3.3. Reaction unit or manifold

6.3.4. Colorimetric detector

6.3.5. Data system

6.4. Special apparatus

6.4.1. Heating Unit

6.4.2. Block Digestor/75 mL (Lachat Part. No. 1800-000)

6.4.3. 5 mL and 20 mL repipet dispensers

6.4.4. Vortex mixer

7. REAGENTS AND STANDARDS

7.1. PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

Degassing with Helium

To prevent bubble formation, the water carrier is degassed with helium. Use He at 20 lb/in^2 through a helium degassing wand. Bubble He vigorously through the solution for one minute. If air spikes continue to be a problem, the buffer can also be degasssed.

Reagent 1. Mercuric Sulfate Solution

To a 100 mL volumetric flask add approximately 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.

Reagent 2. Digestion Solution

In 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. Prepare fresh monthly.

Reagent 3. Buffer

By Volume: In a **1 L** volumetric flask containing **900 mL water** completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ($Na_2HPO_4 \cdot 7H_2O$). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** (NaOH), dilute to the mark and invert to mix. Degas weekly and prepare fresh monthly.

By Weight: To a tared **1 L** container add **958 g water** and completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ($Na_2HPO_4 \cdot 7H_2O$). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** (NaOH). Stir or shake until dissolved. Degas weekly and prepare fresh monthly.

Reagent 4. 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 invert to mix. Store in a dark bottle and prepare fresh monthly.

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

Reagent 5. 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. Prepare fresh daily.

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 DI water**. Shake to mix. Prepare fresh daily.

Reagent 6. Diluent 5.0% (V/V) Sulfuric Acid

NOTE: Diluent is prepared to dilute off scale samples. This reagent is not used on-line.

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared **1 L** container, add **760 g water** and **250 mL Reagent 2 (Digestion Solution)**. Invert to mix.

7.2. PREPARATION OF STANDARDS

Prepare standards in DI water daily or preserve them with 2 mL/L sulfuric acid. Once preserved, standards may be stored for 28 days. Standards in digest matrix may be stored for up to 28 days. If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.

Digested Standards

NOTE: Working standards prepared in DI water are digested per the procedure in section 8.

Standard 1: Stock Standard 250 mg N/L

In a **1 L** volumetric flask dissolve **0.9540 g ammonium chloride** (NH_4Cl) that has been dried for two hours at 110°C in about **800 mL DI water**. Dilute to the mark and invert to mix. As an alternative, primary standard grade ammonium sulfate is available from Fisher Scientific, cat. no. A938-500 (use 1.18g).

Standard 2. Working Stock Standard 5.0 mg N/L

By Volume: In a **250 mL** volumetric flask, dilute **5.0 mL Stock Standard 1** to the mark with **DI water**. Invert to mix.

By Weight: To a tared **1 L** container add about **20 g Stock Standard 1**. Divide the exact weight of the standard solution by **0.02** and dilute up to this resulting total weight with **DI water**. Shake to mix.

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	5.00	2.00	1.00	0.50	0.25	0.10	0.00

By Volume

Volume (mL) of **Standard 2** diluted to 100 mL with DI water	100	40	20	10	5	2	0

By Weight

Weight (g) of **Standard 2** diluted to final weight (~250 g) divide by **factor** below with DI water.	250.0	100	50	25	12.5	5	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.40	0.20	0.10	0.05	0.02	0

Non-Digested Standards

Standard 3. Blank in Digestion Matrix (0.00 mg N/L)

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared **1 L** container, add **760 g water** and **250 mL Reagent 2 (Digestion Solution)**. Invert to mix.

Standard 4. High Standard in Digestion Matrix (5.00 mg N/L)

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Add **20 mL** of Standard 1 (250 mg N/L). Allow the solution to cool and dilute to the mark with DI water. Invert to mix. Prepare fresh monthly.

By Weight: To a tared **1 L** container, add **740 g water** and **250 mL Reagent 2 (Digestion Solution)**. Add **20 g** of Standard 1 (250 mg N/L) and shake to mix.

Note: Non-Digested standards will need to be labeled to reflect the changing concentration or dilution which occurs during the digestion procedure. The following formula can be used to calculate the adjustment. For example, using a final volume of 21 mL for the digestate and an initial sample volume of 20 mL results in a labeled concentration of a 5.25 mg N/L for a 5.00 mg N/L non-digested standard. If non-digested standards are used to calibrate, the "labeled" concentrations should be entered in the data system.

$$\text{Labeled non-digested standard concentration} = \frac{\text{final digestate volume}}{\text{initial sample volume}} \times \text{standard concentration}$$

Preparation of Non-digested Working Standards

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	5.00	2.00	1.00	0.50	0.25	0.10	0.00

By Volume

Volume (mL) of **Standard 4** diluted to 100 mL with **Standard 3**	100	40	20	10	5	2	0

By Weight

Weight (g) of **Standard 4** diluted to final weight (~250 g) divide by **factor** below with **Standard 3**.	250.0	100	50	25	12.5	5	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.40	0.20	0.10	0.05	0.02	0

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with dilute hydrochloric acid (0.5 M) and then rinsed with reagent water. The volume collected should be sufficient to insure a representative sample, allow for replicate analysis and minimize waste disposal.

8.2. Samples should be preserved to pH < 2 and cooled to 4°C at the time of collection.

8.3. Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 28 days.

9. QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards, the lowest concentration being > 10X MDL. If any determined concentration exceeds the known values by +/- 10%, linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3. Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within +/-10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) spiked at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the spiked reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates, t= 2.528 for twenty one replicates].

S = standard deviation of the replicate analyses.

MDLs should be determined every 6 months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3. ASSESSING LABORATORY PERFORMANCE

9.3.1. Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2. Laboratory Spiked Blank (LSB) -- The laboratory must analyze at least one LSB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3. The laboratory must used LSB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (X) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \overline{X} + 3S$$

$$\text{LOWER CONTROL LIMIT} = \overline{X} - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LSB. These data must be kept on file and be available for review.

9.3.4. Instruments Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/-10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/-10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

9.4. ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1. Laboratory Spiked Sample Matrix (LSM) -- The laboratory must add a known amount of analyte to a minimum of 10% of routine samples. In each case the LSM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory spiked blank.

9.4.2. Calculate the percent recovery for each analyte, corrected for concentrations measured in the unspiked sample, and compare these values to the designated LSM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_S - C}{s} \ X100$$

Where,

R = percent recovery

C_S = spiked sample concentration.

C = sample background concentration.

s = concentration equivalent of analyte added to sample.

9.4.3. If the recovery of any analyte falls outside the designated LSM recovery range and the laboratory performance for that analyte is shown to be in control the recovery problem encountered with the LSM is judged to be either matrix or solution related, not system related.

9.4.4. Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10. CALIBRATION AND STANDARDIZATION

10.1. Prepare a series of 7 standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (suggested range in section 7.2).

10.4. Calibrate the instrument as description in section 11.

10.2. Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

10.3. After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed +/-10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

[illegible]1. PROCEDURE

11.1. DIGESTION PROCEDURE

NOTE: Some laboratories prepare standards in DI water and process them through the digestion as outlined below. Other laboratories calibrate using standards in the digest matrix, i.e., **NOT** digested. Instructions for preparing standards in the digest matrix are given in section 7 of this method, following the instructions for preparing standards in DI water. At a minimum, two blanks and one standard should be prepared in DI water and digested.

11.1.1. Both standards and samples should be carried through this procedure. If samples have been preserved with sulfuric acid, standards should be preserved in the same manner.

11.1.2. To 20.0 mL of sample or standard add 5 mL digestion solution and mix. This is efficiently accomplished using an acid resistant 5 mL repipet device (EM Science, 108033-1, available through major scientific supply companies.)

11.1.3. Add 2 - 4 Hengar granules or 10 - 12 teflon stones to each tube. Hengar (Alundum) granules and teflon stones are effective for smooth boiling. Hengar granules are available from Fisher Scientific. cat. no. S145-500. Teflon stones are available from Markson Science, cat. no. 248-808, (800) 528-5114.

11.1.4. Ensure that the digestion tubes are dry on the outside and that all tubes contain boiling stones. 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 sample should have boiled off before increasing the temperature in step 5.

11.1.5. Continue to digest for 1.5 additional hours with the controller set to 380°C. This time includes the ramp time for the block temperature to come up to 380°C. The typical ramp time is 50 - 60 minutes. 380°C must be maintained for 30 minutes.

11.1.6. Before removing samples, gather the necessary supplies to dilute the samples with water. Remove the samples from the block and allow exactly 5 minutes to cool. Add water to the samples rapidly so that all samples are diluted within 10 minutes of removal from the block.

11.1.7. Add 19.0 mL DI water to each tube and vortex to mix. The total final volume should be 20 mL. The longer the samples have been allowed to cool, the longer the samples should be vortexed. For samples diluted at 5 minutes, 10 seconds of vortexing is sufficient. For samples which have cooled for greater than 10 minutes, up to 30 seconds of vortexing may be necessary.

11.1.8. If samples are not run immediately they should be diluted, vortexed and covered with lab film or capped tightly.

11.2. SYSTEM START-UP PROCEDURE

11.2.1. Prepare reagent and standards as described in section 7.

11.2.2. Set up manifold as shown in section 17.1.

11.2.3. Input peak timing and integration window parameters as specified in section 17.

11.2.4. Pump DI 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.

11.2.5. Place standards in the autosampler, and fill the sample tray. Input the information required by data system. such as concentration, replicates and QC scheme.

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

11.2.7. After a stable baseline has been obtained. start the sampler and perform analysis (please refer to system notes).

11.4. SYSTEM NOTES

11.4.1. Allow at least 15 minutes for the heating unit to warm up to 60°C.

11.4.2. If sample concentrations are greater than the high standard the digested sample should be diluted with **Reagent 6**. When the digital diluter is used, **Reagent 6** should be used as diluent. Do not dilute digested samples or standards with **DI water**.

11.4.3 If the salicylate reagent is merged with a sample containing sulfuric acid in the absence of the buffer solution, the salicylate reagent will precipitate. If this occurs all teflon manifold tubing should be replaced. To prevent this, prime the system by first placing the buffer transmission line in the buffer. Pump until the air bubble introduced during the transfer reaches the "T" fitting on the manifold. Then place all other transmission lines in the proper containers.

11.4.4. In normal operation nitroprusside gives a yellow background color which combines with the blue indosalicylate to give an emerald green color. This is the normal color of the solution in the waste container.

11.4.5. In normal operation the digest blank will result in a peak of about 1/5 the area of the 0.5 mg N/L standard. This peak is due to the acid in the digest and is present in every injection. Since this blank is constant for all samples and standards it will not effect data quality.

11.4.6. If phosphorus is also determined with the Lachat System, a second helium degassing tube should be purchased and the tubes should be dedicated to the individual chemistries.

11.4.7. If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure:

A. Place transmission lines in water and pump to clear reagents (2-5 minutes).

B. Place reagent lines in **1 M hydrochloric acid** (1 volume of HCl added to 11 volumes of water) and pump for several minutes.

C. Place all transmission lines in water and pump for several minutes.

D. Resume pumping reagents.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply the answer by the appropriate dilution factor.

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

12.3. Report results in mg N/L.

13. METHOD PERFORMANCE

13.1. The method performance data are presented as method support data in section 19.2. This data was generated according to Lachat Standard Operating Procedure J001, Lachat FIA Support Data Generation.

14. POLLUTION PREVENTION

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

14.2. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Society's Department of Government Regulations and Science Policy," 115 16Th Street N. W., Washington D. C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

15.1. The Environmental Protection Agency (USEPA) requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waster discharge permit and regulations, and by complying with all solid and hazardous waste regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the

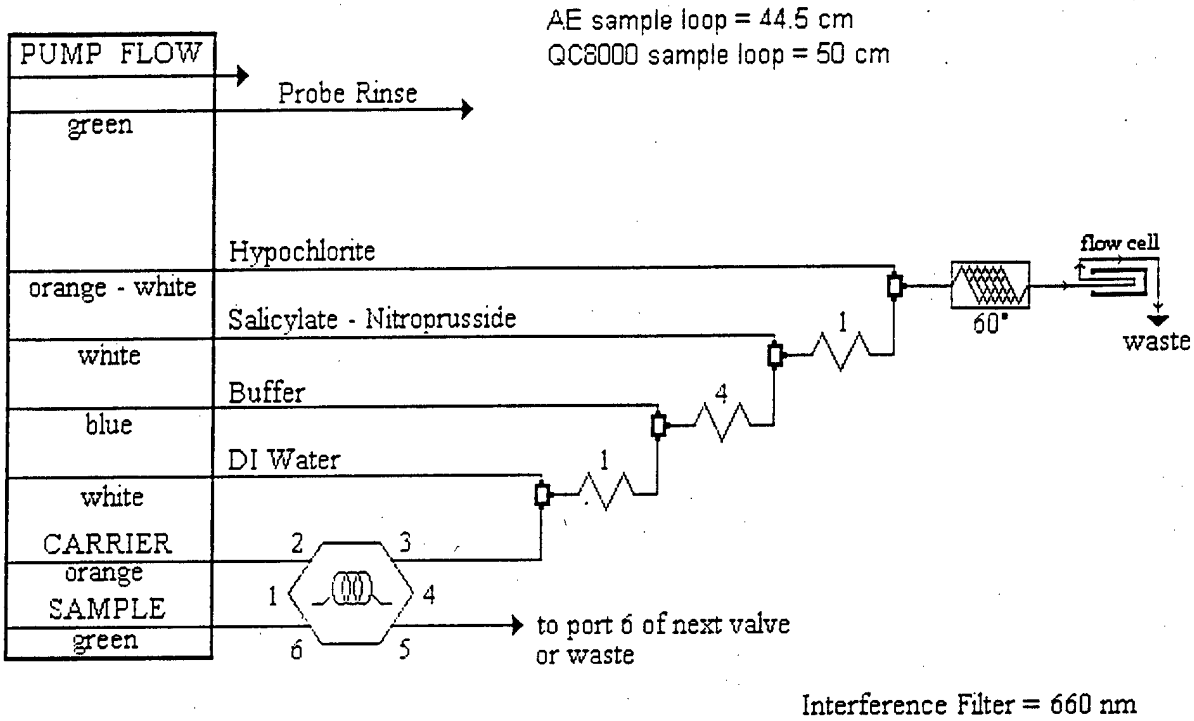
"Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Sect. 14.3.

16. REFERENCES

1. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**. EPA-600/4-79-020, Revised March 1983, Method 351.2
2. ASTM, Water(I), Volume 11.01, Method D3590-89, Test Methods for Kjeldahl Nitrogen in Water, p. 447
3. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**. EPA-600/4-79-020, Revised March 1983, Method 350.1
4. Code of Federal Regulations 40, Chapter 1, Part 136, Appendix B.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

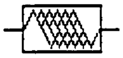
17.1. TOTAL KJELDAHL NITROGEN MANIFOLD DIAGRAM:



CARRIER is helium degassed water.

1 is **70 cm** of tubing on a **1 inch** coil support

4 is **255 cm** of tubing on a **4 inch** coil support

Apparatus: Standard valve, flow cell, and detector head modules are used. The  shows **650 cm of heated tubing**. All manifold tubing is **0.8 mm (0.032 in) i.d.** This is **5.2 uL/cm.**

MANIFOLD DIAGRAM REVISION DATE: 15 July 1992 by D. Diamond - 26Jul94 lc

17.2. DATA SYSTEM PARAMETERS FOR QUIKCHEM AE

Sample throughput: 90 samples/hour; 60 s/sample
Pump speed: 35
Cycle Period: 45 s

Inject to start of peak period: 38 s

Presentation. Data Window

Top Scale Response: 0.25 abs

Bottom Scale Response: 0.00 abs

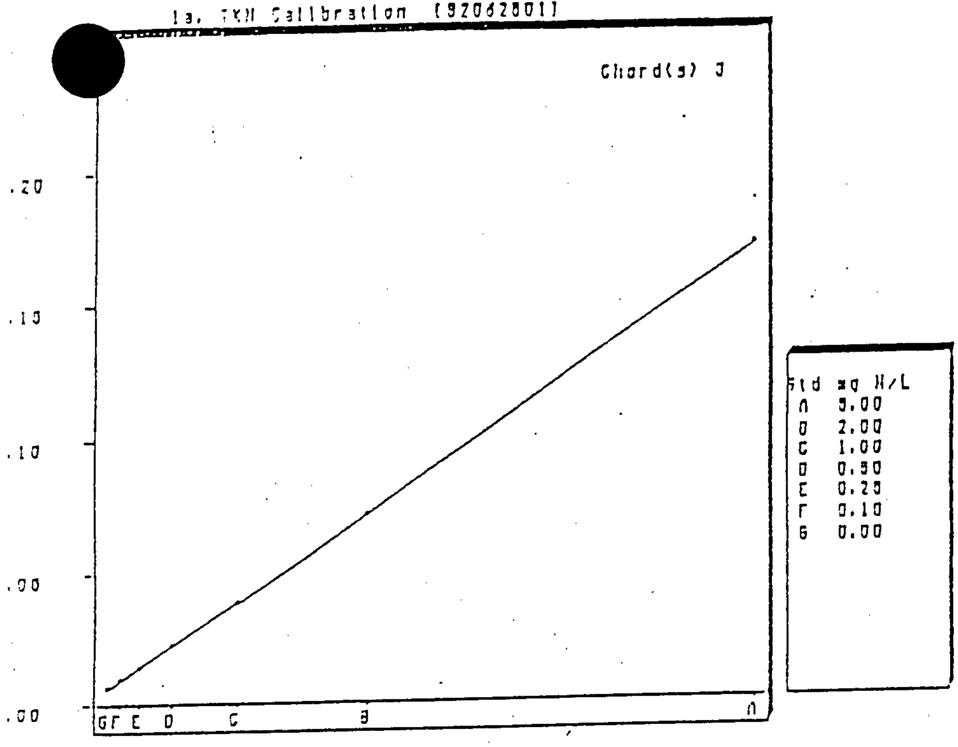
Segment/Boundaries: A: 5.00 mg N/L

C: 1 mg N/L

G: 0.00 mg N/L

Series 4000/System IV Settings: Gain = 570 x 1

17.3. QUIKCHEM AE SUPPORT DATA



```
                    CalibrationStatistics Report
Cal Ref: 92062501                                   06/25/92 01:43 pm
Method: TKN-F

                             Channel: TKN

                        Correlation Coefficients

Seg Stds      Full     Chord 1    Chord 2   Chord 3   Chord 4   Chord 5
 1  A-C     1.0000     0.9992     0.9997    1.0000    0.9999    1.0000
*2  C-G     0.9906     0.9384 ?   0.9980    0.9998    0.9959    0.4112 ?

                   Percent Standard Deviation in Slope

 1  A-C       0.3        2.8        1.8       0.5       0.8       0.4
 2  C-G       2.2       25.3        2.6       0.8       3.7      69.1 ?
```

```
------------------------------------------------------------------------
      QuikChem AE Calibration Report for Calibration 92062501
                        Method: TKN
           This calibration was done on 06/25/92 at  12:25 pm
           This report prepared on 06/25/92 at  01:33 pm
------------------------------------------------------------------------

                                      -- Average Concentrations --   Baseline Corrected
                Analyte       .Units   Known   Determined  % Residual  Average Absorbance
Standard A, TKN                mg N/L  5.000    5.003      -0.05        0.1715

                                      -- Average Concentrations --   Baseline Corrected
                Analyte        Units   Known   Determined  % Residual  Average Absorbance
Standard B, TKN                mg N/L  2.000    1.989       0.54        0.0711

                                      -- Average Concentrations --   Baseline Corrected
                Analyte        Units   Known   Determined  % Residual  Average Absorbance
Standard C, TKN                mg N/L  1.000    1.004      -0.36        0.0384

                                      -- Average Concentrations --   Baseline Corrected
                Analyte        Units   Known   Determined  % Residual  Average Absorbance
Standard D, TKN                mg N/L  0.500    0.496       0.78        0.0220

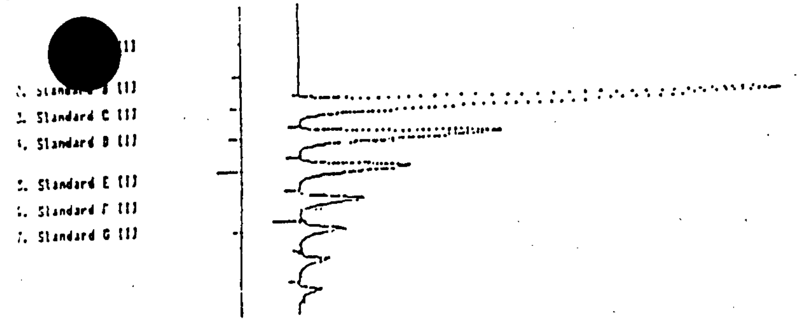
                                      -- Average Concentrations --   Baseline Corrected
                Analyte        Units   Known   Determined  % Residual  Average Absorbance
Standard E, TKN                mg N/L  0.250    0.242       3.27        0.0137

                                      -- Average Concentrations --   Baseline Corrected
                Analyte        Units   Known   Determined  % Residual  Average Absorbance
Standard F, TKN                mg N/L  0.100    0.102      -1.77        0.0092

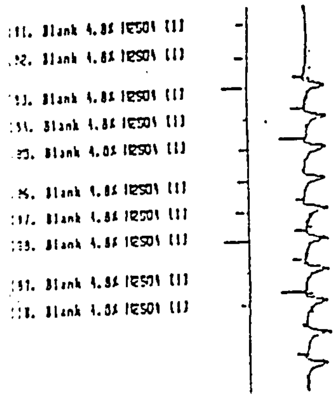
                                      -- Average Concentrations --   Baseline Corrected
                Analyte        Units   Known   Determined  % Residual  Average Absorbance
Standard G, TKN                mg N/L  0.000    0.006      ***.**       0.0061

----------- End of Calibration Report For Calibration 92062501 -----------
```

Calibration 06/25/1992, 12:17 pm

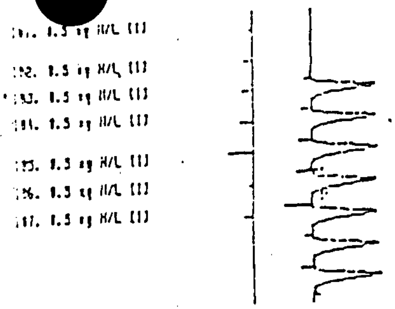


Rack 1 (Ref: 92062502) 06/25/1992, 12:25 pm



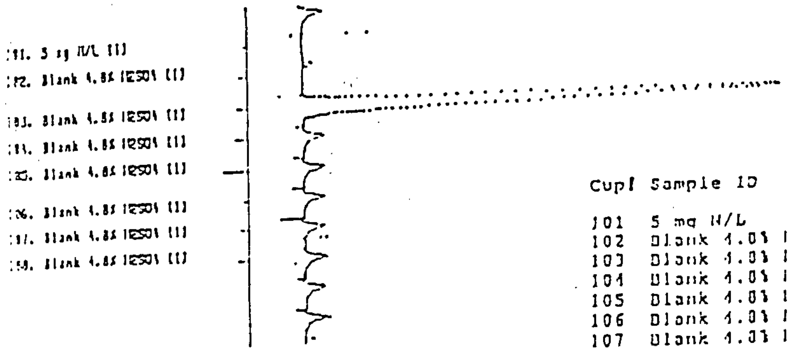
```
                                  1
                                 TKN
 Cup| Sample ID                 mg N/L
                                 0.013<        EMDL Determination
 101   Blank 4.0% H2SO4          0.012<        ------------------
 102   Blank 4.0% H2SO4          0.009<          mean = 0.009
 103   Blank 4.0% H2SO4          0.003<             s = 0.016
 104   Blank 4.0% H2SO4          0.049<      4.65 (s) = 0.076 mg N/L
 105   Blank 4.0% H2SO4         -0.007<
 106   Blank 4.0% H2SO4          0.010<
 107   Blank 4.0% H2SO4          0.009<
 108   Blank 4.0% H2SO4          0.009<
 109   Blank 4.0% H2SO4         -0.013<
 110   Blank 4.0% H2SO4
      --------- End of Report for Tray 92062502.RS -----------
```

[illegible] (Ref: 92062504) 06/25/1992, 12:41 pm



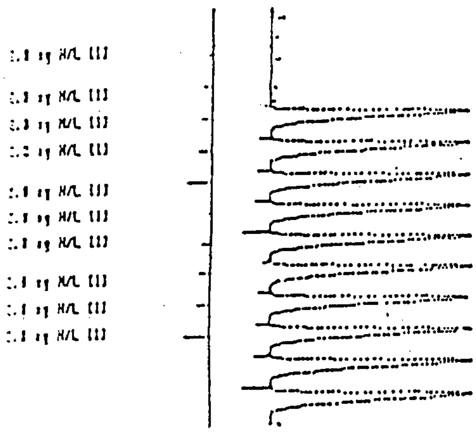
```
                                      1
                                     TKN
 Cup| Sample ID                     mg N/L
                                      0.489
 101   0.5 mg N/L                     0.486          MDL Study
 102   0.5 mg N/L                     0.488         -----------
 103   0.5 mg N/L                     0.491        mean = 0.487
 104   0.5 mg N/L                     0.478           s = 0.008
 105   0.5 mg N/L                     0.492      (3.14) s = 0.024
 106   0.5 mg N/L                     0.490
 107   0.5 mg N/L
      --------- End of Report for Tray 92062504.RS -----------
```

Rack 1 (Ref: 92062506) 06/25/1992, 12:54 pm



```
                                  1
                                 TKN
 Cup| Sample ID                 mg N/L
                                  4.052
 101   5 mg N/L                   0.003<        Carry-over Study
 102   Blank 4.0% H2SO4           0.002<        ----------------
 103   Blank 4.0% H2SO4           0.001<        mean = 0.001
 104   Blank 4.0% H2SO4          -0.001<           s = 0.005
 105   Blank 4.0% H2SO4           0.009<   95% CI ( -0.004 to 0.006)
 106   Blank 4.0% H2SO4           0.004<           passes
 107   Blank 4.0% H2SO4          -0.009<
 108   Blank 4.0% H2SO4
      --------- End of Report for Tray 92062506.RS -----------
```

```
Ch 1 (Ref: 92062507)   06/25/1992, 01:04 pm
```

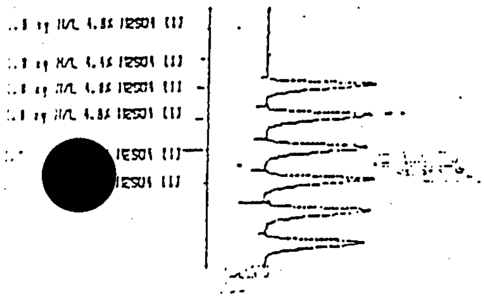


```
                                       1
                                       TKN
Cup Sample ID                          mg N/L

101  2.0 mg N/L                          1.923
102  2.0 mg N/L                          1.926      Precision at Midscale
103  2.0 mg N/L                          1.925      ---------------------
104  2.0 mg N/L                          1.926        mean = 1.929
105  2.0 mg N/L                          1.947           s = 0.023
106  2.0 mg N/L                          1.912        %RSD = 1.18
107  2.0 mg N/L                          1.883
108  2.0 mg N/L                          1.933
109  2.0 mg N/L                          1.955
110  2.0 mg N/L                          1.963

   --------- End of Report for Tray 92062507.RS -----------
```

```
Ch 1 (Ref: 92062503)   06/25/1992, 12:35 pm
```

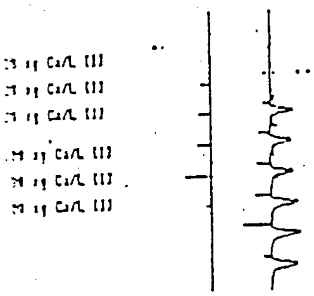


```
                                              1
                                              TKN
Cup Sample ID                                 mg N/L

101  1.0 mg N/L 4.0% H2SO4                    0.966        Acid Effect
102  1.0 mg N/L 4.4% H2SO4                    0.960        -----------
103  1.0 mg N/L 4.8% H2SO4                    0.894    mean (4.0%) = 0.971
104  1.0 mg N/L 4.0% H2SO4                    0.975    mean (4.4%) = 0.943
105  1.0 mg N/L 4.4% H2SO4                    0.925    mean (4.8%) = 0.891
106  1.0 mg N/L 4.8% H2SO4                    0.888

   --------- End of Report for Tray 92062503.RS -----------
```

```
Ch 1 (Ref: 92062508)   06/25/1992, 01:14 pm
```



```
                                        1
                                        TKN
Cup Sample ID                           mg N/L

101  20 mg Ca/L                         -0.013<
102  20 mg Ca/L                          0.005<    Calcium Interference Study
103  20 mg Ca/L                          0.020<    --------------------------
104  100 mg Ca/L                         0.052<     mean (20 mg/L) = 0.004
105  100 mg Ca/L                         0.058<    mean (100 mg/L) = 0.059
106  100 mg Ca/L                         0.068<       <MDL at 100 mg/L

   --------- End of Report for Tray 92062508.RS -----------
```

17.4. DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput:	90 samples/hour; 60 s/sample
Pump speed:	35
Cycle Period:	45 s

Analyte data:

Peak Base Width:	31 s
% Width Tolerance:	100
Threshold:	25000
Inject to Peak Start:	42 s
Chemistry:	Direct

Calibration Data:

Levels	1	2	3	4	5	6	7
Concentrations mg P/L	5.00	2.00	1.00	0.50	0.25	0.10	0.00

Calibration Fit Type: 1st Order Polynomial

Weighting Method: None

Sampler Timing:

Min. Probe in Wash Period: 14 s

Probe in Sample Period: 20 s

Valve Timing:

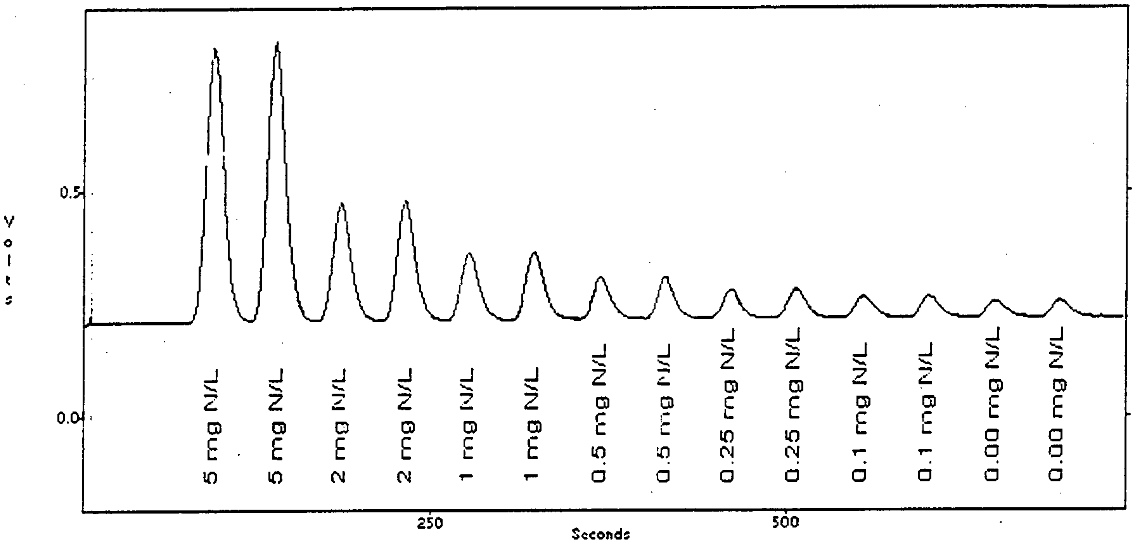
Load Period: 20 s.

Inject Period: 25 s

Load Time: 0.0 s

17.5 QUIKCHEM 8000 SUPPORT DATA

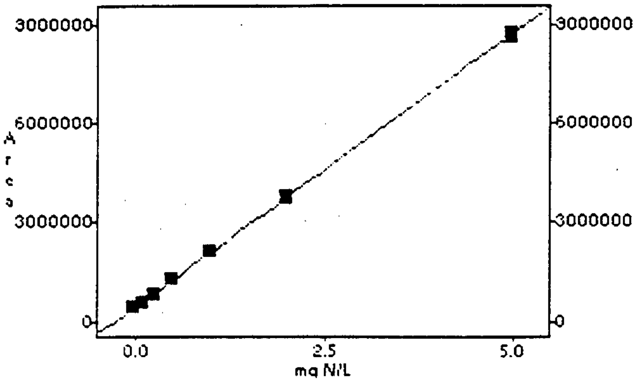
Figure 1. Calibration Graph and Statistics for Total Kjeldahl Nitrogen



ACQ. TIME: Aug 15, 1994 15:00:17
DATA FILENAME: C:\OMNION\DATA\1010762E\081594C1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1010762E\1010762E.met

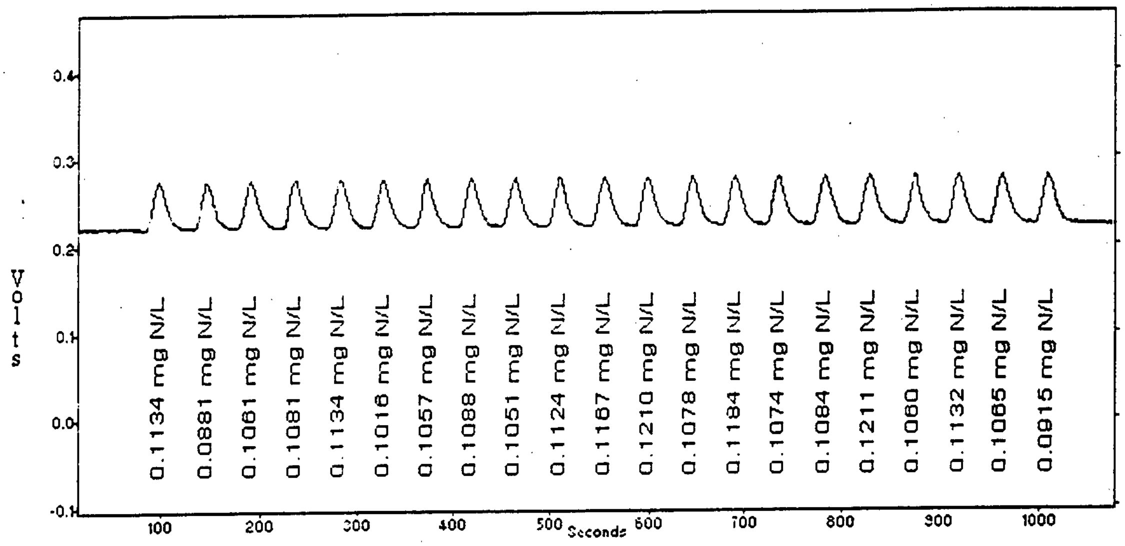
Calibration Graph and Statistics

Level	Area	mg N/L	Determined	Rep 1	Rep 2	Replic STD	Replic RSD	% residual
1	8778128	5	5.000	8851078	8705178	1003166.9	1.2	0.0
2	3809886	2	2.002	3832259	3787513	31640.2	0.8	-0.1
3	2159521	1	1.006	2157189	2161852	3296.9	0.2	-0.6
4	1326319	0.5	0.5035	1335357	1317280	12782.8	1.0	-0.7
5	896683	0.25	0.2445	890758	902609	8380.0	0.9	2.2
6	643806	0.1	0.108	648879	638732	7174.7	1.1	8.0
7	498016	0	0	5071570	488876	12926.6	2.6	---



Scaling: None
Weighting: None
1st Order Poly
Conc = 6.032e-007Area-2.964e-001
$R^2 = 1.000$

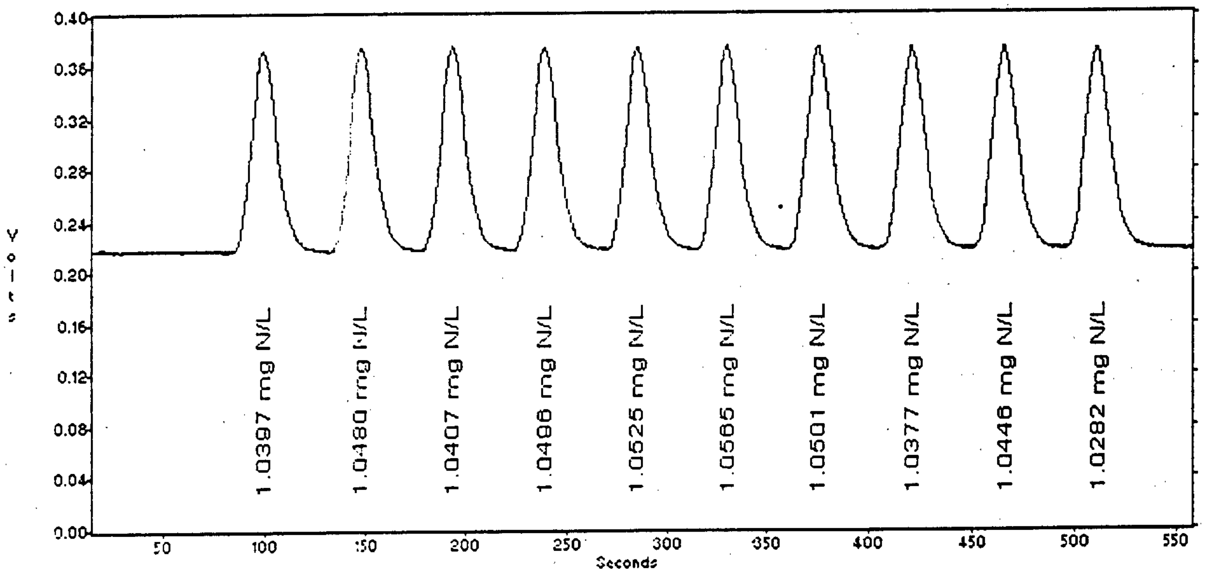
Figure 2. Method Detection Limit



MDL = 0.020 mg N/L

ACQ. TIME: Aug 18, 1994 8:52:31
DATA FILENAME: C:\OMNION\DATA\1010762E\081894M1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1010762E\1010762E.met

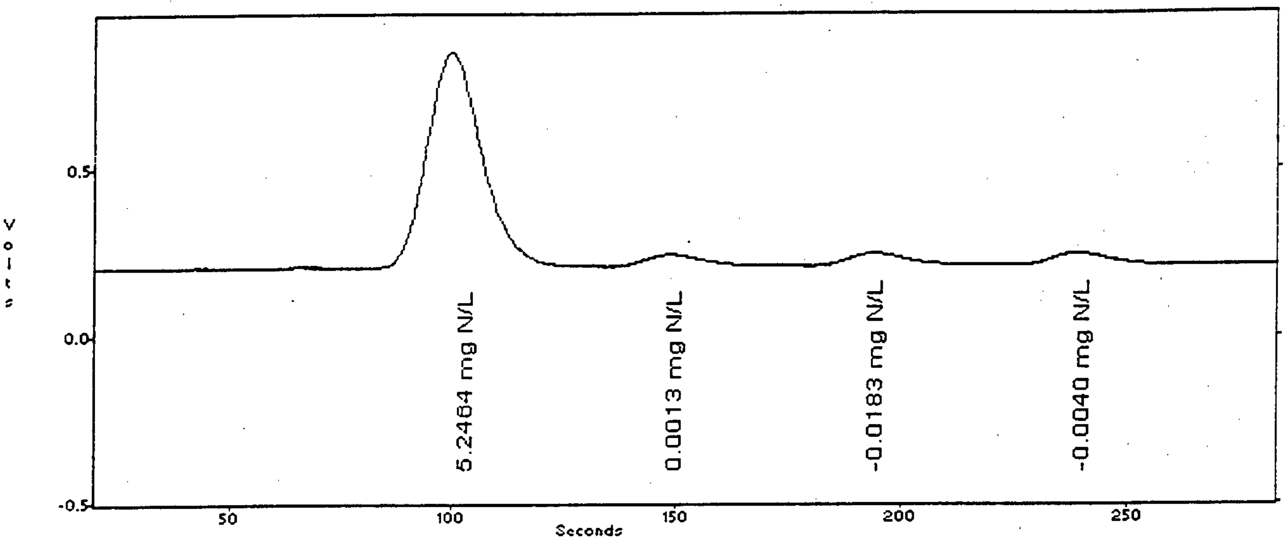
Figure 3. Precision



Precision = 0.796 % RSD

ACQ. TIME: Aug 15, 1994 15:50:24
DATA FILENAME: C:\OMNION\DATA\1010762E\081594P1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1010762E\1010762E.met

Figure 4. Carryover



Carryover passed

ACQ. TIME:	Aug 15, 1994 16:01:01
DATA FILENAME:	C:\OMNION\DATA\1010762E\081594R1.FDT
METHOD FILENAME:	C:\OMNION\METHODS\1010762E\1010762E.met

Technicon™ AutoAnalyzer™ II

INDUSTRIAL METHOD No. 334-74W/B+ RELEASED: JANUARY, 1976/REVISED: MARCH 1977

INDIVIDUAL/SIMULTANEOUS* DETERMINATION OF NITROGEN AND/OR PHOSPHORUS IN BD ACID DIGESTS

RANGE: Nitrogen 1-50 mg/l; 20-1000 mg/l
Phosphorus 1-50 mg/l; 20-1000 mg/l
BD-20/BD-40 (DIALYZER)

GENERAL DESCRIPTION

NITROGEN

The determination of nitrogen is based on a colorimetric method in which an emerald-green color is formed by the reaction of ammonia, sodium salicylate, sodium nitroprusside and sodium hypochlorite (chlorine source) in a buffered alkaline medium at a pH of 12.8-13.0. The ammonia-salicylate complex is read at 660 nm.

PHOSPHORUS

The determination of phosphorus is based on the colorimetric method in which a blue color is formed by the reaction of ortho phosphate, molybdate ion and antimony ion followed by reduction with ascorbic acid at an acidic pH. The phosphomolybdenum complex is read at 660 nm.

The acid digest samples are prepared by digestion with the Technicon™ BD-40 or BD-20 Block Digestor. Refer to Manual No. TA4-0323-11 for sample preparation.

PERFORMANCE AT 40 SAMPLES PER HOUR

MANUALLY PREPARED STANDARDS

NITROGEN	1-50 mg/l	20-1000 mg/l
Sensitivity	at 50 mg/l 0.20 absorbance unit	at 1000 mg/l 1.00 absorbance unit
Coefficient of Variation	at 25 mg/l ±0.6%	at 500 mg/l ±0.4%
Detection Limit	1.0 mg/l	20 mg/l

PHOSPHORUS	1-50 mg/l	20-1000 mg/l
Sensitivity	at 50 mg/l 0.20 absorbance unit	at 1000 mg/l 0.60 absorbance unit
Coefficient of Variation	at 25 mg/l ±0.5%	at 500 mg/l ±0.6%
Detection Limit	1.0 mg/l	20 mg/l

*See Operating Note 7.

TECHNICON INDUSTRIAL SYSTEMS / TARRYTOWN, N.Y. 10591
A DIVISION OF TECHNICON INSTRUMENTS CORPORATION

REAGENTS

Unless otherwise specified, all reagents should be of ACS quality or equivalent.

GENERAL REAGENTS

TRITON X-100 SOLUTION (50% in Methanol)

Triton X-100** (Technicon No. T21-0188)	50 ml
Methanol (CH_3OH)	50 ml

Preparation:

Add 50 ml of Triton X-100 to 50 ml of methanol and mix thoroughly.

SYSTEM WASH WATER SOLUTION (For System Shut-Down and Start-Up Only)

Triton X-100 Solution	1.0 ml
Distilled Water	1000 ml

Preparation:

Add 1.0 ml of Triton X-100 solution to one liter of distilled water and mix.

SAMPLER IV WASH RECEPTACLE SOLUTION

Distilled Water

Note: This reagent contains *no* wetting agent.

NITROGEN REAGENTS

STOCK SODIUM HYDROXIDE SOLUTION, 20%

Sodium Hydroxide Solution, 50% w/w	400 g
Distilled Water, q.s.	1000 ml

Preparation:

To 600 ml of distilled water, add 400 g of sodium hydroxide solution, 50% w/w. Cool to room temperature and dilute to one liter with distilled water.

STOCK SODIUM POTASSIUM TARTRATE SOLUTION, 20%

Sodium Potassium Tartrate ($NaKC_4H_4O_6 \cdot 4H_2O$)	200 g
Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 200 g of sodium potassium tartrate in about 600 ml of distilled water. Dilute to one liter with distilled water and mix thoroughly.

STOCK BUFFER SOLUTION 0.5*M*

Sodium Phosphate, Dibasic, crystal ($Na_2HPO_4 \cdot 7H_2O$	134 g
[Sodium Phosphate, Dibasic, anhydrous (Na_2HPO_4)]	[71 g]
Sodium Hydroxide Solution, 50% w/w	40 g
Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 134 g of sodium phosphate, dibasic, crystal (or 71 g of sodium phosphate, dibasic, anhydrous) in about 800 ml of distilled water. Add 40 g of sodium hydroxide solution, 50% w/w, dilute to one liter with distilled water and mix thoroughly.

WORKING BUFFER SOLUTION

Stock Buffer Solution, 0.5*M*	200 ml
Stock Sodium Potassium Tartrate Solution, 20%	250 ml
Stock Sodium Hydroxide Solution, 20%	250 ml
Distilled Water, q.s.	1000 ml
Brij-35,*** 30% Solution (Technicon No. T21-0110)	1.0 ml

Preparation:

Combine the reagents in the stated order: add 250 ml of stock sodium potassium tartrate solution, 20%, to 200 ml of stock buffer solution, 0.5*M*, with swirling. Slowly, with swirling, add 250 ml of sodium hydroxide solution, 20%. Dilute to one liter with distilled water, add 1.0 ml of Brij-35. 30% solution, (20-25 drops) and mix thoroughly.

SULFURIC ACID/SODIUM CHLORIDE SOLUTION

Sulfuric Acid, 95-98% (H_2SO_4)	7.5 ml
Sodium Chloride (NaCl)	100 g
Distilled Water, q.s.	1000 ml
Brij-35, 30% Solution	1.0 ml

Preparation:

Dissolve 100 g of sodium chloride in about 600 ml of distilled water. Add 7.5 ml of sulfuric acid and dilute to one liter with distilled water. Add 1.0 ml of Brij-35 (about 20 drops) and mix thoroughly.

SODIUM SALICYLATE/SODIUM NITROPRUSSIDE SOLUTION

Sodium Salicylate ($NaC_7H_5O_3$)	150 g
Sodium Nitroprusside [$Na_2Fe(CN)_5NO \cdot 2H_2O$]	0.30 g
Distilled Water, q.s.	1000 ml
Brij-35, 30% solution	1.0 ml

**Trademark of Rohm and Haas Company.

***Trademark of Atlas Chemical Industries, Inc.

Preparation:

Dissolve 150 g of sodium salicylate and 0.30 g of sodium nitroprusside in about 600 ml of distilled water. Filter through fast filter paper into a one liter volumetric flask and dilute to volume with distilled water. Add 1.0 ml of Brij-35 and mix thoroughly. Store in a light-resistant container.

SODIUM HYPOCHLORITE SOLUTION, 0.315%

Sodium Hypochlorite Solution, 5.25%	6.0 ml
Distilled Water, q.s.	100 ml
Brij-35, 30% Solution	0.1 ml

Preparation:

Dilute 6.0 ml of sodium hypochlorite solution to 100 ml with distilled water. Add 0.1 ml (2 drops) of Brij-35 and mix thoroughly. Prepare fresh daily. [Any commercial bleach solution (e.g. Clorox) containing 5.25% available chlorine is satisfactory.]

PHOSPHORUS REAGENTS

SULFURIC ACID SOLUTION, 4.0*N*

Sulfuric Acid, 95-98% (H_2SO_4)	111 ml
Distilled Water, q.s.	1000 ml
Triton X-100 Solution	1.0 ml

Preparation:

While swirling, cautiously add 111 ml of sulfuric acid to about 600 ml of distilled water. Cool to room temperature and dilute to one liter with distilled water. Add 1.0 ml of Triton X-100 solution and mix thoroughly.

SODIUM CHLORIDE SOLUTION, 0.25%

Sodium Chloride (NaCl)	2.5 g
Distilled Water, q.s.	1000 ml
Aerosol-22****	5.0

Preparation:

Dissolve 2.5 g of sodium chloride in about 600 ml of distilled water. Dilute to one liter with distilled water. Add 5.0 ml of Aerosol-22 and mix thoroughly.

MOLYBDATE/ANTIMONY SOLUTION

Ammonium Molybdate $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$	10.0 g
Antimony Potassium Tartrate $[K(SbO)C_4H_4O_6 \cdot 1/2H_2O]$	0.15 g
Sulfuric Acid, 95-98% (H_2SO_4)	60 ml
Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 10.0 g of ammonium molybdate and 0.15 g of antimony potassium tartrate in about 800 ml of distilled water. While swirling, cautiously add 60 ml of sulfuric acid. Cool to room temperature, dilute to one liter with distilled water and mix thoroughly. Transfer to a light-resistant container. This solution is stable for about one month.

ASCORBIC ACID SOLUTION, 1.0%

Ascorbic Acid ($C_6H_8O_6$) — OR — Araboascorbic Acid ($C_6H_8O_6$)	2.0 g
Distilled Water, q.s.	200 ml

Preparation:

Dissolve 2.0 g of ascorbic acid or araboascorbic acid in about 150 ml of distilled water. Dilute to 200 ml with distilled water and mix thoroughly. Transfer to a light-resistant container. If kept refrigerated and tightly stoppered when not in use, this solution is stable for at least two days.

OPERATING NOTES

1. **Start-Up**
   a. Check the level of all reagents to ensure an adequate supply.
   b. Excluding the salicylate and molybdate/antimony lines, place all reagent lines in their respective containers.
   c. When reagents have been pumping for at least five minutes, place the salicylate and molybdate/antimony lines in their respective containers and allow the system to equilibrate for 10 minutes.

   NOTE: If a precipitate appears after the addition of salicylate, immediately stop the proportioning pump and flush the coils with water using a syringe. Precipitation of salicylic acid is caused by a low pH. Before restarting the system, check the concentration of the sulfuric acid solution and/or the working buffer solution.

   d. To prevent precipitation of salicylic acid in the waste tray (which can clog the tray outlet), keep the nitrogen flowcell pump tube and the nitrogen colorimeter TO WASTE tube separate from all other lines or keep tap water flowing in the waste tray.

2. **Shut-Down**
   a. Remove the salicylate and molybdate/antimony lines from their containers and allow them to pump air. When the air bubbles enter the analytical system, place all reagent lines (excluding the Sampler IV Wash Receptacle Solution line) in the System Wash Water Solution.
   b. After 15 minutes, stop the proportioning pump and remove the platen.

****Trademark of American Cyanamid Company.

3. **System Operation**
   a. Be sure the plastic cover of the analytical cartridge is in place when operating the system.
   b. At STD CAL settings of 6.00 or more, the system may be operated in the DAMP 1 position, if necessary.

4. **Manifold Connections**

   To avoid the possibility of airborne contamination, the air lines of the nitrogen channel should be attached to an air scrubber containing dilute sulfuric acid (10% v/v).

5. **Reagent Background Color**
   a. Place all lines in the system wash water container and start the proportioning pump. After making the necessary adjustments on the colorimeters set the STD CAL control of the nitrogen colorimeter to 1.00 and the STD CAL control of the phosphorus colorimeter to 2.90. Adjust the water baseline on both colorimeters to zero with the BLANK control.
   b. Following the start-up procedure, place all reagent lines in the proper order in their respective containers and allow the system to equilibrate.
   c. The reading of the reagents compared to distilled water should not be more than 14 units (0.140 absorbance) for the nitrogen channel and not more than 5 units (0.25 absorbance) for the phosphorus channel. If the absorbance of either channel is much higher than the above values, one or more of the reagents or the water used to make up the reagents is probably contaminated.

6. **Concentration Ranges**
   a. All concentration ranges refer to the concentration of components in the digestion tube after diluting to volume with distilled water.
   b. **Nitrogen Channel**
      1. Concentration ranges from 1-50 mg/l to 20-1000 mg/l can be accommodated by changing the size of the flowcell and the sample, resample and diluent lines as designated in the concentration ranges table (refer to Figure 1 and flow diagram).
      2. For any one manifold configuration, an approximate five-fold change in concentration can be accommodated by use of the STD CAL control. The system is linear when operated at a STD CAL setting of 1.00 or higher.
   c. **Phosphorus Channel**
      1. Concentration ranges from 1-50 mg/l to 20-1000 mg/l can be accommodated by changing the size of the sample, resample and diluent lines as designated in the concentration ranges table (refer to Figure and flow diagram).
      2. For any one manifold configuration, an approximate three-fold change in concentration can be accommodated by use of the STD CAL control. The system is linear when operated at a STD CAL setting of 2.00 or higher.

7. **Manifold Configurations**
   a. **Individual Determination of N or P**

      When N or P is being determined individually, the PT fitting is omitted and the sample line is attached directly to the sample probe of the Sampler IV.
   b. **Simultaneous Determination of N and P**

      When N and P are being determined simultaneously, both initial sample lines are connected to a PT stream-splitter fitting which is in turn connected to the sample probe on the Sampler IV.

8. **Sample Probe and PT Stream-Splitter**

   Because stainless steel is susceptible to attack by sulfuric acid solutions, this method utilizes special Kel-F sample probe (Technicon No. 17 0745) and a special PT stream-splitter with plat inum nipples (Technicon No. 116-B331).

9. **Phosphorus Channel (only)**
   a. **Cleansing Procedure**

      Before initially operating the system, the following procedure should be performed to cleanse the system. Once a week thereafter, this procedure should be repeated during system start-up.

      With the exception of the ascorbic acid and molybdate/antimony lines, place all phosphorus reagent lines into their respective containers. Start the proportioning pump and allow five minute pumping time. Place both the ascorbic acid and molybdate/antimony lines in sodium hydroxide solution, 20% for five minutes, then into hydrogen peroxide, 50% for five minutes, then into distilled water. After five minutes follow the start-up procedure (Operating Note 1) and allow the system to equilibrate.

b. **Conditioning Procedure**

After the initial cleansing of the system is performed, condition the phosphorus channel as described below. Once this channel has been conditioned, there is no need to repeat the procedure; only the cleansing procedure need be performed once each week during start-up.

Following the Start-Up procedure (Operating Note #1), place all reagent lines for phosphorus in their respective containers and allow the system to equilibrate. Place three sample cups containing midscale standard solution on the Sampler IV tray (with a stop-pin at the third cup) and start the sampler. Aspirate the set of standards three times, allowing five minutes of wash between each set. After the Recorder traces the last standard peak, wait ten minutes and adjust the baseline tracing to zero using the BASELINE control.

10. **Crude Protein Determination – AOAC**

When this methodology is utilized to assay acid digestates for the determination of Crude Protein in Feeds by the official AOAC procedure, the following hardware changes **must** be incorporated into the system:

a. **Sampler IV** – Sampler IV cam must be 40/hour with a sample-to-wash ratio of 2:1 (cam is included in the accessories and spares kit).

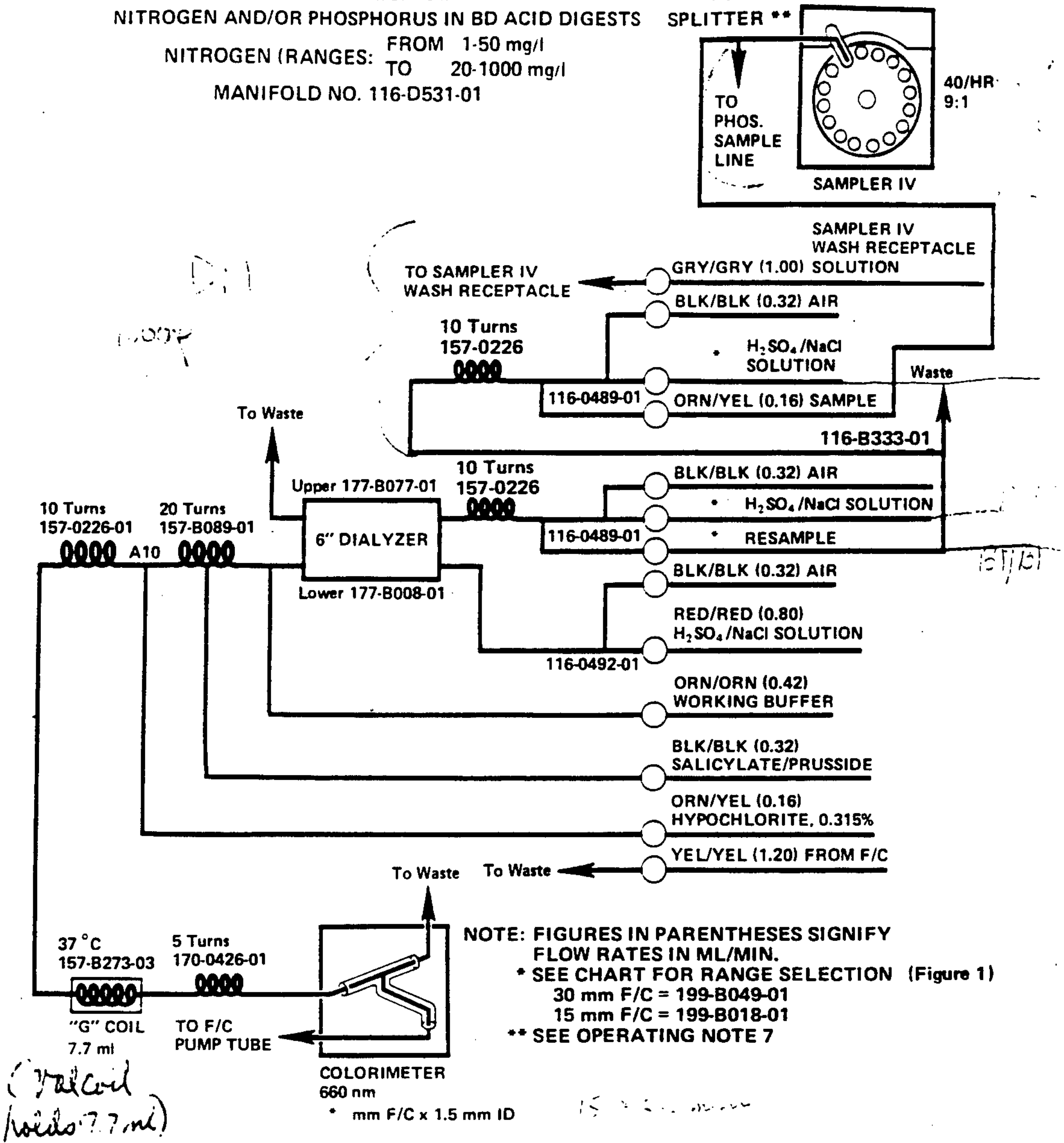
). **Analytical Cartridge** – dilution loop pump tubes **must** be of the following size:

INITIAL SAMPLE DILUTION	
Sample Line	0.16 ml/min (Orn/Yel)
H_2SO_4/NaCl Line	1.20 ml/min (Yel/Yel)

RESAMPLE DILUTION	
Resample Line	0.16 ml/min (Orn/Yel)
H_2SO_4/NaCl Line	0.80 ml/min (Red/Red)

c. **Colorimeter** – must be equipped with 15 mm pathlength flowcell (1.5 or 2.0 mm ID).

INDIVIDUAL/SIMULTANEOUS
NITROGEN AND/OR PHOSPHORUS IN BD ACID DIGESTS
NITROGEN (RANGES: FROM 1-50 mg/l
TO 20-1000 mg/l
MANIFOLD NO. 116-D531-01



(Total Coil
holds 7.7 ml)

NOTE: FIGURES IN PARENTHESES SIGNIFY FLOW RATES IN ML/MIN.
* SEE CHART FOR RANGE SELECTION (Figure 1)
30 mm F/C = 199-B049-01
15 mm F/C = 199-B018-01
** SEE OPERATING NOTE 7

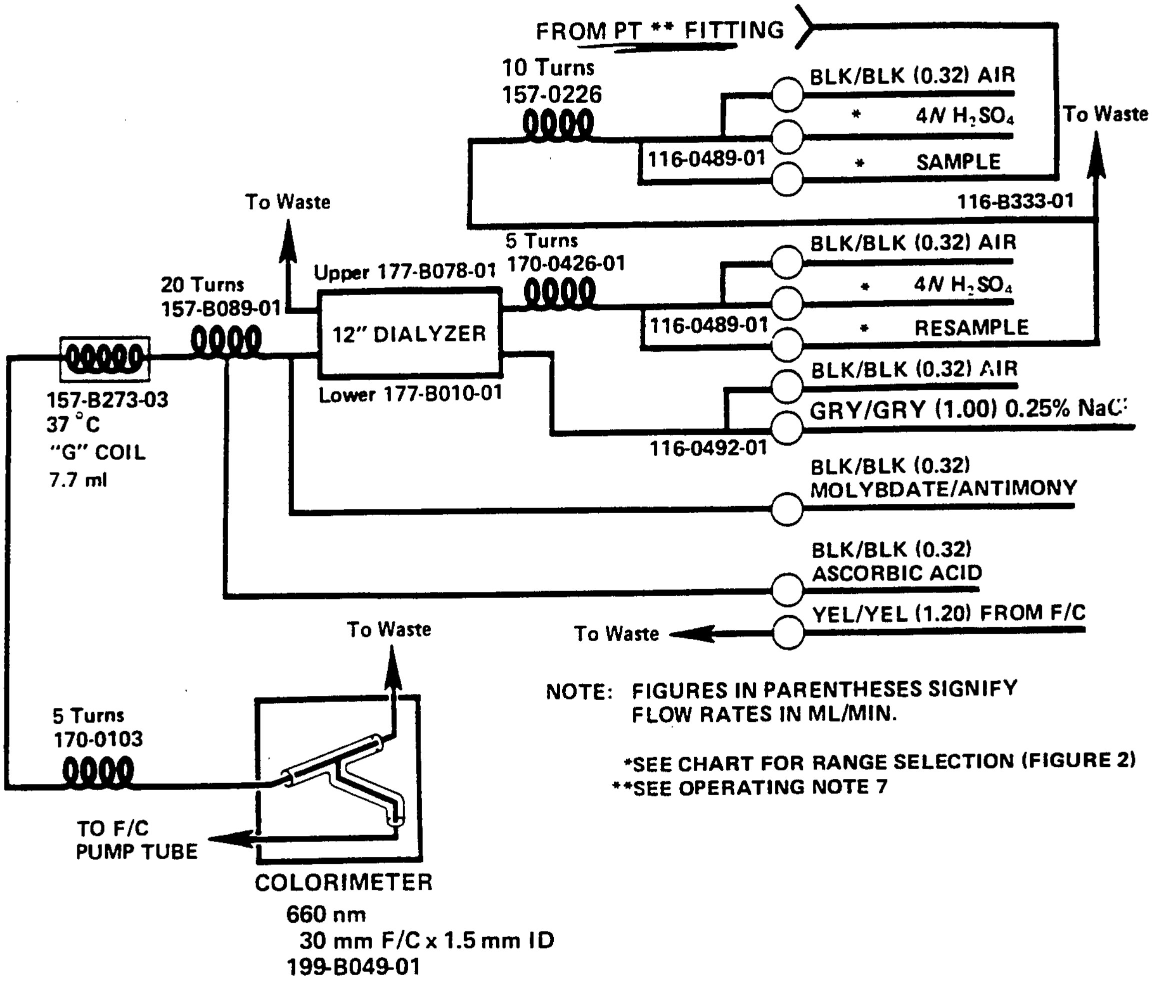
Figure 1. CONCENTRATION RANGES (NITROGEN)

DILUTION LOOPS				FLOWCELL PATH LENGTH (mm)	APPROX. STD CAL SETTING	RANGE PPM N (±10%)
INITIAL SAMPLE		RESAMPLE				
SAMPLE LINE	H_2SO_4/NaCl LINE	RESAMPLE LINE	H_2SO_4/NaCl LINE			
.16 (Orn/Yel)	1.20 Yel/Yel)	.32 (Blk/Blk)	0.80 (Red/Red)	30	7.30	.7-35
					1.00	1.4-170
.16 (Orn/Yel)	1.20 Yel/Yel)	.16 (Orn/Yel)	1.00 (Gry/Gry)	30	7.30	1.2-60
					1.00	-300
.16 (Orn/Yel)	2.00 (Grn/Grn)	.16 (Orn/Yel)	1.00 (Gry/Gry)	30	7.30	2-100
					1.00	10-500
.16 (Orn/Yel)	1.20 (Yel/Yel)	.32 (Blk/Blk)	0.80 (Red/Red)	15	7.30	1.2-60
					1.00	6-300
.16 (Orn/Yel)	1.20 (Yel/Yel)	.16 (Orn/Yel)	1.00 (Gry/Gry)	15	7.30	2.4-120
					1.00	12-600
.16 (Orn/Yel)	2.00 (Grn/Grn)	.16 (Orn/Yel)	1.00 (Gry/Gry)	15	7.30	4-200
					1.00	20-1000

INDIVIDUAL/SIMULTANEOUS NITROGEN AND/OR PHOSPHORUS IN BD ACID DIGESTS

PHOSPHORUS RANGES: FROM 1-50 mg/l TO 20-1000 mg/l

MANIFOLD NO. 116-D541-01



NOTE: FIGURES IN PARENTHESES SIGNIFY FLOW RATES IN ML/MIN.

*SEE CHART FOR RANGE SELECTION (FIGURE 2)

**SEE OPERATING NOTE 7

Figure 2. CONCENTRATION RANGES (PHOSPHORUS)

DILUTION LOOPS				APPROX. STD CAL SETTING	RANGE mg/l P (±10%)
INITIAL SAMPLE		RESAMPLE			
SAMPLE LINE	4N H_2SO_4 LINE	RESAMPLE LINE	4N H_2SO_4 LINE		
0.32 (Blk/Blk)	1.00 (Gry/Gry)	0.32 (Blk/Blk)	0.80 (Red/Red)	7.30	1-50
				2.20	3-150
0.32 (Blk/Blk)	1.00 (Gry/Gry)	0.16 (Orn/Yel)	1.00 (Gry/Gry)	7.30	2-100
				2.20	6-300
0.16 (Orn/Yel)	1.60 (Blu/Blu)	0.16 (Orn/Yel)	1.00 (Gry/Gry)	7.30	6-300
				2.20	20-1000

AutoAnalyzer Application

Industrial Method - #376-75W/B

DIGESTION AND SAMPLE PREPARATION FOR THE ANALYSIS OF TOTAL KJELDAHL NITROGEN AND/OR TOTAL PHOSPHORUS IN WATER SAMPLES USING THE BRAN + LUEBBE BD-40 BLOCK DIGESTOR

The following procedure is recommended for the analysis of nitrogen and/or phosphorus in water samples. Samples are digested using a Bran + Luebbe BD-40 Block Digestor and assayed using a Bran + Luebbe AutoAnalyzer II Continuous Flow Analytical System.

This procedure should be utilized in conjunction with the Operation Manual for the Block Digestor BD-20/40 (Bran + Luebbe Publication No. TA4-0323-11) and the methodology for Individual/Simultaneous Determination of Nitrogen and/or Phosphorus in BD Acid Digests (Industrial Method No. 329-74W - Revised 11/78).

Introduction

When analyzing water samples with the BD-40, the water present in the digestion tubes must be evaporated before heating the tubes at a temperature that is high enough to affect digestion. When water is added to sulfuric acid, the boiling point of the resultant mixture is considerably lower than that of sulfuric acid alone. If this mixture is placed in the block at a temperature significantly higher than its boiling point, the tube contents will bump out resulting in loss of sample, contamination of adjacent tubes, and most importantly, possible bodily injury.

The automatic (temperature-programmed) mode of the BD-40 is utilized to first evaporate the water and then to raise and maintain the block temperature high enough to affect digestion. At the end of the programmed cycle, the unit automatically shuts down.

Since the concentration levels of N and P in the samples encountered are frequently very low, accuracy and precision can be insured only if good analytical technique is employed during all steps of the procedure -- from sample preparation to assay of the digested samples. Every precaution should be observed to avoid contamination of sample tubes, pipets, reagents, spatulas, etc. The use of de-ionized, distilled water or its equivalent is required throughout the procedure. Contaminated water is the most frequently encountered source of difficulty in running this procedure.

Concentration Ranges and Manifold Configuration

Samples

The choice of manifold configuration depends on the concentration of N and/or P in the sample.

Depending on the manifold configuration and STD CAL setting, the BD-40 related analytical cartridges can accommodate N and/or P in the ranges from 0.024 - 1.2 mg/l to 1.50 - 75 mg/l in the undigested sample.

The methodology (No. 329-74W) gives three configurations for the nitrogen and phosphorus cartridges and the concentration ranges for each of the configurations. Each configuration can accommodate approximately a five-fold change in concentration by varying the STD CAL control from 100 to 700. The range represents the detection limit (2% of full scale) and the full scale concentration for a particular STD CAL setting.

Samples containing higher levels of nitrogen or phosphorus should be diluted with distilled de-ionized water prior to digestion.

Referring back to Figures 1 and 2 of Method 329-74W: Because of the possibility of contamination, it is recommended that configuration #1 be used only for samples containing 15 mg N or P/1 or less; all samples greater than 15 mg/l should be assayed on configuration #2 or #3. The choice of cartridge configuration is best illustrated by example.

Consider a group of samples containing 1 - 5 mg N/l and 5 - 50 mg P/1. The configuration of choice for nitrogen would be #2 adjusted with the STD CAL control to 5 mg/l full scale deflection. This would yield peaks ranging from 20% to full scale. The choice for phosphorus would be configuration #3 adjusted for a full scale deflection of 50 mg/l. This would yield peaks ranging from 10% to full scale.

Occasionally, the range of N or P in the samples will not be as narrow as stated above; i.e., a few samples may be too low or too high for the range that was chosen for the majority of the samples. If so, the sample volume per tube may be adjusted up or down or the full scale deflection may be adjusted by means of the STD CAL.

Whenever possible, a cartridge configuration should be chosen such that adjustment of the STD CAL control will accommodate all the values encountered. Note that the STD CAL setting should not be changed while samples are being assayed; i.e., sample peaks can be compared to standard peaks only when both are run at the same STD CAL setting. If a STD CAL adjustment is anticipated, be sure to have on hand standards which will fall into the anticipated range.

Standards

The recommended standards for use with the BD-40 are aqueous solutions of ammonium sulfate [$(NH_4)_2SO_4$] for nitrogen and potassium dihydrogen phosphate (KH_2PO_4) for phosphorus. The volumes of standard solution to be used depend on the concentration range of the sample.

It is recommended that two standards for each parameter to be run: one at 30 - 40% of full scale and one at 70 - 80% of full scale.

Standards should be handled in exactly the same manner as samples; i.e., they should be pipetted into the BD tubes and carried through the entire digestion procedure.

A series of working standard solutions which can accommodate all the ranges of the method can be prepared utilizing the following stock solutions:

Stock Solution A (2.0 mg N/ml)

Ammonium Sulfate $(NH_4)_2SO_4$	0.9434 g
Distilled Water, q.s.	100 ml

Preparation

Dissolve 0.9434 g of ammonium sulfate in about 60 ml of distilled water. Dilute to 100 ml with distilled water and mix thoroughly.

Stock Solution B (2.0 mg P/ml)

Potassium Dihydrogen Phosphate (KH_2PO_4)	0.8788 g
Distilled Water, q.s.	100 ml

Preparation

Dissolve 0.8788 g of potassium dihydrogen phosphate in about 60 ml of distilled water. Dilute to 100 ml with distilled water and mix thoroughly.

The preparation of standards can be performed most readily if pipets ranging from 1 to 10 ml are available.

In Table #1, the extreme left column indicates the milliliters of stock solution to be diluted to one liter to obtain working standard solutions which will yield the concentration values in the second column. The same volumes diluted to 100 ml, or 10x those volumes diluted to one liter, will yield concentrations 10x these concentration values.

The preparation of standard solutions is best illustrated by an example. Using the example cited previously in the section on Samples, the N range was 1 - 5 mg/l and the P range was 5 - 50 mg/l.

For the N channel, an 80% of full scale standard would be 4.0 mg/l. From Table #1 4.0 mg/l can be obtained by using 8 ml per tube of a working standard solution containing 10 mg/l (Row 5, Column 8). Using 3 ml per tube of the same working solution will give 1.5 mg/l (Row 5, Column 3) or 30% of full scale.

Since 8 ml and 3 ml per tube were chosen for the nitrogen standards, the appropriate amount of P^- must also be present in those aliquots to accommodate the phosphorus channel. An 80% deflection for phosphorus corresponds to 40 mg P/l and a 30% deflection corresponds to 15 mg/l. Checking Column 8, 40 mg/l (10x chart value) can be obtained by using 8 ml stock solution B. The 30% value will automatically fall in range using the 3 ml aliquot. Hence, using 3 ml and 8 ml of a working standard solution prepared by diluting 5 ml of Stock Solution A plus 50 ml of Stock Solution B to one liter will yield the required N and P values.

The following general procedure may be used for preparation of standard solutions. Once the manifold configuration and concentration range have been chosen, choose a value (or 10x a chart value) from Table 1 which corresponds to an 80% deflection and which requires 5 ml per tube or more working standard solution. Using 5 ml or more for the 80% deflection insures that a smaller volume can be found on the chart which approximates the 30% deflection.

On a simultaneous system, either parameter may be determined first. Once the chart value has been chosen for one parameter, choose the value (or 10x a chart value) from the same column that most closely approximates an 80% deflection for the other parameter. Since the values are proportional to volume, the 30% values will automatically fall into range with each other.

Blanks

A duplicate blank determination (all reagents less sample) should be performed with each rack of samples by carrying the blank tubes through the entire digestion procedure.

Operating Procedure

Samples, Standards and Blanks

Samples and standards are pipetted directly into the digestion tubes. Samples should be pipetted in 20 ml aliquots. The amount of standard is determined by the level of the component(s) of interest.

Refer to Section II for guidelines on standard volumes and manifold configuration. Samples may be assayed singly or in duplicate, depending on workload. It is recommended that standards and blanks be assayed in duplicate.

While samples and standards are being prepared, pre-heat the block to 200°C by setting the HIGH TEMP dial to 200°C and depressing the MANUAL button.

Boiling Aids

Plain (not selenized) Hengar chips are utilized to promote smooth boiling during digestion. The addition of 2 - 3 chips per tube is recommended. The use of glass beads or perforated glass beads is not satisfactory to obtain smooth boiling.

Hengar chips are available from Arthur H. Thomas Company, Vine & Third Streets, Philadelphia, PA. 19105. As an alternative, some users report a preference for acid-washed Chemware TFE (teflon) boiling stones. TFE boiling stones are available from Markson Science, Inc., Box 767, Delmar, California 92014.

Catalyst

Red mercuric oxide is recommended as a catalyst for the digestion of water samples. Because mercury can interfere in both chemistries, the amount added per tube is limited to an amount determined by the manifold configuration being utilized; i.e., the more the sample is diluted, the greater the amount of mercury that can be utilized. The amount of mercury recommended is 10 mg/tube. The mercury is most conveniently utilized as a solution of HgO in 10% sulfuric acid.

Preparation

Into a 100 ml volumetric flask, weigh 8.0 of red mercuric oxide. Add about 75 ml of 10% sulfuric acid and stir until dissolved. Dilute to 100 ml with 10% sulfuric acid and mix thoroughly.

Digestion (Salt/Acid/Catalyst) Mixture

To insure uniform blank values from tube to tube, it is recommended that potassium sulfate, sulfuric acid and catalyst be added to each tube as a single mixture rather than as separate components. The procedure below may be used to prepare the digestion mixture.

Prepare and store the mixture in a stoppered container to minimize the possibility of airborne contamination. The mixture may be prepared in as large a quantity as is practical to handle and store.

Preparation

Carefully add 200 ml of concentrated sulfuric acid to 700 ml of de-ionized distilled water. Dissolve 133 g of potassium sulfate into this mixture, add 25 ml of mercuric sulfate catalyst solution and dilute to one liter with de-ionized distilled water.

For most applications, 5 ml of digestion mix per tube is satisfactory.

The utilization of a plunger-type repetitive dispensing device offers a rapid, convenient method of adding the digestion mixture to the tubes. When a plunger-type dispenser is utilized, the dispenser must be broken down, cleaned with water and air dried every three days. Failure to clean the plunger every three days can cause the plunger to freeze in the barrel of the dispenser due to crystallization of potassium sulfate.

Digestion

After samples, standards and reagents have been added to the digestion tubes, the water must be evaporated before high temperature digestion can be performed.

Place the loaded rack into the pre-heated block (200°C) and attach the end plates to the rack. The plates should remain in place until the rack is removed from the block. End plates promote water evaporation during low temperature operation and insure proper refluxing of the acid during high temperature digestion.

When the loaded rack is placed in the block, set the programmer as follows and then depress the AUTO button:

Total Cycle Time:	2 1/2 hours
High Temp °C:	380 °C
Low Temp Time:	1 hour
Low Temp °C:	200 °C

Under these conditions, the until will operate for a total cycle of 2 1/2 hours: 1 hour at 200 °C, about 1 hour to heat up to 380 °C and about 1/2 hour at 380 °C. At the end of 2 1/2 hours, the until will automatically shut down.

Cooling and Dilution

At the end of the program cycle (2 1/2 hours), remove the rack from the block, place it on an asbestos pad or in the cooling rack and remove the metal end plates. Allow the tubes to cool for about 5 minutes before diluting with 20 ml of de-ionized distilled water. Tubes are cool enough to dilute when the white acid fumes have dissipated and the upper half of the tube is cool enough to handle comfortably. The tubes should not be allowed to cool to the point of K_2SO_4 precipitation.

With the aid of a vortex type tube mixer, add to each tube, while swirling, 20 ml of deionized distilled water using a repetitive pipetter. Add the water in one continuous portion at a moderate rate and angle the tube away from the face. Allow the tube contents to mix thoroughly.

The tube contents should be at room temperature before analyzing. The tubes may be cooled rapidly by placing the entire rack into a sink partially filled with cold water.

Analysis

After cooling to room temperature, the digests may be analyzed using Bran + Luebbe Methodology No. 329-74W -- Individual/Simultaneous Determination of Nitrogen and/or Phosphorus in BD Acid Digests, Revised 11/78.

Transfer to glass sample cups, which have been previously acid washed and dried.

Before analyzing the entire set of samples, standards and blanks, run a few standard cups through the system and with the STD CAL control, adjust the standard peaks to the proper chart reading.

TABLE 1: CONCENTRATION OF STANDARD IN DIGESTS

*Ml Stock Solution A or B	Working Standard Conc. Mg N or P/L	Ml Working Standard Solution Per Tube and Resulting Digest Concentration in Mg N or P/L									
		1	2	3	4	5	6	7	8	9	10
1	2	.1	.2	.3	.4	.5	.6	.7	.8	.9	1
2	4	.2	.4	.6	.8	1.	1.2	1.4	1.6	1.8	2
3	6	.3	.6	.9	1.2	1.5	1.8	2.1	2.4	2.7	3
4	8	.4	.8	1.2	1.6	2.0	2.4	2.8	3.2	3.6	4
5	10	.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5
6	12	.6	1.2	1.8	2.4	3.0	3.6	4.2	4.8	5.4	6
7	14	.7	1.4	2.1	2.8	3.5	4.2	4.9	5.6	6.3	7
8	16	.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8
9	18	.9	1.8	2.7	3.6	4.5	5.4	6.3	7.2	8.1	9
10	20	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10

*Dilute to 1000 ml to get chart values

Dilute to 100 ml to get 10X chart values

Stock Solution A = Nitrogen

Stock Solution B = Phosphorus

APPENDIX A-15

Procedure for Nitrate and Nitrite Nitrogen: Method 353 Series

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 1$\underline{N}$ 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.

6.3.5 Syringe filters - Titan nylon 25-mm syringe filters - 0.45 micron. SRI Catalog number 44525-NN or equivalent.

6.3.6 Syringes - 10 cc syringe with Luer Lok, B-D Part 309604 or equivalent. (Smaller volumes are acceptable)

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 Pour samples and standards into vials. If samples have particulate matter, filter them into the sample vial with a syringe and nylon syringe filter. 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

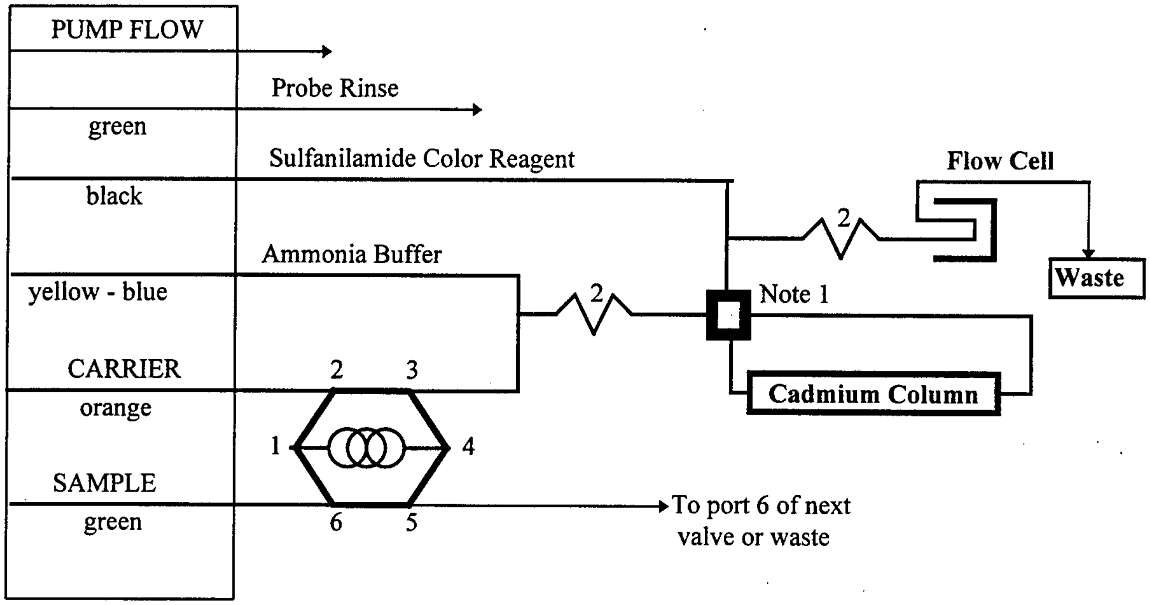
Concentration Scaling:	None
Sampler Timing:	
Method Cycle Period:	50.0
Min. Probe in Wash Period:	9.0
Probe in Sample Period:	25.0
Valve Timing:	
Method Cycle Period:	50.0
Sample Reaches 1st Valve:	18.0
Valve:	On
Load Time:	0.0
Load period	20.0
Inject Period:	30.0
Sample Loop:	Microloop

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

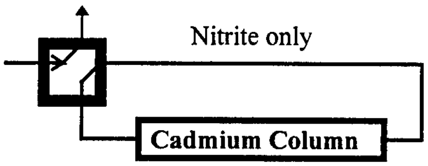
Carrier is DI Water

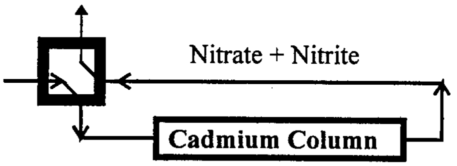
All manifold tubing is **0.8 mm (0.32 in) i.d.** Lachat Part No. 50028. This is **5.2 uL/cm.**

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 A-16

Procedure for Orthophosphate: Method AP-0060

1.0 PURPOSE

This procedure provides a method for the determination of orthophosphate in drinking, ground, and surface waters, and domestic and industrial wastes.

2.0 SCOPE

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

2.2 This method is based on reactions that are specific for the orthophosphate (PO_4^{3-}) ion.

2.3 The applicable range is 0.02 to 4.00 mg P/L.

3.0 SUMMARY

3.1 Only orthophosphate forms a blue color in this test. Polyphosphates and organic phosphorus compounds are not recovered. The sulfuric acid in the molybdate reagent does not have enough time with polyphosphates to hydrolyze them.

3.2 The orthophosphate reacts with ammonium molybdate and potassium tartrate under acidic conditions to form an antimony-phosphomolybdate complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

4.0 REFERENCES

4.1 U.S. Environmental Protection Agency, *Methods for the Determination of Inorganic Substances in Environmental Samples*, EPA-600/R-93/100, August 1993, "Phosphorus, All Forms, Method 365.1 (Colorimetric, Automated, Ascorbic Acid)."

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, Method I-2601-78.

4.3 *Standard Methods for the Examination of Water and Wastewater*, 18th Edition, p. 4 - 116, Method 4500-P F (1992).

4.4 *Guideline and Format for EMSL-Cincinnati Methods*. EPA-600/8-83-020, August 1983.

4.5 Lachat Instruments, *QuickChem Automated Ion Analyzer Methods Manual*, QuickChem Method 10-115-01-1-A, "Determination Of Orthophosphate In Waters By Flow Injection Analysis Colorimetry."

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 (use phosphate-free detergents), acid rinsed with 1:1 HCl, then rinsed with reagent water. The volume collected should be sufficient to ensure a representative sample and allow for quality control analysis (at least 100 mL).

6.1.2 The USEPA recommends that samples be stored at 4° C with a maximum holding time of 48 hours, and that samples for dissolved phosphorus be filtered immediately upon collection.

6.1.3 If samples of high (pH > 8) are suspected add 1 drop of phenolphthalein indicator to a 50 mL aliquot of sample. If a red color develops, add 11 N sulfuric acid (310 mL concentrated H_2SO_4 /L) drop-wise to just discharge the color. If samples have been acidified at collection, match the acid concentration in each calibration standard.

6.2 Limitations and Actions

6.2.1 If the analyte concentration is not within 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 Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant as a silicate concentration of approximately 30 mg SiO_2/L would be required to produce a 0.005 mg P/L positive error in orthophosphate.

6.2.2.2 Concentrations of ferric iron greater than 50 mg/L will cause a negative error due to competition with the complex for the reducing agent ascorbic acid. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.

6.2.2.3 For dissolved orthophosphate, sample turbidity must be removed by filtration prior to analysis. Sample color that absorbs at 880 nm will also interfere. When in doubt about background absorbance, the background concentration should be determined.

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

6.3.5 Syringe filters - Titan nylon 25-mm syringe filters - 0.45 micron. SRI Catalog number 44525-NN or equivalent.

6.3.6 Syringes - 10 cc syringe with Luer Lok, B-D Part 309604 or equivalent. (Smaller volumes are acceptable)

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 the carrier solution and other reagents as noted 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. Stock Ammonium Molybdate Solution**

By Volume: In a **1 L** volumetric flask dissolve **40.0 g ammonium molybdate tetrahydrate** $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$ in approximately **800 mL water**. Dilute to mark with **water** and stir for **two hours**. Store in plastic and refrigerate.

By Weight: To a tared **1 L** container add **40.0 g ammonium molybdate tetrahydrate** $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$ and **983 g water**. Stir for **two hours**. Store in plastic and refrigerate.

6.4.1.2 **Reagent 2. Stock Antimony Potassium Tartrate Solution**

By Volume: In a **1 L** volumetric flask dissolve **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$) in approximately **800 mL of water**. Dilute to mark with **water** and mix. Store in a dark bottle and refrigerate.

By Weight: To a **1 L dark** tared container add **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$) and **995 g water**. Mix. Store in a dark bottle and refrigerate.

6.4.1.3 **Reagent 3. Molybdate Color Reagent**

By Volume: To a **1 L** volumetric flask add about **500 mL water**, then add **35.0 mL concentrated sulfuric acid** (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add **72.0 mL Stock Antimony Potassium Tartrate Solution** (Reagent 2) and **213 mL Stock Ammonium Molybdate Solution** (Reagent 1). Dilute to the mark with **water** and mix. Degas with helium and refrigerate.

By Weight: To a tared **1 L** container add **680 g water**, then **64.4 g concentrated sulfuric acid** (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add **72.0 g Stock Antimony Potassium Tartrate Solution** (Reagent 2) and **213 g Stock Ammonium Molybdate Solution** (Reagent 1). Mix and degas with helium. Refrigerate.

6.4.1.4 **Reagent 4. Ascorbic Acid Reducing Solution, 0.33 M**

By Volume: In a **1 L** volumetric flask dissolve **60.0 g granular ascorbic acid** in about **700 mL water.** Dilute to the mark with **water**, mix and degas. After degassing add **1.0 g dodecyl sodium sulfate** ($CH_3(CH_2)_{11}OSO_3Na$). Refrigerate. Discard if solution becomes yellow.

By Weight: To a tared **1 L** container add **60.0 g granular ascorbic acid** and **975 g water**. Stir until dissolved then degas. After degassing add **1.0 g dodecyl sodium sulfate** ($CH_3(CH_2)_{11}OSO_3Na$). Refrigerate. Discard if solution becomes yellow.

6.4.1.5 **Reagent 5. Sodium Hydroxide - EDTA Rinse**

Dissolve **65 g sodium hydroxide** (NaOH) and **6 g tetrasodium ethylenediamine tetraacetic acid** (Na_4EDTA) in **1.0 L** or **1.0 kg water**.

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 $\underline{N}$ H_2SO_4 added per 100 mL, add **5 mL of 1$\underline{N}$ 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 **1$\underline{N}$ 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 **1$\underline{N}$ 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

7.1.1 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 9.2.

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 37°C. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

7.1.6 Pour samples and standards into vials. If samples have particulate matter, filter them into the sample vial with a syringe and nylon syringe filter. 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, remove all transmission lines from reagents and place them in water. Pump for about five minutes.

7.1.11 Place the color reagent and ascorbic acid transmission lines into the NaOH - EDTA solution (Reagent 5). Pump for about 5 minutes to remove any precipitated reaction products.

7.1.12 Remove the reagent lines from the NaOH - EDTA solution and place them in water. Pump for an additional 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 PO_4-P/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:	Orthophosphate (PO_4)-P
Concentration Units:	mg PO_4-P/L
Chemistry:	Direct
Inject to Peak Start (s):	10.0
Peak Base Width (s):	23.000
% Width Tolerance:	100.000
Threshold:	5000.000
Autodilution Trigger:	Off
QuickChem Method:	10-115-01-1-A

Calibration Data:

Levels: (mg NO_3-N/L) 3: 4.000 4: 2.500 5: 1.000

6: 0.100 7: 0.020 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

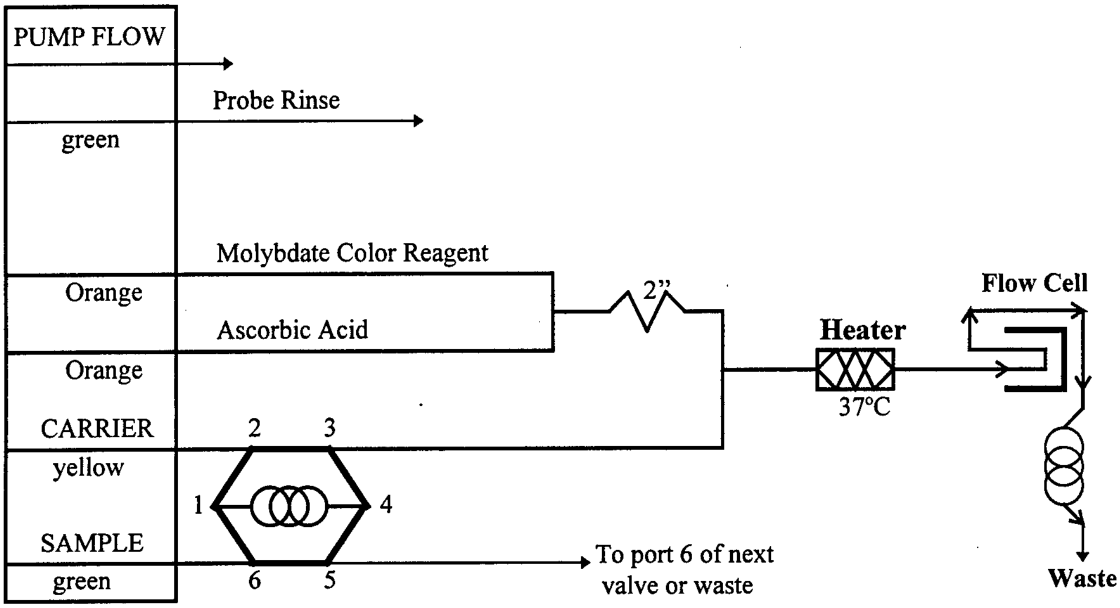
Load Time: 0.0

Load period 25.0

Inject Period: 45.0

Sample Loop: 75.5 cm

9.2 Orthophosphate Manifold Diagram



Sample Loop = 75.5 cm

Interference Filter = 880 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 **135 cm** of tubing on a **7 cm** coil support.

Apparatus: The  includes 175 cm of tubing wrapped around the heater block at 37°C. An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

10.0 ATTACHMENTS AND APPENDICES

None

End of Procedure

APPENDIX A-17

Procedure for Toxicity using Fathead Minnow Larvae (*Pimephales promelas*): EPA Method 1000.0

SECTION 11

TEST METHOD

FATHEAD MINNOW, *PIMEPHALES PROMELAS*, LARVAL SURVIVAL AND GROWTH TEST

METHOD 1000.0

11.1 SCOPE AND APPLICATION

11.1.1 This method estimates the chronic toxicity of effluents and receiving water to the fathead minnow, *Pimephales promelas*, using newly hatched larvae in a seven-day, static renewal test. The effects include the synergistic, antagonistic, and additive effects of all the chemical, physical, and biological components which adversely affect the physiological and biochemical functions of the test organisms.

11.1.2 Daily observations on mortality make it possible to also calculate acute toxicity for desired exposure periods (i.e., 24-h, 48-h, 96-h LC50s).

11.1.3 Detection limits of the toxicity of an effluent or pure substance are organism dependent.

11.1.4 Brief excursions in toxicity may not be detected using 24-h composite samples. Also, because of the long sample collection period involved in composite sampling, and because the test chambers are not sealed, highly degradable or highly volatile toxicants present in the source may not be detected in the test.

11.1.5 This test method is commonly used in one of two forms: (1) a definitive test, consisting of a minimum of five effluent concentrations and a control, and (2) a receiving water test(s), consisting of one or more receiving water concentrations and a control.

11.2 SUMMARY OF METHOD

11.2.1 Fathead minnow, *Pimephales promelas*, larvae are exposed in a static renewal system for seven days to different concentrations of effluent or to receiving water. Test results are based on the survival and weight of the larvae.

11.3. INTERFERENCES

11.3.1 Toxic substances may be introduced by contaminants in dilution water, glassware, sample hardware, and testing equipment (see Section 5, Facilities, Equipment and Supplies).

11.3.2 Adverse effects of low dissolved oxygen (DO) concentrations, high concentrations of suspended and/or dissolved solids, and extremes of pH, alkalinity, or hardness, may mask the presence of toxic substances.

11.3.3 Improper effluent sampling and sample handling may adversely affect test results (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests).

11.3.4 Pathogenic and/or predatory organisms in the dilution water and effluent may affect test organism survival and confound test results.

11.3.5 Food added during the test may sequester metals and other toxic substances and confound test results. Daily renewal of solutions, however, will reduce the probability of reduction of toxicity caused by feeding.

11.4 SAFETY

11.4.1 See Section 3, Health and Safety.

11.5 APPARATUS AND EQUIPMENT

11.5.1 Fathead minnow and brine shrimp culture units -- see USEPA, 1985a and USEPA, 1993b. This test requires 180-360 larvae. It is preferable to obtain larvae from an in-house fathead minnow culture unit. If it is not feasible to culture fish in-house, embryos or newly hatched larvae can be shipped in well oxygenated water in insulated containers.

11.5.2 Samplers -- automatic sampler, preferably with sample cooling capability, that can collect a 24-h composite sample of 5 L.

11.5.3 Sample containers -- for sample shipment and storage (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests).

11.5.4 Environmental chamber or equivalent facility with temperature control (25 ± 1°C).

11.5.5 Water purification system -- MILLIPORE MILLI-Q®, deionized water or equivalent (see Section 5, Facilities, Equipment, and Supplies).

11.5.6 Balance -- analytical, capable of accurately weighing to 0.00001 g.

11.5.7 Reference weights, Class S -- for checking performance of balance. Weights should bracket the expected weights of the weighing pans and the expected weights of the pans plus fish.

11.5.8 Test chambers -- four (minimum of three) borosilicate glass or non-toxic disposable plastic test chambers are required for each concentration and control. Test chambers may be 1 L, 500 mL or 250 mL beakers, 500 mL plastic cups, or fabricated rectangular (0.3 cm thick) glass chambers, 15 cm x 7.5 cm x 7.5 cm. To avoid potential contamination from the air and excessive evaporation of test solutions during the test, the chambers should be covered with safety glass plates or sheet plastic (6 mm thick).

11.5.9 Volumetric flasks and graduated cylinders -- Class A, borosilicate glass or non-toxic plastic labware, 10-1000 mL for making test solutions. 5.10

11.5.10 Volumetric pipets -- Class A, 1-100 mL.

11.5.11 Serological pipets -- 1-10 mL, graduated.

11.5.12 Pipet bulbs and fillers -- PROPIPET®, or equivalent.

11.5.13 Droppers, and glass tubing with fire polished edges, 4 mm ID -- for transferring larvae.

11.5.14 Wash bottles -- for rinsing small glassware and instrument electrodes and probes.

11.5.15 Thermometers, glass or electronic, laboratory grade -- for measuring water temperatures.

11.5.16 Bulb-thermograph or electronic-chart type thermometers -- for continuously recording temperature.

11.5.17 Thermometers, National Bureau of Standards Certified (see USEPA Method 170.1, USEPA, 1979b) -- to calabrate laboratory themometers.

11.5.18 Meters, pH, DO, and specific conductivity -- for routine physical and chemical measurements.

11.5.19 Drying oven -- 50-105°C range for drying larvae.

11.6 REAGENTS AND CONSUMABLE MATERIALS

11.6.1 Sample containers -- for sample shipment and storage (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests).

11.6.2 Data sheets (one set per test) -- for recording data.

11.6.3 Vials, marked -- 18-24 per test, containing 4% formalin or 70% ethanol to preserve larvae (optional).

11.6.4 Weighing boats, aluminum -- 18-24 per test.

11.6.5 Tape, colored -- for labelling test chambers.

11.6.6 Markers, waterproof -- for marking containers, etc.

11.6.7 Reagents for hardness and alkalinity tests -- see USEPA Methods 130.2 and 310.1, USEPA, 1979b.

11.6.8 Buffers, pH 4, pH 7, and pH 10 (or as per instructions of instrument manufacturer) -- for instrument calibration (see USEPA Method 150.1, USEPA, 1979b).

11.6.9 Specific conductivity standards -- see USEPA Method 120.1, USEPA, 1979b.

11.6.10 Membranes and filling solutions for DO probe (see USEPA Method 360.1, USEPA, 1979b), or reagents -- for modified Winkler analysis.

11.6.11 Laboratory quality control samples and standards -- for calibration of the above methods.

11.6.12 Reference toxicant solutions (see Section 4, Quality Assurance).

11.6.13 Ethanol (70%) or formalin (4%) -- for use as a preservative for the fish larvae.

11.6.14 Reagent water -- defined as distilled or deionized water that does not contain substances which are toxic to the test organisms (see Section 5, Facilities, Equipment, and Supplies).

11.6.15 Effluent, receiving water, and dilution water -- see Section 7, Dilution Water; and Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests.

11.6.16 Brine Shrimp, *Artemia*, Nauplii -- for feeding cultures and test organisms

11.6.16.1 Newly-hatched *Artemia* nauplii are used as food (see USEPA, 1993b) for fathead minnow, *Pimephales promelas*, larvae in toxicity tests and frozen brine shrimp and flake food are used in the maintenance of continuous stock cultures. Although there are many commercial sources of brine shrimp cysts, the Brazilian or Colombian strains are currently preferred because the supplies examined have had low concentrations of chemical residues and produce nauplii of suitably small size. For commercial sources of brine shrimp, *Artemia*, cysts, see Table 2 of Section 5, Facilities, Equipment, and Supplies and Section 4, Quality Assurance.

11.6.16.2 Each new batch of brine shrimp, *Artemia*, cysts must be evaluated for size (Vanhaecke and Sorgeloos, 1980, and Vanhaecke et al., 1980) and nutritional suitability (see Leger et al., 1985; Leger et al., 1986) against known suitable reference cysts by performing a side by side larval growth test using the "new" and "reference" cysts. The "reference" cysts used in the suitability test may be a previously tested and acceptable batch of cysts, or may be obtained from the Quality Assurance Branch, Environmental Monitoring Systems Laboratory, Cincinnati, OH 45268; 513-569-7325. A sample of newly-hatched *Artemia* nauplii from each new batch of cysts should be chemically analyzed. The *Artemia* cysts should not be used if the concentration of total organochlorine exceeds 0.15 μg/g wet weight or the total concentration of organochlorine pesticides plus PCBs exceeds 0.30 μg/g wet weight. (For analytical methods see USEPA, 1982).

11.6.16.3 *Artemia* nauplii are obtained as follows:

1. Add 1 L of seawater, or a solution prepared by adding 35.0 g uniodized salt (NaCl) or artificial sea salts to 1 L deionized water, to a 2-L separatory funnel, or equivalent.
2. Add 10 mL *Artemia* cysts to the separatory funnel and aerate for 24-h at 27°C. (Hatching time varies with incubation temperature and the geographic strain of *Artemia* used) (see USEPA, 1991b; USEPA, 1993b and ASTM, 1993).
3. After 24 h, cut off the air supply in the separatory funnel. *Artemia* nauplii are phototactic, and will concentrate at the bottom of the funnel if it is covered for 5-10 min. To prevent mortality, do not leave the concentrated nauplii at the bottom of the funnel more than 10 min without aeration.
4. Drain the nauplii into a beaker or funnel fitted with a ≤ 150 µm Nitex® or stainless steel screen, and rinse with seawater, or equivalent, before use.

11.6.16.4 Testing *Artemia* nauplii as food for toxicity test organisms.

11.6.16.4.1 The primary criterion for acceptability of each new supply of brine shrimp cysts is the ability of the nauplii to support good survival and growth of the fathead minnow larvae (see SubSection 11.12). The larvae used to evaluate the suitability of the brine shrimp nauplii must be of the same geographical origin, species, and stage of development as those used routinely in the toxicity tests. Sufficient data to detect differences in survival and growth should be obtained by using three replicate test vessels, each containing a minimum of 15 larvae, for each type of food.

11.6.16.4.2 The feeding rate and frequency, test vessels, volume of control water, duration of the test, and age of the nauplii at the start of the test, should be the same as used for the routine toxicity tests.

11.6.16.4.3 Results of the brine shrimp nutrition assay, where there are only two treatments, can be evaluated statistically by use of a t test. The "new" food is acceptable if there are no statistically significant differences in the survival and growth of the larvae fed the two sources of nauplii.

11.6.17 TEST ORGANISMS, FATHEAD MINNOWS, *PIMEPHALES PROMELAS*

11.6.17.1 Newly hatched fish less than 24 h old should be used for the test. If organisms must be shipped to the testing site, fish up to 48 h old may be used, all hatched within a 24-h window.

11.6.17.2 If the fish are kept in a holding tank or container, most of the water should be siphoned off to concentrate the fish. The fish are then transferred one at a time randomly to the test chambers until each chamber contains ten fish. Alternately, fish may be placed one or two at a time into small beakers or plastic containers until they each contain five fish. Three (minimum of two) of these beakers/plastic containers are then assigned to randomly-arranged control and exposure chambers.

11.6.17.2.1 The fish are transferred directly to the test vessels or intermediate beakers/plastic containers, using a large-bore, fire-polished glass tube (6 mm to 9 mm I.D. X 30 cm long) equipped with a rubber bulb, or a large volumetric pipet with tip removed and fitted with a safety type bulb filler. The glass or plastic containers should only contain a small volume of dilution water.

11.6.17.2.2 It is important to note that larvae should not be handled with a dip net. Dipping small fish with a net may result in damage to the fish and cause mortality.

11.6.17.3 The test is conducted with four (minimum of three) test chambers at each toxicant concentration and control. Fifteen (minimum of ten) embryos are placed in each replicate test chamber. Thus 60 (minimum of 30) fish are exposed at each test concentration.

11.6.17.4 Sources of organisms

11.6.17.4.1 Fathead minnows, *Pimephales promelas*, may be obtained from commercial biological supply houses. Fish obtained from outside sources for use as brood stock or in toxicity tests may not always be of suitable age and quality. Fish provided by supply houses should be guaranteed to be of (1) the correct species, (2) disease free, (3) in the requested age range, and (4) in good condition. This can be done by providing the record of the date on which the eggs were laid and hatched, and information on the sensitivity of contemporary fish to reference toxicants.

11.6.17.5 Inhouse Sources of Fathead Minnows, *Pimephales promelas*

11.6.17.5.1 Problems in obtaining suitable fish from outside laboratories can be avoided by developing an inhouse laboratory culture facility. Fathead minnows, *Pimephales promelas*, can be easily cultured in the laboratory from eggs to adults in static, recirculating, or flow-through systems. The larvae, juveniles, and adult fish should be kept in 60 L (15 gal) or 76 L (20 gal) rearing tanks supplied with reconstituted water, dechlorinated tap water, or natural water. The water should be analyzed for toxic metals and organics quarterly (see Section 4, Quality Assurance).

11.6.17.5.1.1 If a static or recirculating system is used, it is necessary to equip each tank with an outside activated carbon filter system, similar to those sold for tropical fish hobbyists (or one large activated carbon filter system for a series of tanks) to prevent the accumulation of toxic metabolic wastes (principally nitrite and ammonia) in the water.

11.6.17.5.2 Flow-through systems require large volumes of water and may not be feasible in some laboratories. The culture tanks should be shielded from extraneous disturbances using opaque curtains, and should be isolated from toxicity testing activities to prevent contamination.

11.6.17.5.3 To avoid the possibility of inbreeding of the inhouse brood stock, fish from an outside source should be introduced yearly into the culture unit.

11.6.17.5.4 Dissolved oxygen -- The DO concentration in the culture tanks should be maintained near saturation, using gentle aeration with 15 cm air stones if necessary. Brungs (1971), in a carefully controlled long-term study, found that the growth of fathead minnows was reduced significantly at all dissolved oxygen concentrations below 7.9 mg/L. Soderberg (1982) presented an analytical approach to the re-aeration of flowing water for culture systems.

11.6.17.5.5 Culture Maintenance

11.6.17.5.5.1 Adequate procedures for culture maintenance must be followed to avoid poor water quality in the culture system. The spawning and brood stock culture tanks should be kept free of debris (excess food, detritus, waste, etc.) by siphoning the accumulated materials (such as dead brine shrimp nauplii or cysts) from the bottom of the tanks daily with a glass siphon tube attached to a plastic hose leading to the floor drain. The tanks are more thoroughly cleaned as required. Algae, mostly diatoms and green algae, growing on the glass of the spawning tanks are left in place, except for the front of the tank, which is kept clean for observation. To avoid excessive build-up of algal growth, the walls of the tanks are periodically scraped. The larval culture tanks are cleaned once or twice a week to reduce the mass of fungus growing on the bottom of the tank.

11.6.17.5.5.2 Activated charcoal and floss in the tank filtration systems should be changed weekly, or more often if needed. Culture water may be maintained by preparation of reconstituted water or use of dechlorinated tap water. Distilled or deionized water is added as needed to compensate for evaporation.

11.6.17.5.5.3 Before new fish are placed in tanks, salt deposits are removed by scraping or with 5% acid solution, the tanks are washed with detergent, sterilized with a hypochlorite solution, and rinsed well with hot tap water and then with laboratory water.

11.6.17.5.6 Obtaining Embryos for Toxicity Tests

11.6.17.5.6.1 Embryos can be shipped to the laboratory from an outside source or obtained from adults held in the laboratory as described below.

11.6.17.5.6.2 For breeding tanks, it is convenient to use 60 L (15 gal) or 76 L (20 gal) aquaria. The spawning unit is designed to simulate conditions in nature conducive to spawning, such as water temperature and photoperiod. Spawning tanks must be held at a temperature of 25 ± 2°C. Each aquarium is equipped with a heater, if necessary, a continuous filtering unit, and spawning substrates. The photoperiod for the culture system should be maintained at 16 h light and 8 h darkness. For the spawning tanks, this photoperiod must be rigidly controlled. A convenient photoperiod is 5:00 AM to 9:00 PM. Fluorescent lights should be suspended about 60 cm above the surface of the water in the brood and larval tanks. Both DURATEST® and cool-white fluorescent lamps have been used, and produce similar results. An illumination level of 50 to 100 ft-c is adequate.

11.6.17.5.6.3 To simulate the natural spawning environment, it is necessary to provide substrates (nesting territories) upon which the eggs can be deposited and fertilized, and which are defended and cared for by the males. The recommended spawning substrates consist of inverted half-cylinders, 7.6 cm x 7.6 cm (3 in x 3 in) of Schedule 40 PVC pipe. The substrates should be placed equi-distant from each other on the bottom of the tanks.

11.6.17.5.6.4 To establish a breeding unit, 15-20 pre-spawning adults six to eight months old are taken from a "holding" or culture tank and placed in a 76-L spawning tank. At this point, it is not possible to distinguish the sexes. However, after less than a week in the spawning tank, the breeding males will develop their distinct coloration and territorial behavior, and spawning will begin. As the breeding males are identified, all but two are removed, providing a final ratio of 5-6 females per male. The excess spawning substrates are used as shelter by the females.

11.6.17.5.6.5 Sexing of the fish to ensure a correct female/male ratio in each tank can be a problem. However, the task usually becomes easier as experience is gained (Flickinger, 1966). Sexually mature females usually have large bellies and a tapered snout. The sexually mature males are usually distinguished by their larger overall size, dark vertical color bands, and the spongy nuptial tubercles on the snout. Unless the males exhibit these secondary breeding characteristics, no reliable method has been found to distinguish them from females. However, using the coloration of the males and the presence of enlarged urogenital structures and other characteristics of the females, the correct selection of the sexes can usually be achieved by trial and error.

11.6.17.5.6.6 Sexually immature males are usually recognized by their aggressive behavior and partial banding. These undeveloped males must be removed from the spawning tanks because they will eat the eggs and constantly harass the mature males, tiring them and reducing the fecundity of the breeding unit. Therefore, the fish in the spawning tanks must be carefully checked periodically for extra males.

11.6.17.5.6.7 A breeding unit should remain in their spawning tank about four months. Thus, each brood tank or unit is stocked with new spawners about three times a year. However, the restocking process is rotated so that at any one time the spawning tanks contain different age groups of brood fish.

11.6.17.5.6.8 Fathead minnows spawn mostly in the early morning hours. They should not be disturbed except for a morning feeding (8:00 AM) and daily examination of substrates for eggs in late morning or early afternoon. In nature, the male protects, cleans, and aerates the eggs until they hatch. In the laboratory, however, it is necessary to remove the eggs from the tanks to prevent them from being eaten by the adults, for ease of handling, for purposes of recording embryo count and hatchability, and for the use of the newly hatched young fish for toxicity tests.

11.6.17.5.6.9 Daily, beginning six to eight hours after the lights are turned on (11:00 AM - 1:00 PM), the substrates in the spawning tanks are each lifted carefully and inspected for embryos. Substrates without embryos are immediately returned to the spawning tank. Those with embryos are immersed in clean water in a collecting tray, and replaced with a clean substrate. A daily record is maintained of each spawning site and the estimated number of embryos on the substrate.

11.6.17.5.6.10 Three different methods are described for embryo incubation.

1. Incubation of Embryos on the Substrates: Several (2-4) substrates are placed on end in a circular pattern (with the embryos on the innerside) in 10 cm of water in a tray. The tray is then placed in a constant temperature water bath, and the embryos are aerated with a 2.5 cm airstone placed in the center of the circle. The embryos are examined daily, and the dead and fungused embryos are counted, recorded, and removed with forceps. At an incubation temperature of 25°C, 50% hatch occurs in five days. At 22°C embryos incubated on aerated tiles require 7 days for 50% hatch.

2. Incubation of Embryos in a Separatory Funnel: The embryos are removed from the substrates with a rolling action of the index finger ("rolled off") (Gast and Brungs, 1973), their total volume is measured, and the number of embryos is calculated using a conversion factor of approximately 430 embryos/mL. The embryos are incubated in about 1.5 L of water in a 2 L separatory funnel maintained in a water bath. The embryos are stirred in the separatory funnel by bubbling air from the tip of a plastic micro-pipette placed at the bottom, inside the separatory funnel. During the first two days, the embryos are taken from the funnel daily, those that are dead and fungused are removed, and those that are alive are returned to the separatory funnel in clean water. The embryos hatch in four days at a temperature of 25°C. However, usually on day three the eyed embryos are removed from the separatory funnel and placed in water in a plastic tray and gently aerated with an air stone. Using this method, the embryos hatch in five days. Hatching time is greatly influenced by the amount of agitation of the embryos and the incubation temperature. If on day three the embryos are transferred from the separatory funnel to a static, unaerated container, a 50% hatch will occur in six days (instead of five) and a 100% hatch will occur in seven days. If the culture system is operated at 22°C, embryos incubated on aerated tiles require seven days for 50% hatch.

3. Incubation in Embryo Incubation Cups: The embryos are "rolled off" the substrates, and the total number is estimated by determining the volume. The embryos are then placed in incubation cups attached to a rocker arm assembly (Mount, 1968). Both flow-through and static renewal incubation have been used. On day one, the embryos are removed from the cups and those that are dead and fungused are removed. After day one only dead embryos are removed from the cups. During the incubation period, the eggs are examined daily for viability and fungal growth, until they hatch. Unfertilized eggs, and eggs that have become infected by fungus, should be removed with forceps using a table top magnifier-illuminator. Non-viable eggs become milky and opaque, and are easily recognized. The non-viable eggs are very susceptible to fungal infection, which may then spread throughout the egg mass. Removal of fungus should be done quickly, and the substrates should be returned to the incubation tanks as rapidly as possible so that the good eggs are not damaged by desiccation. Hatching takes four to five days at an optimal temperature of

25°C. Hatching can be delayed several (two to four) days by incubating at lower temperatures. A large plastic tank receiving recirculating water from a temperature control unit, can be used as a water bath for incubation of embryos.

11.6.17.5.6.11 Newly-hatched larvae are transferred daily from the egg incubation apparatus to small rearing tanks, using a large bore pipette, until the hatch is complete. New rearing tanks are set up on a daily basis to separate fish by age group. Approximately 1500 newly hatched larvae are placed in a 60-L (15 gal) or 76-L (20 gal) all-glass aquarium for 30 days. A density of 150 fry per liter is suitable for the first four weeks. The water temperature in the rearing tanks is allowed to follow ambient laboratory temperatures of 20-25°C, but sudden, extreme variations in temperature must be avoided.

11.6.17.5.7 Food and Feeding

11.6.17.5.7.1 The amount of food and feeding schedule affects both growth and egg production. The spawning fish and pre-spawners in holding tanks usually are fed all the adult frozen brine shrimp and tropical fish flake food or dry commercial fish food (No. 1 or No. 2 granules) that they can eat (ad libitum) at the beginning of the work day and in the late afternoon (8:00 AM and 4:00 PM). The fish are fed twice a day (twice a day with dry food and once a day with adult shrimp) during the week and once a day on weekends.

11.6.17.5.7.2 Fathead minnow larvae are fed freshly-hatched brine shrimp (*Artemia*) nauplii twice daily until they are four weeks old. Utilization of older (larger) brine shrimp nauplii may result in starvation of the young fish because they are unable to ingest the larger food organisms (see SubSection 11.6.16 or USEPA, 1993b for instructions on the preparation of brine shrimp nauplii).

11.6.17.5.7.3 Fish older than four weeks are fed frozen brine shrimp and commercial fish starter (#1 and #2), which is ground fish meal enriched with vitamins. As the fish grow, larger pellet sizes are used, as appropriate. (Starter, No. 1 and N. 2 granules, U.S. Fish and Wildlife Service Formulation Specification Diet SD9-30, can be obtained from Zeigler Bros., Inc., P.O. Box 90, Gardners, PA 17324). Newly hatched brine shrimp nauplii, and frozen adult brine shrimp (San Francisco Bay Brand) are fed to the fish cultures in volumes based on age, size, and number of fish in the tanks.

11.6.17.5.7.4 Fish in the larval tanks (from hatch to 30 days old) are fed commercial starter fish food at the beginning and end of the work day, and newly hatched brine shrimp nauplii (from the brine shrimp culture unit) once a day, usually mid-morning and mid-afternoon.

11.6.17.5.7.5 Attempts should be made to avoid introducing *Artemia* cysts and empty shells when the brine shrimp nauplii are fed to the fish larvae. Some of the mortality of the larval fish observed in cultures could be caused from the ingestion of these materials.

11.6.17.5.8 Disease Control

11.6.17.5.8.1 Fish are observed daily for abnormal appearance or behavior. Bacterial or fungal infections are the most common diseases encountered. However, if normal precautions are taken, disease outbreaks will rarely, if ever, occur. Hoffman and Mitchell (1980) have put together a list of some chemicals that have been used commonly for fish diseases and pests.

11.6.17.5.8.2 In aquatic culture systems where filtration is utilized, the application of certain antibacterial agents should be used with caution. A treatment with a single dose of antibacterial drugs can interrupt nitrate reduction and stop nitrification for various periods of time, resulting in changes in pH, and in ammonia, nitrite and nitrate concentrations (Collins et al., 1976). These changes could cause the death of the culture organisms.

11.6.17.5.8.3 Do not transfer equipment from one tank to another without first disinfecting tanks and nets. If an outbreak of disease occurs, any equipment, such as nets, airlines, tanks, etc., which has been exposed to diseased fish should be disinfected with sodium hypochlorite. Also to avoid the contamination of cultures or spread of disease, each time nets are used to remove live or dead fish from tanks, they are first sterilized with sodium hypochlorite or formalin, and rinsed in hot tap water. Before a new lot of fish is transferred to culture tanks, the tanks are cleaned and sterilized as described above.

11.6.17.5.8.4 It is recommended that chronic toxicity tests be performed monthly with a reference toxicant. Newly hatched fathead minnow larvae less than 24 h old are used to monitor the chronic toxicity of the reference toxicant to the test fish produced by the culture unit (see Section 4, Quality Assurance).

11.6.17.5.9 Record Keeping

11.6.17.5.9.1 Records, kept in a bound notebook, include: (1) type of food and time of feeding for all fish tanks; (2) time of examination of the tiles for embryos, the estimated number of embryos on the tile, and the tile position number; (3) estimated number of dead embryos and embryos with fungus observed during the embryonic development stages; (4) source of all fish; (5) daily observation of the condition and behavior of the fish; and (6) dates and results of reference toxicant tests performed (see Section 4, Quality Assurance).

11.7 EFFLUENT AND RECEIVING WATER COLLECTION, PRESERVATION, AND STORAGE

11.7.1 See Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests.

11.8 CALIBRATION AND STANDARDIZATION

11.8.1 See Section 4, Quality Assurance.

11.9 QUALITY CONTROL

11.9.1 See Section 4, Quality Assurance.

11.10 TEST PROCEDURES

11.10.1 TEST SOLUTIONS

11.10.1.1 Receiving Waters

11.10.1.1.1 The sampling point is determined by the objectives of the test. Receiving water toxicity is determined with samples used directly as collected or after samples are passed through a 60 µm NITEX® filter and compared without dilution, against a control. Using four replicate chambers per test, each containing 250 mL, and 400 mL for chemical analyses, would require approximately 1.5 L or more of sample per test per day.

11.10.1.2 Effluents

11.10.1.2.1 The selection of the effluent test concentrations should be based on the objectives of the study. A dilution factor of 0.5 is commonly used. A dilution factor of 0.5 provides precision of ± 100%, and testing of concentrations between 6.25% and 100% effluent using only five effluent concentrations (6.25%, 12.5%, 25%, 50%, and 100%). Test precision shows little improvement as the dilution factor is increased beyond 0.5, and declines rapidly if a smaller dilution factor is used. **Therefore, USEPA recommends the use of the ≥ 0.5 dilution factor.**

11.10.1.2.2 If the effluent is known or suspected to be highly toxic, a lower range of effluent concentrations should be used (such as 25%, 12.5%, 6.25%, 3.12%, and 1.56%). If a high rate of mortality is observed during the first 1 to 2 h of the test, additional dilutions should be added at the lower range of effluent concentrations.

11.10.1.2.3 The volume of effluent required for daily renewal of four replicates per concentration, each containing 250 mL of test solution, is approximately 2.5 L. Sufficient test solution (approximately 1500 mL) is prepared at each effluent concentration to provide 400 mL additional volume for chemical analyses at the high, medium, and low test concentrations. If the sample is used for more than one daily renewal of test solutions, the volume must be increased proportionately.

11.10.1.2.4 Tests should begin as soon as possible, preferably within 24 h of sample collection. The maximum holding time following retrieval of the sample from the sampling device should not exceed 36 h for off-site toxicity tests unless permission is granted by the permitting authority. In no case should the sample be used for the first time in a test more than 72 h after sample collection (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests).

11.10.1.2.5 Just prior to test initiation (approximately 1 h) the temperature of sufficient quantity of the sample to make the test solutions should be adjusted to the test temperature and maintained at that temperature during the addition of dilution water.

11.10.1.2.6 The DO of the test solutions should be checked prior to the test initiation. If any of the solutions are supersaturated with oxygen or any solution has a DO concentration below 4.0 mg/L, all of the solutions and the control must be gently aerated.

11.10.1.3 Dilution Water

11.10.1.3.1 Dilution water may be uncontaminated receiving water, a standard synthetic (reconstituted) water, or some other uncontaminated natural water (see Section 7, Dilution Water).

11.10.2 START OF THE TEST

11.10.2.1 Label the test chambers with a marking pen. Use of color-coded tape to identify each treatment and replicate is helpful. A minimum of five effluent concentrations and a control are used for each effluent test. Each treatment (including the control) should have four (minimum of three) replicates.

11.10.2.2 Tests performed in laboratories that have in-house fathead minnow breeding cultures should use larvae less than 24 h old. When eggs or larvae must be shipped to the test site from a remote location, it may be necessary to use larvae older than 24 h because of the difficulty in coordinating test organism shipments with field operations. However, in the latter case, the larvae should not be more than 48 h old at the start of the test and should all be within 24 h of the same age.

11.10.2.3 Randomize the position of test chambers at the beginning of the test (see Appendix A). Maintain the chambers in this configuration throughout the test. Preparation of a position chart may be helpful.

11.10.2.4 The larvae are pooled and placed one or two at a time into each randomly arranged test chamber or intermediate container in sequential order, until each chamber contains 15 (minimum of 10) larvae, for a total of 60 larvae (minimum of 30) for each concentration (see Appendix A). The test organisms should come from a pool of larvae consisting of at least three separate spawnings. The amount of water added to the chambers when transferring the larvae should be kept to a minimum to avoid unnecessary dilution of the test concentrations.

11.10.2.4.1 The chambers may be placed on a light table to facilitate counting the larvae.

11.10.3 LIGHT, PHOTOPERIOD, AND TEMPERATURE

11.10.3.1 The light quality and intensity should be at ambient laboratory levels, which is approximately 10-20 $\mu E/m^2/s$, or 50 to 100 foot candles (ft-c), with a photoperiod of 16 h of light and 8 h of darkness. The water temperature in the test chambers should be maintained at 25 ± 1°C.

11.10.4 DISSOLVED OXYGEN (DO) CONCENTRATION

11.10.4.1 Aeration may affect the toxicity of effluents and should be used only as a last resort to maintain satisfactory DO concentrations. The DO concentrations should be measured in the new solutions at the start of the test (Day 0) and before daily renewal of the test solutions on subsequent days. The DO concentrations should not fall below 4.0 mg/L (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests). If it is necessary to aerate, all concentrations and the control should be aerated. The aeration rate should not exceed 100 bubbles/min, using a pipet with an orifice of approximately 1.5 mm, such as a 1-mL, KIMAX® serological pipet, No. 37033, or equivalent. Care should be taken to ensure that turbulence resulting from aeration does not cause undue physical stress to the fish.

11.10.5 FEEDING

11.10.5.1 The fish in each test chamber are fed 0.1 g (approximately 700 to 1000) of a concentrated suspension of newly hatched (less than 24-h old) brine shrimp nauplii three times daily at 4-h intervals or, as a minimum, 0.15 g are fed twice daily at an interval of 6 h. Equal amounts of nauplii must be added to each replicate chamber to reduce variability in larval weight. Sufficient numbers of nauplii should be provided to assure that some remain alive in the test chambers at the next feeding, but not in excessive amounts which will result in depletion of DO below acceptable levels (below 4.0 mg/L).

11.10.5.2 The feeding schedule will depend on when the test solutions are renewed. If the test is initiated after 12:00 PM, the larvae may be fed only once the first day. On following days, the larvae normally would be fed at the beginning of the work day, at least 2 h before test solution renewal, and at the end of the work day, after test solution renewal. However, if the test solutions are changed at the beginning of the work day, the first feeding would be after test solution renewal in the morning, and the remaining feeding(s) would be at the appropriate intervals. The larvae are not fed during the final 12 h of the test.

11.10.5.3 The nauplii should be rinsed with freshwater to remove salinity before use (see USEPA, 1993b). At feeding time pipette about 5 mL (5 g) of concentrated newly hatched brine shrimp nauplii into a 120 mesh nylon net or plastic cup with nylon mesh bottom. Slowly run freshwater through the net or rinse by immersing the cup in a container of fresh water several times. Resuspend the brine shrimp in 10 mL of fresh water in a 30 mL beaker or simply set the cup of washed brine shrimp in ¼ inch of fresh water so that the cup contains about 10 mL of water. Allow the container to set for a minute or two to allow dead nauplii and empty cysts to settle or float to the surface before collecting the brine shrimp from just below the surface in a pipette for feeding. Distribute 2 drops (0.1 g) of the brine shrimp to each test chamber. If the survival rate in any test chamber falls below 50%, reduce the feeding in that chamber to 1 drop of brine shrimp at each subsequent feeding.

11.10.6 OBSERVATIONS DURING THE TEST

11.10.6.1 Routine Chemical and Physical Determinations

11.10.6.1.1 DO is measured at the beginning and end of each 24-h exposure period in at least one test chamber at each test concentration and in the control.

11.10.6.1.2 Temperature and pH are measured at the end of each 24-h exposure period in at least one test chamber at each test concentration and in the control. Temperature should also be monitored continuously or observed and recorded daily for at least two locations in the environmental control system or the samples. Temperature should be measured in a sufficient number of test vessels at least at the end of the test to determine the temperature variation in the environmental chamber.

11.10.6.1.3 The pH is measured in the effluent sample each day before new test solutions are made.

11.10.6.1.4 Conductivity, alkalinity and hardness are measured in each new sample (100% effluent or receiving water) and in the control.

11.10.6.1.5 Record all the measurements on the data sheet (Figure 1).

11.10.6.2 Routine Biological Observations

11.10.6.2.1 The number of live larvae in each test chamber are recorded daily (Figure 2), and the dead larvae are discarded.

11.10.6.2.2 Protect the larvae from unnecessary disturbance during the test by carrying out the daily test observations, solution renewals, and removal of dead larvae, carefully. Make sure the larvae remain immersed during the performance of these operations.

11.10.7 DAILY CLEANING OF TEST CHAMBERS

11.10.7.1 Before the daily renewal of test solutions, uneaten and dead *Artemia*, dead fish larvae, and other debris are removed from the bottom of the test chambers with a siphon hose. Alternately, a large pipet (50 mL) fitted with a rubber bulb can be used. Because of their small size during the first few days of the tests, larvae are easily drawn into the siphon tube or pipet when cleaning the test chambers. By placing the test chambers on a light box, inadvertent removal of larvae can be greatly reduced because they can be more easily seen. If the water siphoned from the test chambers is collected in a white plastic tray, the larvae caught up in the siphon can be retrieved and returned to the chambers. Any incidence of removal of live larvae from the test chambers during cleaning, and subsequent return to the chambers, should be noted in the records.

11.10.8 TEST SOLUTION RENEWAL

11.10.8.1 Freshly prepared solutions are used to renew the tests daily immediately after cleaning the test chambers. For on-site toxicity studies, fresh effluent or receiving water samples should be collected daily, and no more than 24 h should elapse between collection of the samples and their use in the tests (see Section 8, Effluent and Receiving Water Sampling, Sample Holding, and Sample Preparation for Toxicity Tests). For off-site tests, a minimum of three samples are collected, preferably on days one, three, and five. Maintain the samples in the refrigerator at 4°C until used.

11.10.8.2 For test solution renewal, the water level in each chamber is lowered to a depth of 7 to 10 mm, which leaves 15 to 20% of the test solution. New test solution (250 mL) should be added slowly by pouring down the side of the test chamber to avoid excessive turbulence and possible injury to the larvae.

11.10.9 TERMINATION OF THE TEST

11.10.9.1 The test is terminated after seven days of exposure. At test termination, dead larvae are removed and discarded. The surviving larvae in each test chamber (replicate) are counted and immediately prepared as a group for dry weight determination, or are preserved as a group in 70% ethanol or 4% formalin. Preserved organisms are dried and weighed within 7 days. For safety, formalin should be used under a hood.

11.10.9.2 For immediate drying and weighing, place live larvae onto a 500 μm mesh screen in a large beaker to wash away debris that might contribute to the dry weight. Each group of larvae is rinsed with deionized water to remove food particles, transferred to a tared weighing boat that has been properly labeled, and dried at 60°C, for 24 h or at 100°C for a minimum of 6 h. Immediately upon removal from the drying oven, the weighing boats are placed in a dessicator until weighed, to prevent the absorption of moisture from the air. All weights should be measured to the nearest 0.01 mg and recorded on data sheets (Figure 3). Subtract tare weight to determine the dry weight of the larvae in each replicate. For each test chamber, divide the final dry weight by the number of original larvae in the test chamber to determine the average individual dry weight and record on the data sheet (Figure 3). For the controls, also calculate the mean weight per surviving fish in the test chamber to evaluate if weights met test acceptability criteria (See Section 11.11). Average weights should be expressed to the nearest 0.001 mg.

11.10.9.3 Prepare a summary table as illustrated in Figure 4.

11.11 **SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA**

11.11.1 A summary of test conditions and test acceptability criteria is presented in Table 1.

TABLE 1. SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR FATHEAD MINNOW, *PIMEPHALES PROMELAS*, LARVAL SURVIVAL AND GROWTH TOXICITY TESTS WITH EFFLUENTS AND RECEIVING WATERS

1. Test type:	Static renewal
2. Temperature (°C):	25 ± 1°C
3. Light quality:	Ambient laboratory illumination
4. Light intensity:	10-20 $\mu E/m^2/s$ (50-100 ft-c)(ambient laboratory levels)
5. Photoperiod:	16 h light, 8 h darkness
6. Test chamber size:	500 mL (minimum)
7. Test solution volume:	250 mL (minimum)
8. Renewal of test solutions:	Daily
9. Age of test organisms:	Newly hatched larvae less than 24 h old. If shipped, not more than 48 h old, 24 h range in age
10. No. larvae per test chamber:	15 (minimum of 10)
11. No. replicate chambers per concentration:	4 (minimum of 3)
12. No. larvae per concentration:	60 (minimum of 30)
13. Source of food:	Newly hatched *Artemia* nauplii (less than 24 h old)
14. Feeding regime:	Feed 0.1 g newly hatched (less than 24-h old) brine shrimp nauplii three times daily at 4-h intervals or, as a minimum, 0.15 g twice daily, 6 h between feedings (at the beginning of the work day prior to renewal, and at the end of the work day following renewal). Sufficient nauplii are added to provide an excess. Larvae fish are not fed during the final 12 h of the test

TABLE 1. SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR FATHEAD MINNOW, *PIMEPHALES PROMELAS*, LARVAL SURVIVAL AND GROWTH TOXICITY TESTS WITH EFFLUENTS AND RECEIVING WATERS (CONTINUED)

15. Cleaning:	Siphon daily, immediately before test solution renewal
16. Aeration:	None, unless DO concentration falls below 4.0 mg/L. Rate should not exceed 100 bubbles/min
17. Dilution water:	Uncontaminated source of receiving or other natural water, synthetic water prepared using MILLIPORE MILLI-Q® or equivalent deionized water and reagent grade chemicals, or DMW (see Section 7, Dilution Water)
18. Test concentrations:	Effluents: Minimum of 5 and a control Receiving Water: 100% receiving water or minimum of 5 and a control
19. Dilution factor	Effluents: ≥ 0.5 Receiving waters: None or ≥ 0.5
20. Test duration:	7 days
21. Endpoints:	Survival and growth (weight)
22. Test acceptability criteria:	80% or greater survival in controls; average dry weight per surviving organism in control chambers equals or exceeds 0.25 mg
23. Sampling requirements:	For on-site tests, samples collected daily, and used within 24 h of the time they are removed from the sampling device; For off-site tests, a minimum of three samples collected on days one, three and five with a maximum holding time of 36 h before first use (see Section 8,
24. Sample volume required:	2.5 L/day

11.12 ACCEPTABILITY OF TEST RESULTS

11.12.1 For the test results to be acceptable, survival in the controls must be at least 80%. The average dry weight per surviving control larvae at the end of the test should equal or exceed 0.25 mg.

TABLE 13. PRECISION OF THE FATHEAD MINNOW, *PIMEPHALES PROMELAS*, EMBRYO-LARVAL SURVIVAL AND TERATOGENICITY STATIC-RENEWAL TEST CONDUCTED WITH TRICKLING FILTER EFFLUENT[1,2,3]

Test No.	NOEC (% Effluent)	LOEC (% Effluent)
1	7	11
2	7	11
3	7	11
4	7	11

[1] Data provided by Timothy Neiheisel, Bioassessment and Ecotoxicology Branch, EMSL, USEPA, Cincinnati, OH.

[2] Effluent concentrations used: 3, 5, 7, 11 and 16%.

[3] Maximum precision achieved in terms of NOEC-LOEC interval. For a discussion of the precision of data from chronic toxicity tests (see Section 4, Quality Assurance).

APPENDIX A-18

Procedure for Toxicity using Daphnid (*Ceriodaphnia dubia*): EPA Method 1002.0

SECTION 13

TEST METHOD

DAPHNID, *CERIODAPHNIA DUBIA*, SURVIVAL AND REPRODUCTION TEST

METHOD 1002.0

13.1 SCOPE AND APPLICATION

13.1.1 This method measures the chronic toxicity of effluents and receiving water to the daphnid, *Ceriodaphnia dubia*, using less than 24 h old neonates during a three-brood (seven-day), static renewal test. The effects include the synergistic, antagonistic, and additive effects of all the chemical, physical, and biological components which adversely affect the physiological and biochemical functions of the test organisms.

13.1.2 Daily observations on mortality make it possible to also calculate acute toxicity for desired exposure periods (i.e., 24-h, 48-h, and 96-h LC50s).

13.1.3 Detection limits of the toxicity of an effluent or pure substance are organism dependent.

13.1.4 Brief excursions in toxicity may not be detected using 24-h composite samples. Also, because of the long sample collection period involved in composite sampling, and because the test chambers are not sealed, highly degradable or highly volatile toxicants in the source may not be detected in the test.

13.1.5 This test method is commonly used in one of two forms: (1) a definitive test, consisting of a minimum of five effluent concentrations and a control, and (2) a receiving water test(s), consisting of one or more receiving water concentrations and a control.

13.2 SUMMARY OF METHOD

13.2.1 *Ceriodaphnia dubia* are exposed in a static renewal system to different concentrations of effluent, or to receiving water, until 60% of surviving control organisms have three broods of offspring. Test results are based on survival and reproduction. If the test is conducted as described, the surviving control organisms should produce 15 or more young in three broods. If these criteria are not met at the end of 8 days, the test must be repeated.

13.3 INTERFERENCES

13.3.1 Toxic substances may be introduced by contaminants in dilution water, glassware, sample hardware, and testing equipment (see Section 5, Facilities, Equipment, and Supplies).

13.3.2 Improper effluent sampling and handling may adversely affect test results (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests).

13.3.3 Pathogenic and/or predatory organisms in the dilution water and effluent may affect test organism survival and confound test results.

13.3.4 The amount and type of natural food in the effluent or dilution water may confound test results.

13.3.5 Food added during the test may sequester metals and other toxic substances and confound test results. Daily renewal of solutions, however, will reduce the probability of reduction of toxicity caused by feeding.

13.4 SAFETY

13.4.1 See Section 3, Health and Safety.

13.5 APPARATUS AND EQUIPMENT

13.5.1 *Ceriodaphnia* and algal culture units -- See *Ceriodaphnia* and algal culturing methods below and algal culturing methods in Section 14 and USEPA, 1993b.

13.5.2 Samplers -- automatic sampler, preferably with sample cooling capability, capable of collecting a 24-h composite sample of 5 L or more.

13.5.3 Sample containers -- for sample shipment and storage (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests).

13.5.4 Environmental chambers, incubators, or equivalent facilities with temperature control (25 ± 1°C).

13.5.5 Water purification system -- MILLIPORE MILLI-Q®, deionized water or equivalent (see Section 5, Facilities, Equipment, and Supplies).

13.5.6 Balance -- analytical, capable of accurately weighing 0.00001 g.

13.5.7 Reference weights, Class S -- for checking performance of balance. Weights should bracket the expected weights of the material to be weighed.

13.5.8 Test chambers -- 10 test chambers are required for each concentration and control. Test chambers such as 30-mL borosilicate glass beakers or disposable polystyrene cups are recommended because they will fit in the viewing field of most stereoscopic microscopes. The glass beakers and plastic cups are rinsed thoroughly with dilution water before use. To avoid potential contamination from the air and excessive evaporation of the test solutions during the test, the test vessels should be covered with safety glass plates or sheet plastic (6 mm thick).

13.5.9 Mechanical shaker or magnetic stir plates -- for algal cultures.

13.5.10 Light meter -- with a range of 0-200 $\mu E/m^2/s$ (0-1000 ft-c).

13.5.11 Fluorometer (optional) -- equipped with chlorophyll detection light source, filters, and photomultiplier tube (Turner Model 110 or equivalent).

13.5.12 UV-VIS spectrophotometer (optional) -- capable of accommodating 1-5 cm cuvettes.

13.5.13 Cuvettes for spectrophotometer -- 1-5 cm light path.

13.5.14 Electronic particle counter (optional) -- Coulter Counter, ZBI, or equivalent, with mean cell (particle) volume determination.

13.5.15 Microscope with 10X, 45X, and 100X objective lenses, 10X ocular lenses, mechanical stage, substage condensor, and light source (inverted or conventional microscope) -- for determining sex and verifying identification.

13.5.16 Dissecting microscope, stereoscopic, with zoom objective, magnification to 50X -- for examining and counting the neonates in the test vessels.

13.5.17 Counting chamber -- Sedgwick-Rafter, Palmer-Maloney, or hemocytometer.

13.5.18 Centrifuge (optional) -- plankton, or with swing-out buckets having a capacity of 15-100 mL.

13.5.19 Centrifuge tubes -- 15-100 mL, screw-cap.

13.5.20 Filtering apparatus -- for membrane and/or glass fiber filters.

13.5.21 Racks (boards) -- to hold test chambers. It is convenient to use a piece of styrofoam insulation board, 50 cm x 30 cm x 2.5 cm (20 in x 12 in x 1 in), drilled to hold 60 test chambers, in six rows of 10 (see Figure 1).

13.5.22 Light box -- for illuminating organisms during examination.

13.5.23 Volumetric flasks and graduated cylinders -- class A, borosilicate glass or non-toxic plastic labware, 10-1000 mL, for culture work and preparation of test solutions.

13.5.24 Pipettors, adjustable volume repeating dispensers -- for feeding. Pipettors such as the Gilson REPETMAN®, Eppendorf, Oxford, or equivalent, provide a rapid and accurate means of dispensing small volumes (0.1 mL) of food to large numbers of test chambers.

13.5.25 Volumetric pipets -- class A, 1-100 mL.

13.5.26 Serological pipets -- 1-10 mL, graduated.

13.5.27 Pipet bulbs and fillers -- PROPIPET®, or equivalent.

13.5.28 Disposable polyethylene pipets, droppers, and glass tubing with fire-polished edges, ≥ 2mm ID -- for transferring organisms.

13.5.29 Wash bottles -- for rinsing small glassware and instrument electrodes and probes.

13.5.30 Thermometer, glass or electronic, laboratory grade, -- for measuring water temperatures.

13.5.31 Bulb-thermograph or electronic-chart type thermometers -- for continuously recording temperature.

13.5.32 Thermometer, National Bureau of Standards Certified (see USEPA Method 170.1, USEPA 1979b) -- to calibrate laboratory thermometers.

13.5.33 Meters, DO, pH, and specific conductivity -- for routine physical and chemical measurements.

13.6 **REAGENTS AND CONSUMABLE MATERIALS**

13.6.1 Sample containers -- for sample shipment and storage (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests).

13.6.2 Data sheets (one set per test) -- for recording the data.

13.6.3 Vials, marked -- for preserving specimens for verification (optional).

13.6.4 Tape, colored -- for labeling test vessels.

13.6.5 Markers, waterproof -- for marking containers.

13.6.6 Reagents for hardness and alkalinity tests -- see USEPA Methods 130.2 and 310.1, USEPA, 1979b.

13.6.7 Buffers, pH 4, pH 7, and pH 10 (or as per instructions of instrument manufacturer) -- for instrument calibration check (see USEPA Method 150.1, USEPA, 1979b).

13.6.8 Specific conductivity standards -- see USEPA Method 120.1, USEPA, 1979b.

13.6.9 Membranes and filling solutions for DO probe (see USEPA Method 360.1, USEPA, 1979b), or reagents -- for modified Winkler analysis.

13.6.10 Laboratory quality control samples and standards -- for calibration of the above methods.

13.6.11 Reference toxicant solutions -- see Section 4, Quality Assurance.

13.6.12 Reagent water -- defined as distilled or deionized water that does not contain substances which are toxic to the test organisms (see Section 5, Facilities, Equipment, and Supplies).

13.6.13 Effluent, surface water, and dilution water -- see Section 7, Dilution Water; and Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests.

13.6.14 Trout chow, yeast, and CEROPHYL® food (or substitute food) -- for feeding the cultures and test organisms.

13.6.14.1 Digested trout chow, or substitute flake food (TETRAMIN®, BIORIL®, or equivalent), is prepared as follows:

1. Preparation of trout chow or substitute flake food requires one week. Use starter or No. 1 pellets prepared according to current U.S. Fish and Wildlife Service specifications. Suppliers of trout chow include Zeigler Bros., Inc., P.O. Box 95, Gardners, PA, 17324 (717-780-9009); Glencoe Mills, 1011 Elliott, Glencoe, MN, 55336 (612-864-3181); and Murray Elevators, 118 West 4800 South, Murray, UT 84107 (800-521-9092).
2. Add 5.0 g of trout chow pellets or substitute flake food to 1 L of MILLI-Q® water. Mix well in a blender and pour into a 2-L separatory funnel. Digest prior to use by aerating continuously from the bottom of the vessel for one week at ambient laboratory temperature. Water lost due to evaporation is replaced during digestion. Because of the offensive odor usually produced during digestion, the vessel should be placed in a fume hood or other isolated, ventilated area.
3. At the end of digestion period, place in a refrigerator and allow to settle for a minimum of 1 h. Filter the supernatant through a fine mesh screen (i.e., NITEX® 110 mesh). Combine with equal volumes of supernatant from CEROPHYLL® and yeast preparations (below). The supernatant can be used fresh, or frozen until use. Discard the sediment.

13.6.14.2 Yeast is prepared as follows:

1. Add 5.0 g of dry yeast, such as FLEISCHMANN'S® Yeast, Lake State Kosher Certified Yeast, or equivalent, to 1 L of MILLI-Q® water.
2. Stir with a magnetic stirrer, shake vigorously by hand, or mix with a blender at low speed, until the yeast is well dispersed.
3. Combine the yeast suspension immediately (do not allow to settle) with equal volumes of supernatant from the trout chow (above) and CEROPHYLL® preparations (below). Discard excess material.

13.6.14.3 CEROPHYLL® is prepared as follows:

1. Place 5.0 g of dried, powdered, cereal or alfalfa leaves, or rabbit pellets, in a blender. Cereal leaves are available as "CEREAL LEAVES," from Sigma Chemical Company, P.O. Box 14508, St. Louis, MO 63178; 800-325-3010; or as CEROPHYLL®, from Ward's Natural Science Establishment, Inc., P.O. Box 92912, Rochester, NY 14692-9012;

716-359-2502. Dried, powdered, alfalfa leaves may be obtained from health food stores, and rabbit pellets are available at pet shops.

2. Add 1 L of MILLI-Q® water.
3. Mix in a blender at high speed for 5 min, or stir overnight at medium speed on a magnetic stir plate.
4. If a blender is used to suspend the material, place in a refrigerator overnight to settle. If a magnetic stirrer is used, allow to settle for 1 h. Decant the supernatant and combine with equal volumes of supernatant from trout chow and yeast preparations (above). Discard excess material.

13.6.14.4 Combined yeast-cerophyl-trout chow (YCT) is mixed as follows:

1. Thoroughly mix equal (approximately 300 mL) volumes of the three foods as described above.
2. Place aliquots of the mixture in small (50 mL to 100 mL) screw-cap plastic bottles and freeze until needed.
3. Freshly prepared food can be used immediately, or it can be frozen until needed. Thawed food is stored in the refrigerator between feedings, and is used for a maximum of two weeks. Do not store frozen over three months.
4. It is advisable to measure the dry weight of solids in each batch of YCT before use. The food should contain 1.7-1.9 g solids/L. Cultures or test solutions should contain 12-13 mg solids/L.

13.6.15 Algal food -- for feeding the cultures and test organisms.

13.6.15.1 Algal Culture Medium is prepared as follows:

1. Prepare (five) stock nutrient solutions using reagent grade chemicals as described in Table 1.
2. Add 1 mL of each stock solution, in the order listed in Table 1, to approximately 900 mL of MILLI-Q® water. Mix well after the addition of each solution. Dilute to 1 L, mix well. The final concentration of macronutrients and micronutrients in the culture medium is given in Table 2.
3. Immediately filter the medium through a 0.45 μm pore diameter membrane at a vacuum of not more than 380 mm (15 in.) mercury, or at a pressure of not more than one-half atmosphere (8 psi). Wash the filter with 500 mL deionized water prior to use.
4. If the filtration is carried out with sterile apparatus, filtered medium can be used immediately, and no further sterilization steps are required before the inoculation of the medium. The medium can also be sterilized by autoclaving after it is placed in the culture vessels.
5. Unused sterile medium should not be stored more than one week prior to use, because there may be substantial loss of water by evaporation.

13.6.15.2 Algal Cultures

13.6.15.2.1 See Section 6, Test Organisms, for information on sources of "starter" cultures of *Selenastrum capricornutum*, *S. minutum*, and *Chlamydomonas reinhardti*.

TABLE 1. NUTRIENT STOCK SOLUTIONS FOR MAINTAINING ALGAL STOCK CULTURES

STOCK SOLUTION	COMPOUND	AMOUNT DISSOLVED IN 500 mL MILLI-Q® WATER
1. MACRONUTRIENTS		
A.	$MgCl_2 \cdot 6H_2O$	6.08 g
	$CaCl_2 \cdot 2H_2O$	2.20 g
	$NaNO_3$	12.75 g
B.	$MgSO_4 \cdot 7H_2O$	7.35 g
C.	K_2HPO_4	0.522 g
D.	$NaHCO_3$	7.50 g
2. MICRONUTRIENTS		
	H_3BO_3	92.8 mg
	$MnCl_2.4H_2O$	208.0 mg
	$ZnCl_2$	1.64 mg[1]
	$FeCl_3 \cdot 6H_2O$	79.9 mg
	$CoCl_2 \cdot 6H_2O$	0.714 mg[2]
	$Na_2MoO_4 \cdot 2H_2O$	3.63 mg[3]
	$CuCl_2 \cdot 2H_2O$	0.006 mg[4]
	$Na_2EDTA \cdot 2H_2O$	150.0 mg
	Na_2SeO_4	1.196 mg[5]

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[1] $ZnCl_2$ - Weigh out 164 mg and dilute to 100 mL. Add 1 mL of this solution to Stock 2, micronutrients.

[2] $CoCl_2 \cdot 6H_2O$ - Weigh out 71.4 mg and dilute to 100 mL. Add 1 mL of this solution to Stock 2, micronutrients.

[3] $Na_2MoO_4 \cdot 2H_2O$ - Weigh out 36.6 mg and dilute to 10 mL. Add 1 mL of this solution to Stock 2, micronutrients.

[4] $CuCl_2 \cdot 2H_2O$ - Weigh out 60.0 mg and dilute to 1000 mL. Take 1 mL of this solution and dilute to 10 mL. Take 1 mL of the second dilution and add to Stock 2, micronutrients.

[5] Na_2SeO_4 - Weigh out 119.6 mg and dilute to 100 mL. Add 1 mL of this solution to Stock 2, micronutrients.

TABLE 2. FINAL CONCENTRATION OF MACRONUTRIENTS AND MICRONUTRIENTS IN THE CULTURE MEDIUM

MACRONUTRIENT	CONCENTRATION (mg/L)	ELEMENT	CONCENTRATION (mg/L)
$NaNO_3$	25.5	N	4.20
$MgCl_2 \cdot 6H_2O$	12.2	Mg	2.90
$CaCl_2 \cdot 2H_2O$	4.41	Ca	1.20
$MgSO_4 \cdot 7H_2O$	14.7	S	1.91
K_2HPO_4	1.04	P	0.186
$NaHCO_3$	15.0	Na	11.0
		K	0.469
		C	2.14

MICRONUTRIENT	CONCENTRATION (μg/L)	ELEMENT	CONCENTRATION (μg/L)
H_3BO_3	185.0	B	32.5
$MnCl_2 \cdot 4H_2O$	416.0	Mn	115.0
$ZnCl_2$	3.27	Zn	1.57
$CoCl_2 \cdot 6H_2O$	1.43	Co	0.354
$CuCl_2 \cdot 2H_2O$	0.012	Cu	0.004
$Na_2MoO_4 \cdot 2H_2O$	7.26	Mo	2.88
$FeCl_3 \cdot 6H_2O$	160.0	Fe	33.1
$Na_2EDTA \cdot 2H_2O$	300.0	--	----
Na_2SeO_4	2.39	Se	0.91

13.6.15.2.2 Two types of algal cultures are maintained: "stock" cultures, and "food" cultures.

13.6.15.2.2.1 Establishing and Maintaining Stock Cultures of Algae:

1. Upon receipt of the "starter" culture (usually about 10 mL), a stock culture is initiated by aseptically transferring one milliliter to each of several 250-mL culture flasks containing 100 mL algal culture medium (prepared as described above). The remainder of the starter culture can be held in reserve for up to six months in a refrigerator (in the dark) at 4°C.
2. The stock cultures are used as a source of algae to initiate "food" cultures for *Ceriodaphnia dubia* toxicity tests. The volume of stock culture maintained at any one time will depend on the amount of algal food required for the *Ceriodaphnia dubia* cultures and tests. Stock culture volume may be rapidly "scaled up" to several liters, if necessary, using 4-L serum bottles or similar vessels, each containing 3 L of growth medium.
3. Culture temperature is not critical. Stock cultures may be maintained at 25°C in environmental chambers with cultures of other organisms if the illumination is adequate (continuous "cool-white" fluorescent lighting of approximately 86 ± 8.6 $\mu E/m^2/s$, or 400 ft-c).
4. Cultures are mixed twice daily by hand.
5. Stock cultures can be held in the refrigerator until used to start "food" cultures, or can be transferred to new medium weekly. One-to-three milliliters of 7-day old algal stock culture, containing approximately 1.5 X 10^6 cells/mL, are transferred to each 100 mL of fresh culture medium. The inoculum should provide an initial cell density of approximately 10,000-30,000 cells/mL in the new stock cultures. Aseptic techniques should be used in maintaining the stock algal cultures, and care should be exercised to avoid contamination by other microorganisms.
6. Stock cultures should be examined microscopically weekly, at transfer, for microbial contamination. Reserve quantities of culture organisms can be maintained for 6-12 months if stored in the dark at 4°C. It is advisable to prepare new stock cultures from "starter" cultures obtained from established outside sources of organisms (see Section 6, Test Organisms) every four to six months.

13.6.15.2.2.2 Establishing and Maintaining "Food" Cultures of Algae:

1. "Food" cultures are started seven days prior to use for *Ceriodaphnia dubia* cultures and tests. Approximately 20 mL of 7-day-old algal stock culture (described in the previous paragraph), containing 1.5 X 10^6 cells/mL, are added to each liter of fresh algal culture medium (i.e., 3 L of medium in a 4-L bottle, or 18 L in a 20-L bottle). The inoculum should provide an initial cell density of approximately 30,000 cells/mL. Aseptic techniques should be used in preparing and maintaining the cultures, and care should be exercised to avoid contamination by other microorganisms. However, sterility of food cultures is not as critical as in stock cultures because the food cultures are terminated in

7-10 days. A one-month supply of algal food can be grown at one time, and stored in the refrigerator.

2. Food cultures may be maintained at 25°C in environmental chambers with the algal stock cultures or cultures of other organisms if the illumination is adequate (continuous "cool-white" fluorescent lighting of approximately 86 ± 8.6 $\mu E/m^2/s$ or 400 ft-c).
3. Cultures are mixed continuously on a magnetic stir plate (with a medium size stir bar) or in a moderately aerated separatory funnel, or are mixed twice daily by hand. If the cultures are placed on a magnetic stir plate, heat generated by the stirrer might elevate the culture temperature several degrees. Caution should be exercised to prevent the culture temperature from rising more than 2-3°C.

13.6.15.2.3 Preparing Algal Concentrate for Use as *Ceriodaphnia dubia* Food:

1. An algal concentrate containing 3.0 to 3.5 X 10^7 cells/mL is prepared from food cultures by centrifuging the algae with a plankton or bucket-type centrifuge, or by allowing the cultures to settle in a refrigerator for at least three weeks and siphoning off the supernatant.
2. The cell density (cells/mL) in the concentrate is measured with an electronic particle counter, microscope and hemocytometer, fluorometer, or spectrophotometer (see Section 14, Green Alga, *Selenastrum capricornutum* Growth Test), and used to determine the dilution (or further concentration) required to achieve a final cell count of 3.0 to 3.5 X 10^7/mL.
3. Assuming a cell density of approximately 1.5 X 10^6 cells/mL in the algal food cultures at 7 days, and 100% recovery in the concentration process, a 3-L, 7-10 day culture will provide 4.5 X 10^9 algal cells. This number of cells would provide approximately 150 mL of algal cell concentrate (1500 feedings at 0.1 mL/feeding) for use as food. This would be enough algal food for four *Ceriodaphnia dubia* tests.
4. Algal concentrate may be stored in the refrigerator for one month.

13.6.15.3 Food Quality

13.6.15.3.1 USEPA recommends Fleishmann's® yeast, Cerophyll®, trout chow, and *Selenastrum capricornutum* as the preferred *Ceriodaphnia dubia* food combination. This recommendation is based on extensive data developed by many laboratories which indicated high *Ceriodaphnia dubia* survival and reproduction in culturing and testing. The use of substitute food(s) is acceptable only after side-by-side tests are conducted to determine that the quality of the substitute food(s) is equal to the USEPA recommended food combination based on survival and reproduction of *Ceriodaphnia dubia*.

13.6.15.3.2 The quality of food prepared with newly acquired supplies of yeast, trout chow, dried cereal leaves, algae, and/or any substitute food(s) should be determined in side-by-side comparisons of *Ceriodaphnia dubia* survival and reproduction, using the new food and food of known, acceptable quality, over a seven-day period in control medium.

13.6.16 TEST ORGANISMS, DAPHNIDS, *CERIODAPHNIA DUBIA*

13.6.16.1 Cultures of test organisms should be started at least three weeks before the brood animals are needed, to ensure an adequate supply of neonates for the test. Only a few individuals are needed to start a culture because of their prolific reproduction.

13.6.16.2 Neonates used for toxicity tests must be obtained from individually cultured organisms. Mass cultures may be maintained, however, to serve as a reserve source of organisms for use in initiating individual cultures and in case of loss of individual cultures.

13.6.16.3 Starter animals may be obtained from commercial sources and may be shipped in polyethylene bottles. Approximately 40 animals and 3 mL of food are placed in a 1-L bottle filled full with culture water for shipment. Animals received from an outside source should be transferred to new culture media gradually over a period of 1-2 days to avoid mass mortality.

13.6.16.4 It is best to start the cultures with one animal, which is sacrificed after producing young, mounted on a microscope slide, and retained as a permanent slide mount to facilitate identification and permit future reference. The species identification of the stock culture should be verified by preparing slide mounts, regardless of the number of animals used to start the culture. The following procedure is recommended for making slide mounts of *Ceriodaphnia dubia* (modified from Beckett and Lewis, 1982):

1. Pipet the animal onto a watch glass.
2. Reduce the water volume by withdrawing excess water with the pipet.
3. Add a few drops of carbonated water (club soda or seltzer water) or 70% ethanol to relax the specimen so that the post-abdomen is extended. (Optional: with practice, extension of the postabdomen may be accomplished by putting pressure on the cover slip).
4. Place a small amount (one to three drops) of mounting medium on a glass microscope slide. The recommended mounting medium is CMCP-9/10 Medium[1], prepared by mixing two parts of CMCP-9 with one part of CMCP-10 stained with enough acid fuchsin dye to color the mixture a light pink. For more viscosity and faster drying, CMC-10 stained with acid fuchsin may be used.
5. Using forceps or a pipet, transfer the animal to the drop of mounting medium on the microscope slide.
6. Cover with a 12 mm round cover slip and exert minimum pressure to remove any air bubbles trapped under the cover slip. Slightly more pressure will extend the postabdomen.
7. Allow mounting medium to dry.
8. Make slide permanent by placing varnish around the edges of the coverslip.

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[1] CMCP-9, CMCP-10 and Acid Fuchsin are available from Polysciences, Inc., Paul Valley Industrial Park, Warrington, PA, 18976, 215-343-6484. Neonates from mass cultures are not to be used directly in toxicity tests (see Subsection 13.10.2.3).

9. Identify to species (see Pennak, 1978; Pennak, 1989; and Berner, 1986).
10. Label with waterproof ink or diamond pencil.
11. Store for permanent record.

13.6.16.5 Mass Culture

13.6.16.5.1 Mass cultures are used only as a "backup" reservoir of organisms.

13.6.16.5.2 One-liter or 2-L glass beakers, crystallization dishes, "battery jars," or aquaria may be used as culture vessels. Vessels are commonly filled to three-fourths capacity. Cultures are fed daily. Four or more cultures are maintained in separate vessels and with overlapping ages to serve as back-up in case one culture is lost due to accident or other unanticipated problems, such as low DO concentrations or poor quality of food or laboratory water.

13.6.16.5.3 Mass cultures which will serve as a source of brood organisms for individual culture should be maintained in good condition by frequent renewal with new culture medium at least twice a week for two weeks. At each renewal, the adult survival is recorded, and the offspring and the old medium are discarded. After two weeks, the adults are also discarded, and the culture is re-started with neonates in fresh medium. Using this schedule, 1-L cultures will produce 500 to 1000 neonate *Ceriodaphnia dubia* each week.

13.6.16.6 Individual Culture

13.6.16.6.1 Individual cultures are used as the immediate source of neonates for toxicity tests.

13.6.16.6.2 Individual organisms are cultured in 15 mL of culture medium in 30-mL (1 oz) plastic cups or 30-mL glass beakers. One neonate is placed in each cup. It is convenient to place the cups in the same type of board used for toxicity tests (see Figure 1).

13.6.16.6.3 Organisms are fed daily (see Subsection 13.6.16.9) and are transferred to fresh medium a minimum of three times a week, typically on Monday, Wednesday, and Friday. On the transfer days, food is added to the new medium immediately before or after the organisms are transferred.

13.6.16.6.4 To provide cultures of overlapping ages, new boards are started weekly, using neonates from adults which produce at least eight young in their third or fourth brood. These adults can be used as sources of neonates until 14 days of age. A minimum of two boards are maintained concurrently to provide backup supplies of organisms in case of problems.

13.6.16.6.5 Cultures which are properly maintained should produce at least 20 young per adult in three broods (seven days or less). Typically, 60 adult females (one board) will produce more than the minimum number of neonates (120) required for two tests.

13.6.16.6.6 Records should be maintained on the survival of brood organisms and number of offspring at each renewal. Greater than 20% mortality of

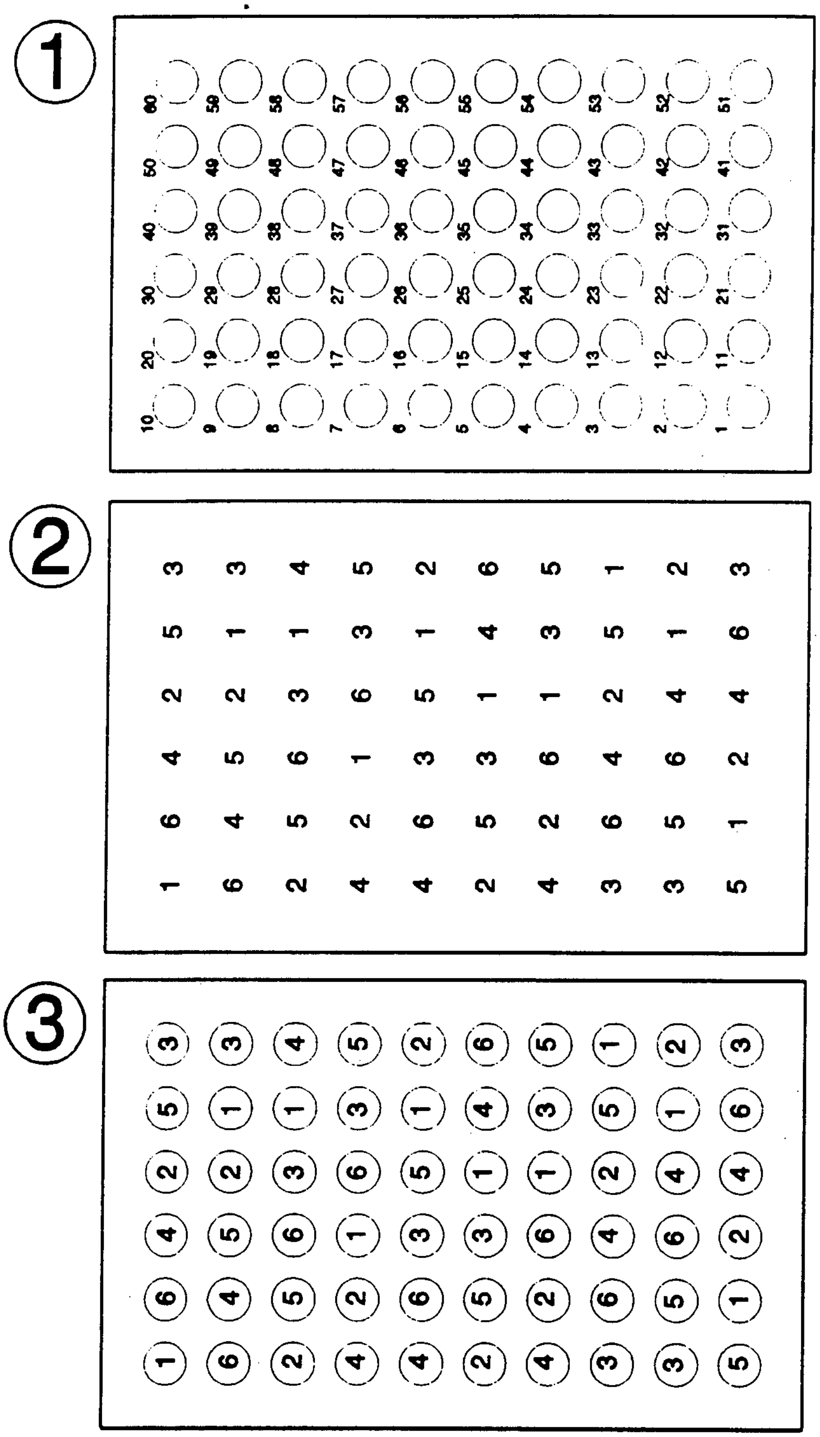

Figure 1. Examples of a test board and randomizing template: (1) test board with positions for six columns of ten replicate test chambers, (2) randomizing template prepared by throwing a single die for each position in each row across the board, and (3) test board placed on the template for the purpose of assigning the position of treatment solutions (cups) within each row on the board.

adults, or less than an average of 20 young per female would indicate problems, such as poor quality of culture media or food. Cultures that do not meet these criteria should not be used as a source of test organisms.

13.6.16.7 Culture Medium

13.6.16.7.1 Moderately hard synthetic water prepared using MILLIPORE MILLI-Q® or equivalent deionized water and reagent grade chemicals or 20% DMW is recommended as a standard culture medium (see Section 7, Dilution Water).

13.6.16.8 Culture Conditions

13.6.16.8.1 The daphnid, *Ceriodaphnia dubia*, should be cultured at a temperature of 25 ± 1°C.

13.6.16.8.2 Day/night cycles prevailing in most laboratories will provide adequate illumination for normal growth and reproduction. A photoperiod of 16-h of light and 8-h of darkness is recommended. Light intensity should be 10-20 $\mu E/m^2/s$ or 50 to 100 ft-c.

13.6.16.8.3 Clear, double-strength safety glass or 6 mm plastic panels are placed on the culture vessels to exclude dust and dirt, and reduce evaporation.

13.6.16.8.4 The organisms are delicate and should be handled as carefully and as little as possible so that they are not unnecessarily stressed. They are transferred with a pipet of approximately 2-mm bore, taking care to release the animals under the surface of the water. Any organism that is injured during handling should be discarded.

13.6.16.9 Food and Feeding

13.6.16.9.1 Feeding the proper amount of the right food is extremely important in *Ceriodaphnia dubia* culturing. The key is to provide sufficient nutrition to support normal reproduction without adding excess food which may reduce the toxicity of the test solutions, clog the animal's filtering apparatus, or greatly decrease the DO concentration and increase mortality. A combination of Yeast, CEROPHYLL®, and Trout chow (YCT), along with the unicellular green alga, *Selenastrum capricornutum*, will provide suitable nutrition if fed daily.

13.6.16.9.2 Other algal species (such as *S. minutum* or *Chlamydomonas reinhardti*), other substitute food combinations (such as Flake Fish Food), or different feeding rates may be acceptable as long as performance criteria are met and side-by-side comparison tests confirm acceptable quality (see Subsection 13.6.15.3).

13.6.16.9.3 Cultures should be fed daily to maintain the organisms in optimum condition so as to provide maximum reproduction. Stock cultures which are stressed because they are not adequately fed may produce low numbers of young, large numbers of males, and/or ephippial females. Also, their offspring may produce few young when used in toxicity tests.

13.6.16.9.4 Feed as follows:

1. If YCT is frozen, remove a bottle of food from the freezer 1 h before feeding time, and allow to thaw.
2. YCT food mixture and algal concentrates should both be thoroughly mixed by shaking before dispensing.
3. Mass cultures are fed daily at the rate of 7 mL YCT and 7 mL algae concentrate/L culture.
4. Individual cultures are fed at the rate of 0.1 mL YCT and 0.1 mL algae concentrate per 15 mL culture.
5. Return unused YCT food mixture and algae concentrate to the refrigerator. Do not re-freeze YCT. Discard unused portion after two weeks.

13.6.16.10 It is recommended that chronic toxicity tests be performed monthly with a reference toxicant. Daphnid, *Ceriodaphnia dubia*, neonates less than 24 h old, and all within 8 h of the same age are used to monitor the chronic toxicity of the reference toxicant to the *Ceriodaphnia dubia* produced by the culture unit (see Section 4, Quality Assurance).

13.6.16.11 Record Keeping

13.6.16.11.1 Records, kept in a bound notebook, include (1) source of organisms used to start the cultures, (2) type of food and feeding times, (3) dates culture were thinned and restarted, (4) rate of reproduction in individual cultures, (5) daily observations of the condition and behavior of the organisms in the cultures, and (6) dates and results of reference toxicant tests performed (see Section 4, Quality Assurance).

13.7 EFFLUENT AND RECEIVING WATER COLLECTION, PRESERVATION, AND STORAGE

13.7.1 See Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests.

13.8 CALIBRATION AND STANDARDIZATION

13.8.1 See Section 4, Quality Assurance.

13.9 QUALITY CONTROL

13.9.1 See Section 4, Quality Assurance.

13.10 TEST PROCEDURES

13.10.1 TEST SOLUTIONS

13.10.1.1 Receiving Waters

13.10.1.1.1 The sampling point is determined by the objectives of the test. Receiving water toxicity is determined with samples used directly as collected or after samples are passed through a 60 μm NITEX® filter and compared without dilution, against a control. For a test consisting of single receiving water and control, approximately 600 mL of sample would be required for each test, assuming 10 replicates of 15 mL, and sufficient additional sample for chemical analysis.

13.10.1.2 Effluents

13.10.1.2.1 The selection of the effluent test concentrations should be based on the objectives of the study. A dilution factor of 0.5 is commonly used. A dilution factor of 0.5 provides precision of ± 100%, and testing of concentrations between 6.25% and 100% effluent using only five effluent concentrations (6.25%, 12.5%, 25%, 50%, and 100%). Improvements in precision decline rapidly if the dilution factor is increased beyond 0.5, and precision declines rapidly if a smaller dilution factor is used. **Therefore, USEPA recommends the use of the ≥ 0.5 dilution factor.**

13.10.1.2.2 If the effluent is known or suspected to be highly toxic, a lower range of effluent concentrations should be used (such as 25%, 12.5%, 6.25%, 3.12%, and 1.56%). If a high rate of mortality is observed during the first 1 to 2 h of the test, additional dilutions should be added at the lower range of effluent concentrations.

13.10.1.2.3 The volume of effluent required for daily renewal of 10 replicates per concentration, each containing 15 mL of test solution, with a dilution series of 0.5, is approximately 1 L/day. A volume of 15 mL of test solution is adequate for the organisms, and will provide a depth in which it is possible to count the animals under a stereomicroscope with a minimum of re-focusing. Ten test chambers are used for each effluent dilution and for the control. Sufficient test solution (approximately 550 mL) is prepared at each effluent concentration to provide 400 mL additional volume for chemical analyses at the high, medium, and low test concentrations.

13.10.1.2.4 Tests should begin as soon as possible, preferably within 24 h of sample collection. The maximum holding time following retrieval of the sample from the sampling device should not exceed 36 h for off-site toxicity tests unless permission is granted by the permitting authority. In no case should the sample be used in a test more than 72 h after sample collection (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests).

13.10.1.2.5 Just prior to test initiation (approximately one h) the temperature of sufficient quantity of the sample to make the test solutions should be adjusted to the test temperature and maintained at that temperature during the preparation of the test solutions.

13.10.1.2.6 The DO of the test solutions should be checked prior to test initiation. If any of the solutions are supersaturated with oxygen or any solution has a DO concentration below 4.0 mg/L, all the solutions and the control must be gently aerated.

13.10.1.3 Dilution Water

13.10.1.3.1 Dilution water may be uncontaminated receiving water, a standard synthetic (reconstituted) water, or some other uncontaminated natural water (see Section 7, Dilution Water).

13.10.2 START OF THE TEST

13.10.2.1 Label the test chambers with a marking pen. Use of color-coded tape to identify each treatment and replicate is helpful. A minimum of five effluent concentrations and a control are used for each effluent test. Each treatment (including the control) must have ten replicates.

13.10.2.2 The test solutions can be randomly assigned to a board using a template (Figure 1) or by using a table of random numbers (see Appendix A). When using the randomized block design, test chambers are randomized only once, at the beginning of the test. A number of different templates should be prepared, so that the same template is not used for every test.

13.10.2.3 Neonates less than 24 h old, and all within 8 h of the same age, are required to begin the test. The neonates are obtained from individual cultures using brood boards, as described above (see Section 6, Test Organisms). Neonates are taken only from adults that have eight or more young in their third or subsequent broods. These adults can be used as brood stock until they are 14 days old. If the neonates are held more than one or two hours before using in the test, they should be fed (0.1 mL YCT and 0.1 mL algal concentrate/15 mL of media).

13.10.2.4 Ten brood cups, each with 8 or more young, are randomly selected from a brood board for use in setting up a test. To start the test, one neonate from the first brood cup is transferred to each of the six test chambers in the first row on the test board (Figure 1). One neonate from the second brood cup is transferred to each of the six test chambers in the second row on the test board. This process is continued until each of the 60 test chambers contains one neonate.

13.10.2.4.1 The cups and test chambers may be placed on a light table to facilitate counting the neonates. However, care must be taken to avoid temperature increase due to heat from the light table.

13.10.2.5 This blocking procedure allows the performance of each female to be tracked. If a female produces one weak offspring or male, the likelihood of producing all weak offspring or all males is greater. By using this known parentage technique, poor performance of young from a given female can be omitted from all concentrations.

13.10.3 LIGHT, PHOTOPERIOD, AND TEMPERATURE

13.10.3.1 The light quality and intensity should be at ambient laboratory levels, approximately 10-20 $\mu E/m^2/s$, or 50 to 100 ft-c, with a photoperiod of 16 h of light and 8 h of darkness.

13.10.3.2 It is critical that the test water temperature be maintained at 25 ± 1°C to obtain three broods in seven days.

13.10.4 DISSOLVED OXYGEN (DO) CONCENTRATION

13.10.4.1 Aeration may affect the toxicity of effluents and should be used only as a last resort to maintain satisfactory DO concentrations. The DO concentrations should be measured in the new solutions at the start of the test (Day 0) and before daily renewal of the test solutions on subsequent days. The DO concentration should not fall below 4.0 mg/L (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests). Aeration is generally not practical during the daphnid, *Ceriodaphnia dubia*, test. If the DO in the effluent and/or dilution water is low, aerate gently before preparing the test solutions. The aeration rate should not exceed 100 bubbles/min using a pipet with an orifice of approximately 1.5 mm, such as a 1 ml KIMAX® serological pipet, No. 37033, or equivalent. Care should be taken to ensure that turbulence resulting from aeration does not cause undue physical stress to the organisms.

13.10.5 FEEDING

13.10.5.1 The organisms are fed when the test is initiated, and daily thereafter. Food is added to the fresh medium immediately before or immediately after the adults are transferred. Each feeding consists of 0.1 mL YCT and 0.1 mL *Selenastrum capricornutum* concentrate/15 mL test solution (0.1 mL of algal concentrate containing 3.0-3.5 X 10^7 cells/mL will provide 2-2.3 X 10^5 cells/mL in the test chamber).

13.10.5.2 The YCT and algal suspension can be added accurately to the test chambers by using automatic pipettors, such as Gilson, Eppendorf, Oxford, or equivalent.

13.10.6 OBSERVATIONS DURING THE TEST

13.10.6.1 Routine Chemical and Physical Determinations

13.10.6.1.1 DO is measured at the beginning and end of each 24-h exposure period in at least one test chamber at each test concentration and in the control.

13.10.6.1.2 Temperature and pH are measured at the end of each 24-h exposure period in at least one test chamber at each test concentration and in the control. Temperature should be monitored continuously or observed and recorded daily for at least two locations in the environmental control system or the samples. Temperature should be measured in sufficient number of test vessels at least at the end of the test to determine the temperature variation in the environmental chamber.

13.10.6.1.3 The pH is measured in the effluent sample each day before new test solutions are made.

13.10.6.1.4 Conductivity, alkalinity and hardness are measured in each new sample (100% effluent or receiving water) and in the control.

13.10.6.1.5 Record the data on data sheet (Figure 2).

13.10.6.2 Routine Biological Observations

13.10.6.2.1 Three or four broods are usually obtained in the controls in a 7-day test conducted at 25 ± 1°C. A brood is a group of offspring released from the female over a short period of time when the carapace is discarded during molting. In the controls, the first brood of two-to-five young is usually released on the third or fourth day of the test. Successive broods are released every 30 to 36 h thereafter. The second and third broods usually consist of eight to 20 young each. The total number of young produced by a healthy control organism in three broods often exceeds 30 per female.

13.10.6.2.2 The release of a brood may be inadvertently interrupted during the daily transfer of organisms to fresh test solutions, resulting in a split in the brood count between two successive days. For example, four neonates of a brood of five might be released on Day 3, just prior to test solution renewal, and the fifth released just after renewal, and counted on Day 4. Partial broods, released over a two-day period, should be counted as one brood.

13.10.6.2.3 Each day, the live adults are transferred to fresh test solutions, and the numbers of live young are recorded (see data form, Figure 3). The young can be counted with the aid of a stereomicroscope with substage lighting. Place the test chambers on a light box over a strip of black tape to aid in counting the neonates. The young are discarded after counting.

13.10.6.2.4 Some of the effects caused by toxic substances include, (1) a reduction in the number of young produced, (2) young may develop in the brood pouch of the adults, but may not be released during the exposure period, and (3) partially or fully developed young may be released, but are all dead at the end of the 24-h period. Such effects should be noted on the data sheets (Figure 3).

13.10.6.2.5 Protect the daphnids, *Ceriodaphnia dubia*, from unnecessary disturbance during the test by carrying out the daily test observations, solution renewals, and transfer of females carefully. Make sure the females remain immersed during the performance of these operations.

13.10.7 DAILY PREPARATION OF TEST CHAMBERS

13.10.7.1 The test is started (Day 0) with new disposable polystyrene cups or precleaned 30-mL borosilicate glass beakers that are labeled and color-coded with tape. Each following day, a new set of plastic cups or precleaned glass beakers is prepared, labeled, and color-coded with tape similar to the original set. New solutions are placed in the new set of test chambers, and the test organisms are transferred from the original test chambers to the new ones with corresponding labels and color-codes. Each day, previously used glass beakers are recleaned (see Section 5, Facilities, Equipment, and Supplies) for the following day, and previously used plastic cups are discarded.

13.10.8 TEST SOLUTION RENEWAL

13.10.8.1 Freshly prepared solutions are used to renew the test daily. For on-site toxicity studies, fresh effluent or receiving water samples should be collected daily, and no more than 24 h should elapse between collection of the samples and their use in the tests (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests). For off-site tests, a minimum of three samples are collected, preferably on days one, three, and five. No more than 36 h should elapse between collection of the sample and the first use in the test. Maintain the samples in the refrigerator at 4°C until used.

13.10.8.2 New test solutions are prepared daily, and the test organisms are transferred to the freshly prepared solutions using a small-bore (2 mm) glass or polyethylene dropper or pipet. The animals are released under the surface of the water so that air is not trapped under the carapace. Organisms that are dropped or injured are discarded.

13.10.9 TERMINATION OF THE TEST

13.10.9.1 Tests should be terminated when 60% of the control organisms have produced their third brood, or at the end of 8 days, whichever occurs first. Because of the rapid rate of development of *Ceriodaphnia dubia*, at test termination all observations on organism survival and numbers of offspring should be completed within two hours. An extension of more than a few hours in the test period would be a significant part of the brood production cycle of the animals, and could result in additional broods.

13.10.9.2 Count the young, conduct required chemical measurements, and complete the data sheets (Figure 3).

13.10.9.3 Any animal not producing young should be examined to determine if it is a male (Berner, 1986). In most cases, the animal will need to be placed on a microscope slide before examining (see Subsection 13.6.16.4).

13.11 SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA

13.11.1 A summary of test conditions and test acceptability criteria is presented in Table 3.

13.12 ACCEPTABILITY OF TEST RESULTS

13.12.1 For the test results to be acceptable, at least 80% of the control organisms must survive, and 60% of surviving adults in the controls must have had at least three broods, with an average total number of 15 or more offspring per surviving adult.

TABLE 3. SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR DAPHNID, *CERIODAPHNIA DUBIA*, SURVIVAL AND REPRODUCTION TOXICITY TESTS WITH EFFLUENTS AND RECEIVING WATERS

1. Test type:	Static renewal
2. Temperature (°C):	25 ± 1°C
3. Light quality:	Ambient laboratory illumination
4. Light intensity:	10-20 $\mu E/m^2/s$, or 50-100 ft-c (ambient laboratory levels)
5. Photoperiod:	16 h light, 8 h dark
6. Test chamber size:	30 mL (minimum)
7. Test solution volume:	15 mL (minimum)
8. Renewal of test solutions:	Daily
9. Age of test organisms:	Less than 24 h; and all released within a 8-h period
10. No. neonates per test chamber:	1
11. No. replicate test chambers per concentration:	10
12. No. neonates per test concentration:	10
13. Feeding regime:	Feed 0.1 mL each of YCT and algal suspension per test chamber daily
14. Cleaning:	Use freshly cleaned glass beakers or new plastic cups daily
15. Aeration:	None
16. Dilution water:	Uncontaminated source of receiving or other natural water, synthetic water prepared using MILLIPORE MILLI-Q® or equivalent deionized water and reagent grade chemicals or DMW (see Section 7, Dilution Water)

TABLE 3. SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR DAPHNID, *CERIODAPHNIA DUBIA*, SURVIVAL AND REPRODUCTION TOXICITY TESTS WITH EFFLUENTS AND RECEIVING WATERS (CONTINUED)

17. Test concentrations:	Effluents: Minimum of 5 and a control Receiving Water: 100% receiving water or minimum of 5 and a control
18. Dilution factor:	Effluents: ≥ 0.5 Receiving Waters: None or ≥ 0.5
19. Test duration:	Until 60% of surviving control organisms have three broods (maximum test duration 8 days)
20. Endpoints:	Survival and reproduction
21. Test acceptability criteria:	80% or greater survival and an average of 15 or more young per surviving female in the control solutions. 60% of surviving control organisms must produce three broods.
22. Sampling requirements:	For on-site tests, samples collected daily, and used within 24 h of the time they are removed from the sampling device. For off-site tests, a minimum of three samples collected on days one, three, and five with a maximum holding time of 36 h before first use (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests, Subsection 8.5.4)
22. Sample volume required:	1 L/day

APPENDIX A-19

Procedure for Toxicity using Amphipods (*Hyalella azteca*):

EPA Method 100.1

Section 11
Test Method 100.1
Hyalella azteca 10-d Survival Test for Sediments

11.1 Introduction

11.1.1 *Hyalella azteca* (Saussure) have many desirable characteristics of an ideal sediment toxicity testing organism including relative sensitivity to contaminants associated with sediment, short generation time, contact with sediment, ease of culture in the laboratory, and tolerance to varying physico-chemical characteristics of sediment. Their response has been evaluated in interlaboratory studies and has been confirmed with natural benthos populations. Many investigators have successfully used *H. azteca* to evaluate the toxicity of freshwater sediments (e.g., Nebeker et al., 1984a; Borgmann and Munwar, 1989; Ingersoll and Nelson, 1990; Ankley et al., 1991a, Ankley et al., 1991b; Burton et al., 1989; Winger and Lasier, 1993; Kemble et al., 1994). *H. azteca* has been used for a variety of sediment assessments (Ankley et al., 1991; West et al., 1993; Hoke et al., 1994; West et al., 1994; and Hoke et al., 1994). *Hyalella azteca* can also be used to evaluate the toxicity of estuarine sediments (up to 15 ‰ salinity; Nebeker and Miller, 1988; Roach et al., 1992, Winger et al., 1993). Endpoints typically monitored in sediment toxicity tests with *H. azteca* include survival and growth.

11.1.2 A specific test method for conducting a 10-d sediment toxicity test is described in Section 11.2 for *H. azteca*. Methods outlined in Appendix A and the literature cited in Table A.2 were used for developing test method 100.1. Results of tests using procedures different from the procedures described in Section 11.2 may not be comparable, and these different procedures may alter contaminant bioavailability. Comparison of results obtained using modified versions of these procedures might provide useful information concerning new concepts and procedures for conducting sediment tests with aquatic organisms. If tests are conducted with procedures different from the procedures described in this manual, additional tests are required to determine comparability of results (Section 1.3).

11.2 Recommended Test Method for Conducting a 10-d Sediment Toxicity Test with *Hyalella azteca*

11.2.1 Recommended conditions for conducting a 10-d sediment toxicity test with *H. azteca* are summarized in Table 11.1. A general activity schedule is outlined in Table 11.2. Decisions concerning the various aspects of experimental design, such as the number of treatments, number of test chambers/treatment, and water-quality characteristics should be based on the purpose of the test and the methods of data analysis (Section 14). The number of replicates and concentrations tested depends in part on the significance level selected and the type of statistical analysis. When variability remains constant, the sensitivity of a test increases as the number of replicates increase.

11.2.2 The recommended 10-d sediment toxicity test with *H. azteca* must be conducted at 23°C with a 16L:8D photoperiod at an illuminance of about 500 to 1000 lux (Table 11.1). Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Ten 7- to 14-d old amphipods are used to start a test. The number of replicates/treatment depends on the objective of the test. Eight replicates are recommended for routine testing (Section 14). Amphipods in each test chamber are fed 1.5 mL of YCT food daily (Appendix C). Each chamber receives 2 volume additions/d of overlying water. Water renewals may be manual or automated, and Appendix B describes water-renewal systems that can be used to deliver overlying water. Overlying water can be culture water, well water, surface water, site water, or reconstituted water. For site-specific evaluations, the characteristics of the overlying water should be as similar as possible to the site where sediment is collected. Requirements for test acceptability are summarized in Table 11.3.

11.3 General Procedures

11.3.1 ***Sediment Into Test Chambers:*** The day before the sediment test is started (Day -1) each sediment should be thoroughly mixed and added to the test chambers (Section 8.3.1). Sediment should be visually inspected to judge the degree of homogeneity. Excess water on the surface of the sediment can indicate separation of solid and liquid components. If a quantitative measure of homogeneity is required, replicate subsamples should be taken from the sediment batch and analyzed for TOC, chemical concentrations, and particle size.

11.3.1.1 Each test chamber should contain the same amount of sediment, determined either by volume or by

Table 11.1 Test Conditions for Conducting a 10-d Sediment Toxicity Test with *Hyalella azteca*

Parameter	Conditions
1. Test type:	Whole-sediment toxicity test with renewal of overlying water
[illegible] Temperature:	23 ± 1°C
3. Light quality:	Wide-spectrum fluorescent lights
4. Illuminance:	About 500 to 1000 lux
5. Photoperiod:	16L:8D
6. Test chamber:	300-mL high-form lipless beaker
7. Sediment volume:	100 mL
8. Overlying water volume:	175 mL
9. Renewal of overlying water:	2 volume additions/d; continuous or intermittent (e.g., one volume addition every 12 h)
10. Age of organisms:	7- to 14-d old at the start of the test
11. Number of organisms/chamber:	10
12. Number of replicate chambers/treatment:	Depends on the objective of the test. Eight replicates are recommended for routine testing (see Section 14)
13. Feeding:	YCT food, fed 1.5 mL daily to each test chamber
14. Aeration:	None, unless dissolved oxygen in overlying water drops below 40% of saturation
15. Overlying water:	Culture water, well water, surface water, site water, or reconstituted water
16. Test chamber cleaning:	If screens become clogged during a test, gently brush the *outside* of the screen (Appendix B)
[illegible] Overlying water quality:	Hardness, alkalinity, conductivity, pH, and ammonia at the beginning and end of a test. Temperature and dissolved oxygen daily
18. Test duration:	10 d
19. Endpoints:	Survival (growth optional)
20. Test acceptability:	Minimum mean control survival of 80% and performance-based criteria specifications outlined in Table 11.3

Table 11.2 General Activity Schedule for Conducting a Sediment Toxicity Test with *Hyalella azteca* [1]

Day	Activity
-7	Separate known-age amphipods from the cultures and place in holding chambers. Begin preparing food for the test.
-6 to -2	Feed and observe isolated amphipods, monitor water quality (e.g., temperature and dissolved oxygen).
-1	Feed and observe isolated amphipods, monitor water quality. Add sediment into each test chamber, place chambers into exposure system, and start renewing overlying water.
0	Measure total water quality (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity, ammonia). Transfer 10 7- to 14-day old amphipods into each test chamber. Release organisms under the surface of the water. Add 1.5 mL of YCT into each test chamber. Archive 20 test organisms for weight or length determination. Observe behavior of test organisms.
1 to 8	Add 1.5 mL of YCT food to each test chamber. Measure temperature and dissolved oxygen. Observe behavior of test organisms.
[illegible]	Same as Day 1. Measure total water quality.
[illegible]	Measure temperature and dissolved oxygen. End the test by collecting the amphipods with a sieve. Count survivors and set aside organisms for weight or length measurements.

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[1] Modified from Call et al., 1994

Table 11.3 Test Acceptability Requirements for a 10-d Sediment Toxicity Test with *Hyalella azteca*

A. It is recommended for conducting a 10-d test with *Hyalella azteca* that the following performance criteria be met:

1. Age of *H. azteca* at the start of the test must be between 7- to 14-d old.
2. Average survival of *H. azteca* in the control sediment must be greater than or equal to 80% at the end of the test.
3. Hardness, alkalinity, pH, and ammonia in the overlying water within a treatment should not vary by more than 50% during the test.

B. Performance-based criteria for culturing *H. azteca* include

1. Laboratories should perform monthly 96-h water-only reference-toxicity tests to assess the sensitivity of culture organisms. If reference-toxicity tests are not conducted monthly, the lot of organisms used to start a sediment test must be evaluated using a reference toxicant (Section 9.16).
2. Laboratories should track parental survival in the cultures and record this information using control charts if known-age cultures are maintained. Records should also be kept on the frequency of restarting cultures and the age of brood organisms.
3. Laboratories should record the following water-quality characteristics of the cultures at least quarterly and the day before the start of a sediment test: pH, hardness, alkalinity, and ammonia. Dissolved oxygen should be measured weekly. Temperature should be recorded daily.
4. Laboratories should characterize and monitor background contamination and nutrient quality of food if problems are observed in culturing or testing organisms.
5. Physiological measurements such as lipid content might provide useful information regarding the health of the cultures.

C. Additional requirements:

1. All organisms in a test must be from the same source.
2. It is desirable to start tests soon after collection of sediment from the field (see Section 8.2 for additional detail).
3. All test chambers (and compartments) should be identical and should contain the same amount of sediment and overlying water.
4. Negative-control sediment and appropriate solvent controls must be included in a test. The concentration of solvent used must not adversely affect test organisms.
5. Test organisms must be cultured and tested at 23°C.
6. The daily mean test temperature must be within ±1°C of the desired temperature. The instantaneous temperature must always be within ±3°C of the desired temperature.
7. Natural physico-chemical characteristics of test sediment collected from the field should be within the tolerance limits of the test organisms.

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weight. Overlying water is added to the chambers in a manner that minimizes suspension of sediment. This can be accomplished by gently pouring water along the sides of the chambers or by pouring water onto a baffle (e.g., a circular piece of Teflon® with a handle attached) placed above the sediment to dissipate the force of the water. Renewal of overlying water is started on Day -1. A test begins when the organisms are added to the test chambers (Day 0).

11.3.2 ***Renewal of overlying water:*** Renewal of overlying water is required during a test. At any particular time during the test, flow rates through any two test chambers should not differ by more than 10%. Mount and Brungs (1967) diluters have been modified for sediment testing, and other automated water delivery systems have also been used (Maki, 1977; Ingersoll and Nelson, 1990; Benoit et al., 1993; Zumwalt et al., 1994). The water-delivery system should be calibrated before a test is started to verify that the system is functioning properly. Renewal of overlying water is started on Day -1 before the addition of test organisms or food on Day 0. Appendix B describes water-renewal systems that can be used for conducting sediment tests.

11.3.2.1 In water-renewal tests with one to four volume additions of overlying water/d, water-quality characteristics generally remain similar to the inflowing water (Ingersoll and Nelson, 1990; Ankley et al., 1993); however, in static tests, water quality may change profoundly during the exposure (Shuba et al., 1978). For example, in static whole-sediment tests, the alkalinity, hardness, and conductivity of overlying water more than doubled in several treatments during a four-week exposure (Ingersoll and Nelson, 1990). Additionally, concentrations of metabolic products (e.g., ammonia) may also increase during static exposures, and these compounds can either be directly toxic to the test organisms or may contribute to the toxicity of the contaminants in the sediment. Furthermore, changes in water-quality characteristics such as hardness may influence the toxicity of many inorganic (Gauss et al., 1985) and organic (Mayer and Ellersieck, 1986) contaminants. Although contaminant concentrations are reduced in the overlying water in water-renewal tests, organisms in direct contact with sediment generally receive a substantial proportion of a contaminant dose directly from either the whole sediment or from the interstitial water.

11.3.3 ***Acclimation:*** Test organisms must be cultured and tested at 23°C. Ideally, test organisms should be cultured in the same water that will be used in testing. However, acclimation of test organisms to the test water is not required.

11.3.4 ***Placing Organisms in Test Chambers:*** Test organisms should be handled as little as possible. Amphipods should be introduced into the overlying water below the air-water interface. Test organisms can be pipetted directly into overlying water (Ankley et al., 1993). Alternatively, test organisms can be placed into 30-mL counting cups that are floated in the test chambers for 15 min before organisms are introduced into the overlying water (Ingersoll and Nelson, 1990). Length or weight should be measured on a subset of at least 20 organisms used to start the test.

11.3.5 ***Monitoring a Test:*** All chambers should be checked daily and observations made to assess test organism behavior such as sediment avoidance. However, monitoring effects on burrowing activity of test organisms may be difficult because the test organisms are often not visible during the exposure. The operation of the exposure system should be monitored daily.

11.3.5.1 ***Measurement of Overlying Water-quality Characteristics:*** Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of a test. Overlying water should be sampled just before water renewal from about 1 to 2 cm above the sediment surface using a pipet. It may be necessary to pool water samples from individual replicates. The pipet should be checked to make sure no organisms are removed during sampling of overlying water. Hardness, alkalinity, pH, conductivity, and ammonia in the overlying water with a treatment should not vary by more than 50% during a test.

11.3.5.1.1 Dissolved oxygen should be measured daily and should be between 40 and 100% saturation (ASTM, 1988a). If a probe is used to measure dissolved oxygen in overlying water, it should be thoroughly inspected between samples to make sure that organisms are not attached and should be rinsed between samples to minimize cross contamination. Aeration can be used to maintain dissolved oxygen in the overlying water above 40% saturation. Dissolved oxygen and pH can be measure directly in the overlying water with a probe.

11.3.5.1.2 Temperature should be measured at least daily in at least one test chamber from each treatment. The temperature of the water bath or the exposure chamber should be continuously monitored. The daily mean test temperature must be within ±1°C of the desired temperature. The instantaneous temperature must always be within ±3°C of the desired temperature.

1.3.6 ***Feeding:*** Without addition of food, the test organisms may starve during exposures. However, the addition of the food may alter the availability of the contaminants in the sediment (Wiederholm et al., 1987; Harkey et al., 1994). Furthermore, if too much food is added to the test chamber or if the mortality of test organisms is high, fungal or bacterial growth may develop on the sediment surface. Therefore, the amount of food added to the test chambers is kept to a minimum.

11.3.6.1 Suspensions of food should be thoroughly mixed before aliquots are taken. If excess food collects on the sediment, a fungal or bacterial growth may develop on the sediment surface, in which case feeding should be suspended for one or more days. A drop in dissolved oxygen below 40% of saturation during a test may indicate that the food added is not being consumed. Feeding should be suspended for the amount of time necessary to increase the dissolved oxygen concentration (ASTM, 1994a). If feeding is suspended in one treatment, it should be suspended in all treatments. Detailed records of feeding rates and the appearance of the sediment surface should be made daily.

11.3.7 ***Ending a Test:*** Any of the surviving amphipods in the water column or on the surface of the sediment can be pipetted from the beaker before sieving the sediment. Immobile organisms isolated from the sediment surface or from sieved material should be considered dead. Ankley et al. (1994a) recommend using a #25 sieve (710 µm mesh) to remove amphipods from sediment. Alternatively, Kemble et al. (1994) recommend sieving sediment using the following procedure: (1) pour about half of the overlying water through a #50 (300 µm) U.S. Standard mesh sieve, (2) swirl the remaining water to suspend the upper 1 cm of sediment, (3) pour this slurry through the #50 mesh sieve and wash the contents of the sieve into an examination pan, (4) rinse the coarser sediment remaining in the test chamber through a #40 (425 µm) mesh sieve and wash the contents of this second sieve into a second examination pan. Surviving test organisms can be removed from the two pans and preserved in 8% sugar formalin solution for growth measurements (Ingersoll and Nelson, 1990). NoTox® (Earth Safe Industries, Belle Mead, NJ) can be used as a substitute for formalin (Unger et al., 1993).

11.3.7.1 A consistent amount of time should be taken to examine sieved material for recovery of test organisms (e.g., 10 min/replicate). Laboratories should demonstrate that their personnel are able to recover an average of at least 90% of the organisms from whole sediment. For example, test organisms could be added to control or test sediments, and recovery could be determined after 1 h (Tomasovic et al., 1994).

11.3.8 ***Test Data:*** Survival is the primary endpoint recorded at the end of the 10-d sediment toxicity test with *H. azteca*. Measuring growth is optional; however, growth of amphipods may be a more sensitive toxicity endpoint compared to survival (Burton and Ingersoll, 1994; Kemble et al., 1994). The duration of the 10-d test started with 7- to 14-d old amphipods is not long enough to determine sexual maturation or reproductive effects.

11.3.8.1 Amphipod body length (±0.1 mm) can be measured from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface. Ingersoll and Nelson (1990) describe the use of a digitizing system and microscope to measure lengths of *H. azteca*. Antennal segment number can also be used to estimate length or weight of amphipods (E.L. Brunson, NBS, Columbia, MO, personal communication). Wet or dry weight measurements have also been used to estimate growth of *H. azteca* (ASTM, 1994a). Dry weight of amphipods should be determined by pooling all living organisms from a replicate and drying the sample at about 60 to 90°C to a constant weight. The sample is brought to room temperature in a desiccator and weighed to the nearest 0.01 mg to obtain mean weight per surviving organism per replicate.

11.4 Interpretation of Results

11.4.1 Section 14 describes general information for interpretation of test results. The following sections describe species-specific information that is useful in helping to interpret the results of sediment toxicity tests with *H. azteca*.

11.4.2 ***Age Sensitivity:*** The sensitivity of *H. azteca* appears to be relatively similar up to at least 24- to 26-d old organisms (Collyard et al., 1994). For example, the toxicity of diazinon, Cu, Cd, and Zn was similar in 96-h water-only exposures starting with 0- to 2-d old organisms through 24- to 26-d old organisms (Figure 11.1). The toxicity of alkylphenol ethoxylate (a surfactant) tended to increase with age. In general, this suggests that tests started with 7- to 14-d old amphipods would be representative of the sensitivity of *H. azteca* up to at least the adult life stage.

11.4.3 ***Grain Size:*** *Hyalella azteca* are tolerant of a wide range of substrates. Physico-chemical characteristics (e.g., grain size or TOC) of sediment were not significantly correlated to the response of *H. azteca* in toxicity tests in which organisms were fed (Section 10.1.1.8; Ankley et al., 1994a).

11.4.4 ***Isolating Organisms at the End of a Test:*** Quantitative recovery of young amphipods (e.g., 0- to 7-d old) is difficult given their small size (Figure 11.2, Tomasovic et al., 1994). Recovery of older and larger amphipods (e.g., 21-d old) is much easier. This was a primary reason for deciding to start 10-d tests with 7- to 14-d old amphipods (organisms are 17- to 24-d old at the end of the 10-d test).

11.4.5 ***Influence of Indigenous Organisms:*** Survival of *H. azteca* in 28-d tests was not reduced in the presence of oligochaetes in sediment samples (Reynoldson et al., 1994). However, growth of amphipods was reduced when high numbers of oligochaetes were placed in a sample. Therefore, it is important to determine the number and biomass of indigenous organisms in field-collected sediment in order to better interpret growth data (Reynoldson et al., 1994). Furthermore, presence of predators may also influence the response of test organisms in sediment (Ingersoll and Nelson, 1990).

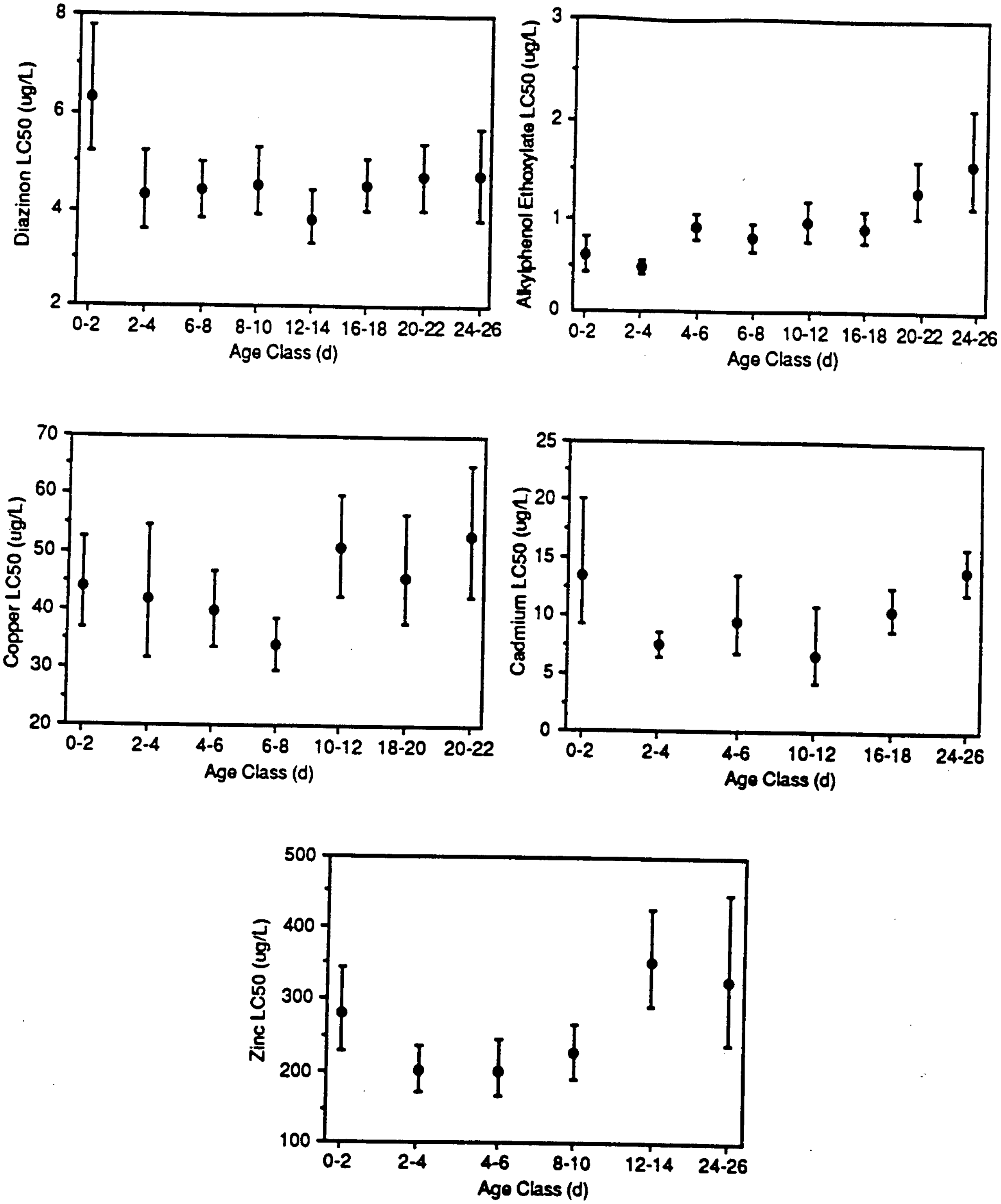


Figure 11.1 Lifestage sensitivity of *Hyalella azteca* in 96-h water-only exposures.

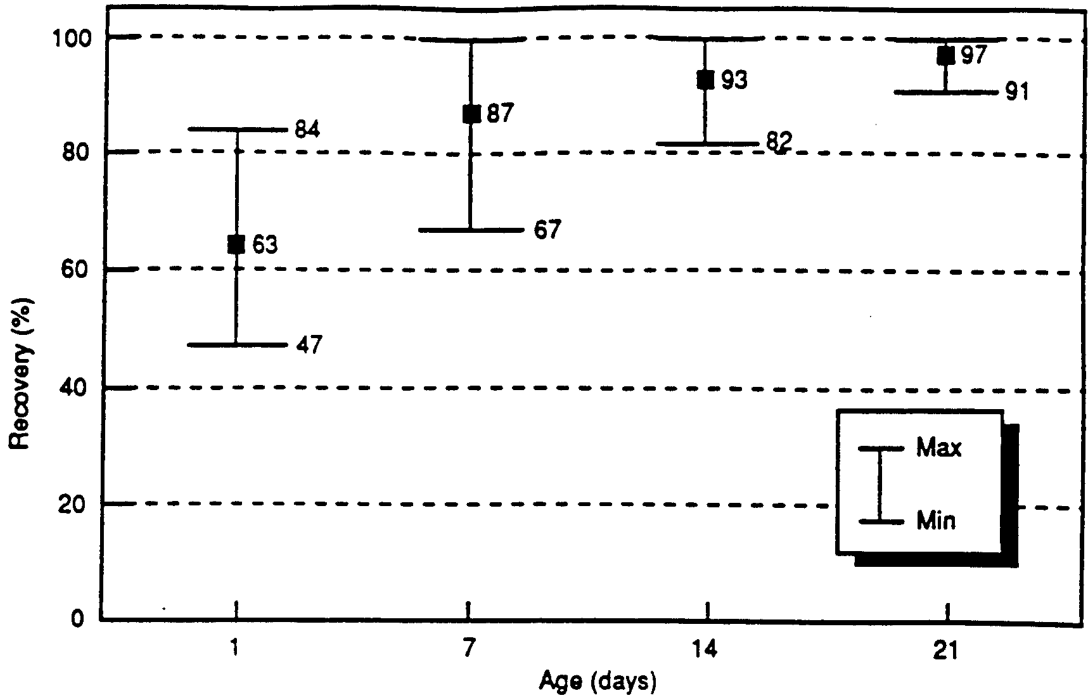


Figure 11.2 **Average recovery of different age *Hyalella azteca* from sediment by 7 individuals.**

APPENDIX A-20

Procedure for Toxicity using Midge (*Chironomus tentans*):

EPA Method 100.2

Section 12
Test Method 100.2
Chironomus tentans 10-d Survival and Growth Test for Sediments

12.1 Introduction

12.1.1 *Chironomus tentans* (Fabricius) have many desirable characteristics of an ideal sediment toxicity testing organism including relative sensitivity to contaminants associated with sediment, contact with sediment, ease of culture in the laboratory, tolerance to varying physico-chemical characteristics of sediment, and short generation time. Their response has been evaluated in interlaboratory studies and has been confirmed with natural benthos populations. Many investigators have successfully used *C. tentans* to evaluate the toxicity of freshwater sediments (e.g., Wentsel et al., 1977; Nebeker et al., 1984a; Nebeker et al., 1988; Adams et al., 1985; Giesy et al., 1988; Hoke et al., 1990; West et al., 1993; Ankley et al., 1993; Ankley et al., 1994a; Ankley et al.,1994b). *C. tentans* has been used for a variety of sediment assessments (West et al., 1993; Hoke et al., 1994; West et al., 1994; Ankley et al., 1994c; and Hoke et al., 1994). Endpoints typically monitored in sediment toxicity tests with *C. tentans* include survival and growth (ASTM, 1994a).

12.1.2 A specific test method for conducting a 10-d sediment toxicity test is described in Section 12.2 for *C. tentans*. Methods outlined in Appendix A and the literature cited in Table A.3 were used for developing test method 100.2. Results of tests using procedures different from the procedures described in Section 12.2 may not be comparable and these different procedures may alter contaminant bioavailability. Comparison of results obtained using modified versions of these procedures might provide useful information concerning new concepts and procedures for conducting sediment tests with aquatic organisms. If tests are conducted with procedures different from the procedures described in this manual, additional tests are required to determine comparability of results (Section 1.3).

12.2 Recommended Test Method for Conducting a 10-d Sediment Toxicity Test with *Chironomus tentans*

2.2.1 Recommended conditions for conducting a 10-d sediment toxicity test with *C. tentans* are summarized in Table 12.1. A general activity schedule is outlined in Table 12.2. Decisions concerning the various aspects of experimental design, such as the number of treatments, number of test chambers/treatment, and water-quality characteristics should be based on the purpose of the test and the methods of data analysis (Section 14). The number of replicates and concentrations tested depends in part on the significance level selected and the type of statistical analysis. When variability remains constant, the sensitivity of a test increases as the number of replicates increases.

12.2.2 The recommended 10-d sediment toxicity test with *C. tentans* must be conducted at 23°C with a 16L:8D photoperiod at an illuminance of about 500 to 1000 lux (Table 12.1). Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Ten third-instar midges are used to start a test. All organisms must be third instar or younger with at least 50% of the organisms at third instar. The number of replicates/treatment depends on the objective of the test. Eight replicates are recommended for routine testing (see Section 14). Midges in each test chamber are fed 1.5 mL of a 4-g/L Tetrafin® suspension daily. Each chamber receives 2 volume additions/d of overlying water. Water renewals may be manual or automated, and Appendix B describes water-renewal systems that can be used to deliver overlying water. Overlying water can be culture water, well water, surface water, site water, or reconstituted water. For site-specific evaluations, the characteristics of the overlying water should be as similar as possible to the site where sediment is collected. Requirements for test acceptability are summarized in Table 12.3.

12.3 General Procedures

12.3.1 ***Sediment Into Test Chambers:*** The day before the sediment test is started (Day -1) each sediment should be thoroughly mixed and added to the test chambers (Section 8.3.1). Sediment should be visually inspected to judge the extent of homogeneity. Excess water on the surface of the sediment can indicate separation of solid and liquid components. If a quantitative measure of homogeneity is required, replicate subsamples should be taken from the sediment batch and analyzed for TOC, chemical concentrations, and particle size.

Table 12.1 Recommended Test Conditions for Conducting a 10-d Sediment Toxicity Test with *Chironomus tentans*

	Parameter	Conditions
1.	Test type:	Whole-sediment toxicity test with renewal of overlying water
2.	Temperature:	23 ± 1°C
3.	Light quality:	Wide-spectrum fluorescent lights
4.	Illuminance:	About 500 to 1000 lux
5.	Photoperiod:	16L:8D
6.	Test chamber:	high-form lipless beaker
7.	Sediment volume:	100 mL
8.	Overlying water volume:	175 mL
9.	Renewal of overlying water:	2 volume additions/d; continuous or intermittent (e.g., one volume addition every 12 h)
10.	Age of organisms:	Third instar larvae (All organisms must be third instar or younger with at least 50% of the organisms at third instar)
11.	Number of organisms/ chamber:	10
12.	Number of replicate chambers/treatment:	Depends on the objective of the test. Eight replicates are recommended for routine testing (see Section 14)
13.	Feeding:	Tetrafin® goldfish food, fed 1.5 mL daily to each test chamber (1.5 mL contains 4.0 mg of dry solids)
14.	Aeration:	None, unless dissolved oxygen in overlying water drops below 40% of saturation
15.	Overlying water:	Culture water, well water, surface water, site water, or reconstituted water
16.	Test chamber cleaning:	If screens become clogged during a test; gently brush the *outside* of the screen (Appendix B)
17.	Overlying water quality:	Hardness, alkalinity, conductivity, pH, and ammonia at the beginning and end of a test. Temperature and dissolved oxygen daily
18.	Test duration:	10 d
19.	Endpoints:	Survival and growth (dry weight)
20.	Test acceptability:	Minimum mean control survival of 70% and mean weight per surviving control organism of 0.6 mg. Performance-based criteria specifications outlined in Table 12.3

2.67/L =

12.3.1.1 Each test chamber should contain the same amount of sediment, determined either by volume or by weight. Overlying water is added to the chambers in a manner that minimizes suspension of sediment. This can be accomplished by gently pouring water along the sides of the chambers or by pouring water onto a baffle (e.g., a circular piece of Teflon with a handle attached) placed above the sediment to dissipate the force of the water. Renewal of overlying water is started on Day -1. A test begins when the organisms are added to the test chambers (Day 0).

12.3.2 ***Renewal of overlying water:*** Renewal of overlying water is required during a test. At any particular time during the test, flow rates through any two test chambers should not differ by more than 10%. Mount and Brungs (1967) diluters have been modified for sediment testing, and other automated water delivery systems have also been used (Maki, 1977; Ingersoll and Nelson, 1990; Benoit et al., 1993; Zumwalt et al., 1994). Each water-delivery system should be calibrated before a test is started to verify that the system is functioning properly. Renewal of overlying water is started on Day -1 before the addition of test organisms or food on Day 0. Appendix B describes water-renewal systems that can be used for conducting sediment tests.

12.3.2.1 In water-renewal tests with one to four volume additions of overlying water/d, water-quality characteristics generally remain similar to the inflowing water (Ingersoll and Nelson, 1990; Ankley et al., 1993); however, in static tests, water quality may change profoundly during the exposure (Shuba et al., 1978). For example, in static whole-sediment tests, the alkalinity, hardness, and conductivity of overlying water more than doubled in several treatments during a four-week exposure (Ingersoll and Nelson, 1990). Additionally, concentrations of metabolic products (e.g., ammonia) may also increase during static exposures, and these compounds can either be directly toxic to the test organisms or may

Table 12.2 General Activity Schedule for Conducting a Sediment Toxicity Test with *Chironomus tentans* [1]

Day	Activity
-14	Isolate adults for production of egg masses.
-13	Place newly deposited egg masses into hatching dishes.
-12	A larval rearing chamber is prepared with new substrate.
-11	Examine egg masses for hatching success. If egg masses have hatched, transfer first instar larvae and any remaining unhatched embryos from the crystallizing dishes into the larval rearing chamber. Feed organisms.
-10	Same as Day -11.
-9 to -2	Feed and observe midges. Measure water quality (e.g., temperature and dissolved oxygen).
-1	Add food to each larval rearing chamber and measure temperature and dissolved oxygen. Add sediment into each test chamber, place chamber into exposure system, and start renewing overlying water.
0	Measure total water quality (temperature, pH, hardness, alkalinity, dissolved oxygen, conductivity, ammonia). Remove third-instar larvae from the culture chamber substrate. Add 1.5 mL of Tetrafin® (4.0 g/L) into each test chamber. Transfer 10 larvae into each test chamber. Release organisms under the surface of the water. Archive 20 test organisms for instar determination and weight or length determination. Observe behavior of test organisms.
1 to 8	Add 1.5 mL of food to each test chamber. Measure temperature and dissolved oxygen. Observe behavior of test organisms.
9	Same as Day 1. Measure total water quality.
10	Measure temperature and dissolved oxygen. End the test by collecting the midges with a sieve. Measure weight or length of surviving larvae.

[1] Modified from Call et al., 1994

contribute to the toxicity of the contaminants in the sediment. Furthermore, changes in water-quality characteristics such as hardness may influence the toxicity of many inorganic (Gauss et al., 1985) and organic (Mayer and Ellersieck, 1986) contaminants. Although contaminant concentrations are reduced in the overlying water in water-renewal tests, organisms in direct contact with sediment generally receive a substantial proportion of a contaminant dose directly from either the whole sediment or from the interstitial water.

12.3.3 ***Acclimation:*** Test organisms must be cultured and tested at 23°C. Ideally, test organisms should be cultured in the same water that will be used in testing. However, acclimation of test organisms to the test water is not required.

12.3.4 ***Placing Organisms in Test Chambers:*** Test organisms should be handled as little as possible. Midges should be introduced into the overlying water below the air-water interface. Test organisms can be pipetted directly into overlying water (Ankley et al., 1993). Alternatively, test organisms can be placed into 30-mL counting cups that are floated in the test chambers for 15 min before organisms are introduced into the overlying water (Ingersoll and Nelson, 1990). Length or weight should be measured on a subset of at least 20 organisms used to start the test. Head capsule width of midges must be measured on this subset of test organisms to determine the instar used to start the test (Table 10.2).

12.3.5 ***Monitoring a Test:*** All chambers should be checked daily and observations made to assess test organism behavior such as sediment avoidance. However, monitoring effects on burrowing activity of test organisms may be difficult because the test organisms are often not visible during the exposure. The operation of the exposure system should be monitored daily.

12.3.5.1 *Measurement of Overlying Water-quality Characteristics:* Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of a test. Overlying water should be sampled just before water renewal from about 1 to 2 cm above the sediment surface using a pipet. It may be necessary to pool water samples from individual replicates. The pipet should be checked to make sure no organisms are removed during sampling of overlying water. Hardness, alkalinity, pH, conductivity, and ammonia in the overlying water within a treatment should not vary by more than 50% during a test.

12.3.5.1.1 Dissolved oxygen should be measured daily and should be between 40 and 100% saturation (ASTM, 1988a). If a probe is used to measure dissolved oxygen in overlying water, it should be thoroughly inspected between samples to make sure that organisms are not attached and should be rinsed between samples to minimize cross contamination. Aeration can be used to maintain dissolved oxygen in the overlying water above 40% saturation. Dissolved oxygen and pH can be measured directly in the overlying water with a probe.

12.3.5.1.2 Temperature should be measured at least daily in at least one test chamber from each treatment. The temperature of the water bath or the exposure

Table 12.3 Test Acceptability Requirements for a 10-d Sediment Toxicity Test with *Chironomus tentans*

A. It is recommended for conducting a 10-d test with *C. tentans* that the following performance criteria be met:

   1. Tests must be started with third-instar and younger larvae. At least 50% of the larvae must be in the third instar at the start of the test.
   2. Average survival of *C. tentans* in the control sediment must be greater than or equal to 70% at the end of the test.
   3. Average size of *C. tentans* in the control sediment must be at least 0.6 mg at the end of the test.
   4. Hardness, alkalinity, pH, and ammonia in the overlying water within a treatment should not vary by more than 50% during the test.

B. Performance-based criteria for culturing *C. tentans* include

   1. Laboratories should perform monthly 96-h water-only reference-toxicity tests to assess the sensitivity of culture organisms. If reference-toxicity tests are not conducted monthly, the lot of organisms used to start a sediment test must be evaluated using a reference toxicant (Section 9.16).
   2. Laboratories should keep a record of time to first emergence for each culture and record this information using control charts. Records should also be kept on the frequency of restarting cultures.
   3. Laboratories should record the following water-quality characteristics of the cultures at least quarterly and the day before the start of a sediment test: pH, hardness, alkalinity, and ammonia. Dissolved oxygen should be measured weekly. Temperature should be recorded daily.
   4. Laboratories should characterize and monitor background contamination and nutrient quality of food if problems are observed in culturing or testing organisms.
   5. Physiological measurements such as lipid content might provide useful information regarding the health of the cultures.

C. Additional requirements:

   1. All organisms in a test must be from the same source.
   2. It is desirable to start tests soon after collection of sediment from the field (see Section 8.2 for additional detail).
   3. All test chambers (and compartments) should be identical and should contain the same amount of sediment and overlying water.
   4. Negative-control sediment and appropriate solvent controls must be included in a test. The concentration of solvent used must not adversely affect test organisms.
   5. Test organisms must be cultured and tested at 23°C.
   6. The daily mean test temperature must be within ±1°C of the desired temperature. The instantaneous temperature must always be within ±3°C of the desired temperature.
   7. Natural physico-chemical characteristics of test sediment collected from the field should be within the tolerance limits of the test organisms.

chamber should be continuously monitored. The daily mean test temperature must be within ±1°C of the desired temperature. The instantaneous temperature must always be within ±3°C of the desired temperature.

12.3.6 ***Feeding:*** Without addition of food, the test organisms may starve during exposures. However, the addition of the food may alter the availability of the contaminants in the sediment (Wiederholm et al., 1987; Harkey et al., 1994). Furthermore, if too much food is added to the test chamber or if the mortality of test organisms is high, fungal or bacterial growth may develop on the sediment surface. Therefore, the amount of food added to the test chambers is kept to a minimum.

12.3.6.1 Suspensions of food should be thoroughly mixed before aliquots are taken. If excess food collects on the sediment, a fungal or bacterial growth may develop on the sediment surface, in which case feeding should be suspended for one or more days. A drop in dissolved oxygen below 40% of saturation during a test may indicate that the food added is not being consumed. Feeding should be suspended for the amount of time necessary to increase the dissolved oxygen concentration (ASTM, 1994a). If feeding is suspended in one treatment, it should be suspended in all treatments. Detailed records of feeding rates and the appearance of the sediment surface should be made daily.

12.3.7 ***Ending a Test:*** Immobile organisms isolated from the sediment surface or from sieved material should be considered dead. Ankley et al. (1994a) recommend using a #25 sieve (710 µm mesh) to remove midges from sediment. Alternatively, Kemble et al. (1994) recommend sieving sediment using the following procedure: (1) pour about half of the overlying water through a #50 (300 µm) U.S. Standard mesh sieve, (2) pour about half of the sediment through the #50 mesh sieve and wash the contents of the sieve into an examination pan, (3) rinse the coarser sediment remaining in the test chamber through a #40 (425 µm) mesh sieve and wash the contents of this second sieve into a second examination pan. Surviving midges can then be isolated from these pans. See Section 12.3.8.1 and 12.3.8.2 for the procedures for measuring weight or length of midges.

12.3.7.1 A consistent amount of time should be taken to examine sieved material for recovery of test organisms (e.g., 10 min/replicate). Laboratories should demonstrate that their personnel are able to recover an average of at least 90% of the organisms from whole sediment. For example, test organisms could be added to control sediment and recovery could be determined after 1 h (Tomasovic et al., 1994).

12.3.8 ***Test Data:*** Dry weight and survival are the endpoints measured at the end of the 10-d sediment toxicity test with *C. tentans*. The duration of the 10-d test starting with third instar larvae is not long enough to determine emergence of adults. Average size of *C. tentans* in the control sediment must be at least 0.6 mg at the end of the test (Ankley et al., 1993; ASTM, 1994b; Section 15).

12.3.8.1 Head capsule width can be measured on surviving midges at the end of the test before dry weight is determined. Dry weight of midges should be determined by pooling all living larvae from a replicate and drying the sample at about 60 to 90°C to a constant weight. The sample is brought to room temperature in a desiccator and weighed to the nearest 0.01 mg to obtain mean weights per surviving organism per replicate. Pupae or adult organisms must not be included in the sample to estimate dry weight.

12.3.8.2 Measurement of length is optional. Separate replicate beakers should be set up to sample lengths of midges at the end of an exposure. An 8% sugar formalin solution can be used to preserve samples for length measurements (Ingersoll and Nelson, 1990). NoTox® (Earth Safe Industries, Belle Mead, NJ) can be used as a substitute for formalin (Unger et al., 1993). Midge body length (±0.1 mm) can be measured from the anterior of the labrum to the posterior of the last abdominal segment (Smock, 1980). Kemble et al. (1994) photographed midges at magnification of 3.5x and measured the images using a computer-interfaced digitizing tablet. A digitizing system and microscope can also be used to measure length (Ingersoll and Nelson, 1990).

12.4 Interpretation of Results

12.4.1 Section 14 describes general information for interpretation of test results. The following sections describe species-specific information that is useful in helping to interpret the results of sediment toxicity tests with *C. tentans*.

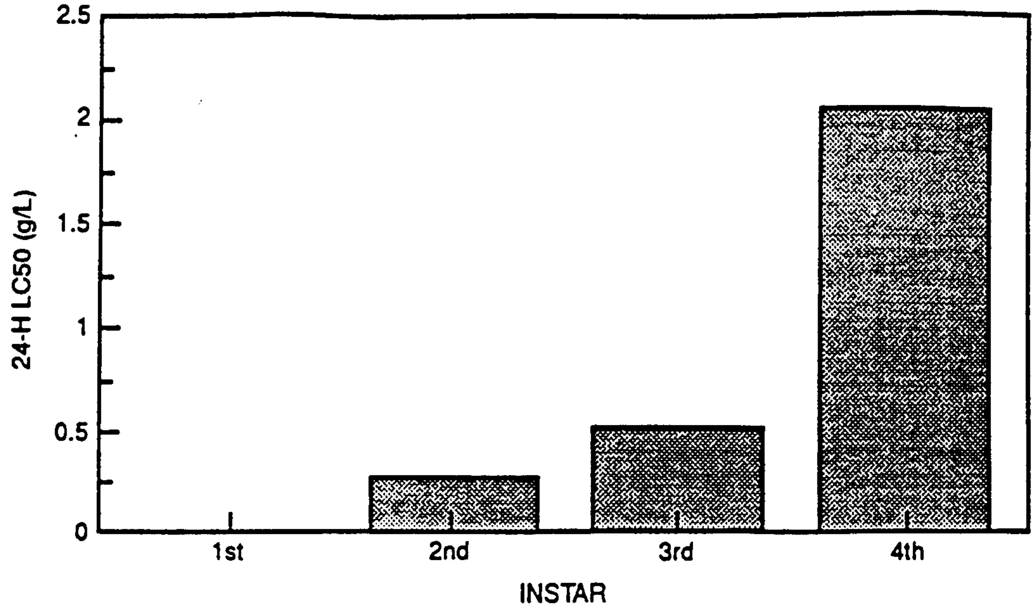
12.4.2 ***Age Sensitivity:*** Midges are perceived to be relatively insensitive organisms in toxicity assessments (Ingersoll, 1994). This conclusion is based on the practice of conducting short-term tests with 4th instar larvae in water-only exposures, a procedure that may underestimate the sensitivity of midges to toxicants. The first and second instars of chironomids are more sensitive to contaminants than the third or fourth instars. For example, first instar *C. tentans* larvae were 6 to 27 times more sensitive than 4th instar larvae to acute copper exposure (Nebeker et al., 1984b; Gauss et al., 1985; Figure 12.1) and first instar *C. riparius* larvae were 127 times more sensitive than second instar larvae to acute cadmium exposure (Williams et al., 1986b; Figure 12.1). In chronic tests with first instar larvae, midges were often as sensitive as daphnids to inorganic and organic compounds (Ingersoll et al., 1990). Sediment tests should be started with uniform age and size midges because of the dramatic differences in sensitivity of midges by age. While third instar midges are not as sensitive as younger organisms, the larger larvae are easier to handle and isolate from sediment at the end of a test.

12.4.3 ***Grain Size:*** *Chironomus tentans* are tolerant of a wide range of substrates. Physicochemical characteristics (e.g., grain size or TOC) of sediment were not significantly correlated to the survival of *C. tentans* in toxicity tests in which organisms were fed. However, linear modeling indicated that growth of *C. tentans* may have been slightly influenced by grain size distribution of the test sediments (Section 10.1.2.3; Ankley et al., 1994a). Survival of *C. tentans* was reduced below 0.91% organic matter in 10-d tests with formulated sediment (Suedel and Rodgers, 1994); however these organisms did not receive a supplemental source of nutrition.

12.4.4 ***Isolating Organisms at the End of a Test:*** Quantitative recovery of larvae at the end of a 10-d sediment test should not be a problem. The larvae are red and typically greater than 5-mm long.

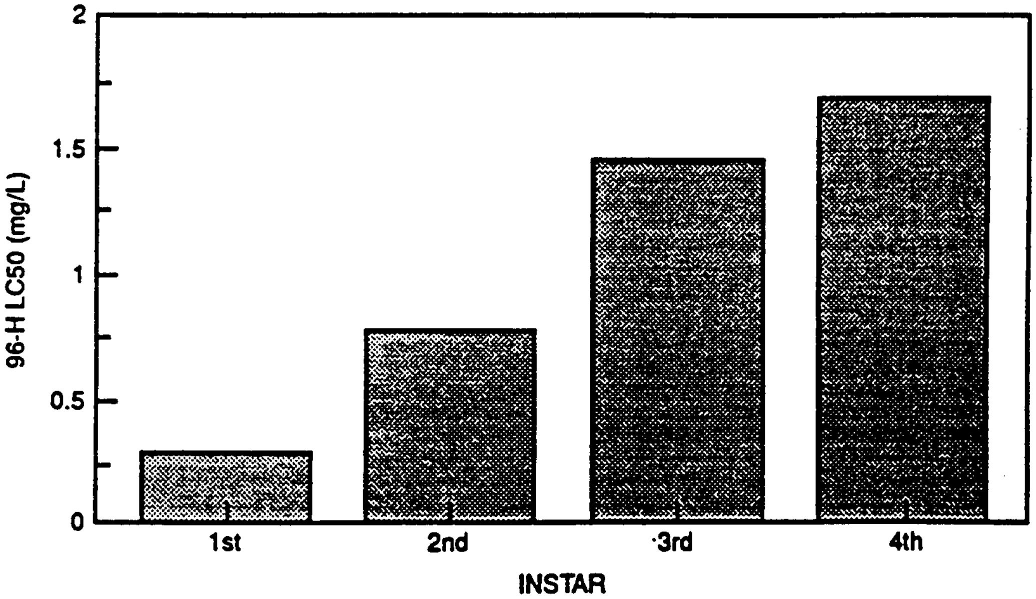
12.4.5 ***Influence of Indigenous Organisms:*** The influence of indigenous organisms on the response of *C. tentans* in sediment tests has not been reported. Survival of a closely related species, *C. riparius* was not reduced in the presence of oligochaetes in sediment samples (Reynoldson et al., 1994). However, growth of *C. riparius* was reduced when high numbers of oligochaetes were placed in a sample. Therefore, it is important to determine the number and biomass of indigenous organisms in field-collected sediment in order to better interpret growth data (Reynoldson et al., 1994). Furthermore, presence of predators may also influence the response of test organisms in sediment (Ingersoll and Nelson, 1990).

A. *Chironomus riparius*: Cadium



Williams et al. (1986)

B. *Chironomus tentans*: Copper



Nebeker et al. (1984)

Figure 12.1 Lifestage sensitivity of chironomids.

APPENDIX A-21

Lab Procedure for 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 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: 2 has been issued to holder on 12/3/96

"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.

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.)

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.

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.

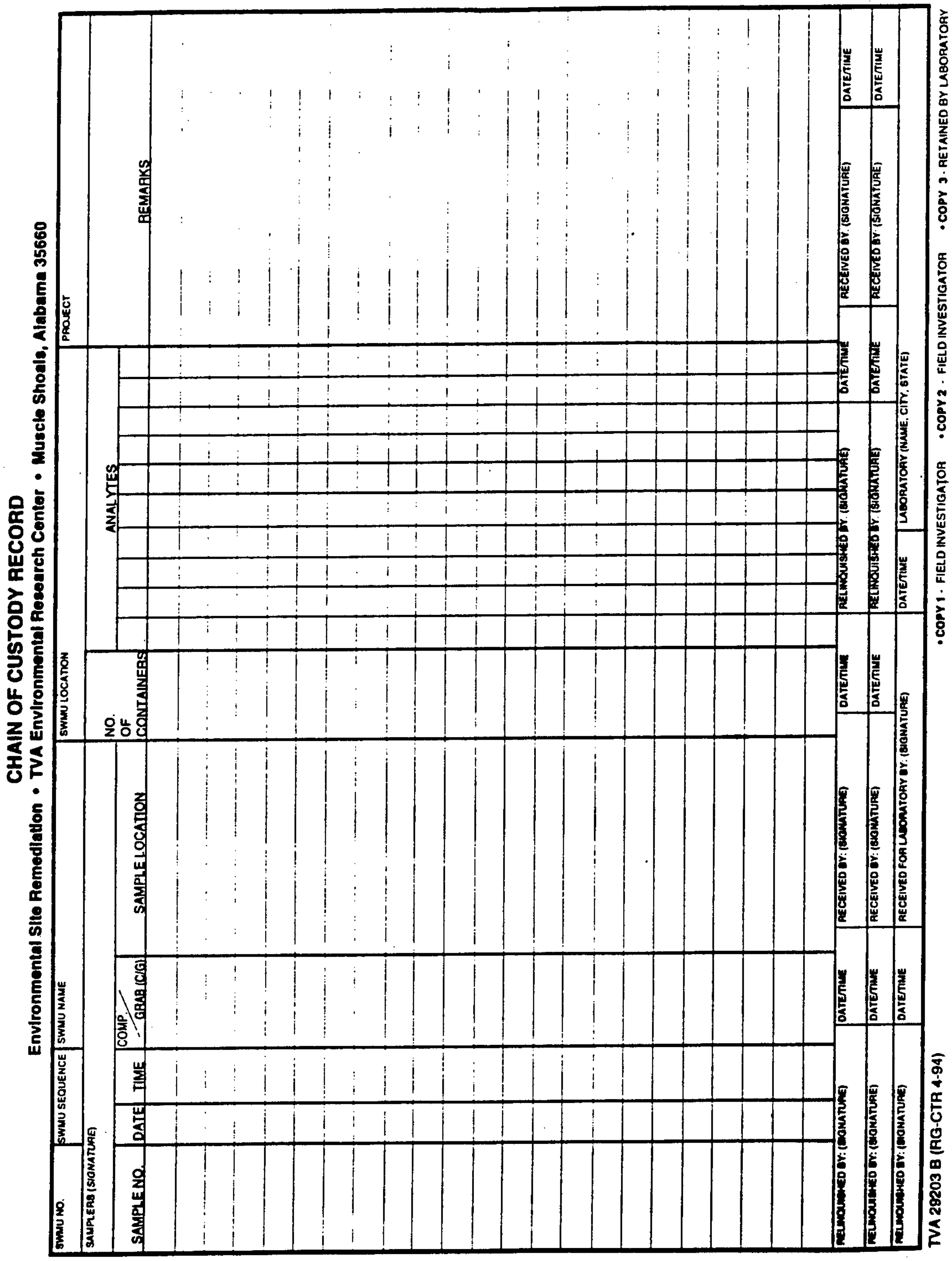
8.6 In handling samples, be aware of spills on outside of containers. Clean the exterior of containers as needed.

9.0 NOTES

None

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	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

Sample Chain of Custody
Tennessee Valley Authority
Environmental Appliations CTR-1K Muscle Shoals, AL

Project

Date of Collection

	Sample ID	Collection Time*	Number of Containers	Analyses Requested	Location*	Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						

	Signatures	**Date and Time**
Collector		
Relinquishing		
Receiving		

* These columns need not be completed if field sampling sheets containing the same information are attached.

END OF PROCEDURE