MARYLAND
DEPARTMENT OF
NATURAL RESOURCES

Martin O'Malley, Governor
Anthony G. Brown, Lt. Governor
John R. Griffin, Secretary
Eric Schwaab, Deputy Secretary

Quality Assurance Project Plan

for the
Maryland Department of Natural Resources
Chesapeake Bay
Water Quality Monitoring Program-
Chemical and Physical Properties Component
for the period July 1, 2008 - June 30, 2009

May 31, 2008

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Quality Assurance Project Plan
for the
Maryland Department of Natural Resources
Chesapeake Bay Water Quality Monitoring Program -
Chemical and Physical Properties Component
for the period July 1, 2009 - June 30, 2009

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Martin O'Malley, Governor Anthony G. Brown, Lt. Governor

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Chemical and Physical Properties Component
for the period July 1, 2007 - June 30, 2008

May 31, 2008

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PREFACE

This document is intended to describe in detail the activities conducted under the Chemical and Physical Properties Component of the Maryland Department of Natural Resources Chesapeake Bay Water Quality Monitoring Program. This is a coordinated program consisting of several components conducted in a similar manner for identical purposes in both the tributaries and mainstem of Maryland's Chesapeake Bay. This program is funded through the Maryland Department of Natural Resources and the U.S. Environmental Protection Agency.

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ACRONYMS AND ABBREVIATIONS

AA - autoanalyzer
Ag - silver
AgCl - silver chloride
AMQAW - Analytical Methods and Quality Assurance Workgroup (a workgroup of the Chesapeake Bay Program's Monitoring Subcommittee)
AP - above pycnocline
ARS - Analysis Request Sheet
Au - gold
B - bottom sample
BP - below pycnocline OR barometric pressure
C - carbon
CBP - EPA's Chesapeake Bay Program
CBPO - EPA's Chesapeake Bay Program Office
CBL - University of Maryland's Chesapeake Biological Laboratory
cm - centimeter
CMC - chlorophyll measurement computer
CSSP - Coordinated Split Sample Program
DHMH - Maryland Department of Health and Mental Hygiene
DI - de-ionized
DNR - Maryland Department of Natural Resources
DO - dissolved oxygen
DOC - dissolved organic carbon
EPA - U.S. Environmental Protection Agency
g - gram
H_2O - dihydrogen oxide (water)
H_2S - hydrogen sulfide
HCL - hydrochloric acid
Hg - mercury
L - liter
m - meter
MDE - Maryland Department of the Environment
$MgCO_3$ - magnesium carbonate
min. - minute
mg - milligram
ml - milliliter
mm - millimeter
MSU - Morgan State University
N - nitrogen
$NaHCO_3$ - sodium bicarbonate
NIST - National Institute of Standards and Technology
nm - nanometer
no. - number

NO_2 - nitrite
NO23 - nitrate + nitrite
NO_3 - nitrate
OD - optical density
P - phosphorus
PC - particulate carbon OR
PC - personal computer
PN - particulate nitrogen
PO_4 - phosphate
PP - particulate phosphorus
ppt - parts per thousand
QAO -Quality Assurance Officer (unless otherwise noted, this refers to the DNR QAO)
QAPP - Quality Assurance Project Plan
RP - replicate
R/V - research vessel
S - surface sample
SAS - Statistic Analysis System
SIF - silica
TDN - total dissolved nitrogen
TDP - total dissolved phosphorus
trib - Bay Tributary
TSS - total suspended solids
USDI - U.S. Department of the Interior
USGS - U.S. Geological Survey

ºC - degrees Celsius

1. INTRODUCTION

1.1 Background

At the completion of the U. S. Environmental Protection Agency's (EPA's) $27 million study of Chesapeake Bay, the Agency published a document entitled *Chesapeake Bay: A Framework for Action* (EPA 1983). This report strongly recommended a long-term water quality monitoring program to serve the Bay's management community by accurately describing the current state of the Bay mainstem and tidal tributaries (baseline or 'status') and detecting long-term changes (trends) resulting from human activities. Management strategies at that time were hindered by the lack of precise information about the Bay and its response to increasing or decreasing pollution.

Managers, scientists, and statisticians recognized that to establish baseline conditions and then begin to identify trends would require a multi-year effort on the order of a decade or more. Long-term data was needed to overcome the natural year-to-year variability that can obscure changes due to human activities. As the EPA study drew to a close, scientists and managers convened in workshops to formulate plans on several topics, including water quality monitoring. The monitoring workshop recommendations for chemical and physical measurements were published in the appendices of *Chesapeake Bay: A Framework for Action* (EPA 1983). The appendices described the chemical/physical monitoring plan in terms of station locations, parameters to be measured, and sampling frequency.

This Quality Assurance Project Plan (QAPP) describes Maryland's implementation of the coordinated Maryland, Virginia, and EPA Chesapeake Bay monitoring program as outlined in *Chesapeake Bay: A Framework for Action* (EPA 1983). This part of Maryland's Chesapeake Bay Water Quality Monitoring Program is known as the "Chemical and Physical Properties Component" and covers monitoring in the Maryland portion of the mainstem as well as the tidal tributaries. Other components of the water quality program measure biological and process oriented indicators of water quality; those components are not described in this document.

1.2 Objectives

The Maryland Department of Natural Resources (DNR) uses the data generated by means of the procedures in this QAPP to meet the five water quality monitoring objectives of the Chesapeake Bay Water Quality Monitoring Program:

1. Characterize the present state of the Bay mainstem and its tributaries (baseline), including spatial and seasonal variation, using key water quality indicators.

2. Determine long-term trends or changes in key water quality indicators in relation to pollution control programs.

3. Integrate the information collected in all components of the monitoring program to gain a more comprehensive understanding of water quality processes and the relationship between water quality and living resources.

4. Track the progress of management strategies to reduce nutrient pollution.

5. Provide data for the Chesapeake Bay watershed and ecological models.

1.3 Sampling Design and Data Quality Objectives

1.3.1 *Parameters*

The scope of work for this component of the coordinated Chesapeake Bay Water Quality Monitoring Program includes the measurement of chemical and physical parameters in the water column. Parameters such as nutrients, total suspended solids, chlorophyll *a*, dissolved oxygen and water transparency were selected to (1) provide information on eutrophication trends; (2) calibrate Bay water quality models; and, (3) correlate living resources data to water quality data. Other parameters such as salinity and temperature are necessary to provide a more rigorous interpretation of these key water quality indicators. The same parameters are collected in the mainstem, large tributaries (Potomac and Patuxent Rivers), and minor tributaries except for dissolved organic carbon and silica. Dissolved organic carbon is no longer collected in the mainstem with one exception. Due to logistical considerations, the Potomac tributary sampling station LE2.3 is sampled during Mainstem cruises.

Starting in July, 2007, silica (SIF) will be collected monthly, from the surface and above pycnocline layers, January through June at the plankton sampling stations (CB1.1, CB2.2, CB3.3C, CB4.3C, CB5.2, TF2.3, RET2.2, LE2.2, TF1.5, TF1.7, LE1.1, ET5.1 and WT5.1). Silica samples will not be collected at any stations July through December 2008. Silica will be sampled January through June 2009.

(A complete list of parameters measured and detection limits is provided in Section 2, Table 2.)

The information gained from analyzing the entire suite of parameters allows managers to determine whether or not water quality goals established for living resources have been met and aids managers in establishing programs to control point and non-point sources of pollutants to the Bay.

1.3.2 *Spatial Aspects*

A total of 21 mainstem stations and 72 tributary stations are included in Maryland's Chemical and Physical Properties Component of the Chesapeake Bay Water Quality Monitoring Program (Figure 1 and Table 1). Station locations were selected to provide data that would satisfy the five objectives of the program stated above for the major tributaries and the mainstem. The following describes the four sets of criteria used to determine the general location for stations:

Primary Selection Criteria. During the initial phases of the Bay Program, EPA developed a segmentation/characterization scheme of the Chesapeake Bay and its tributaries published in the appendices of *Chesapeake Bay: A Profile of Environmental Change* (EPA 1983). This scheme provided guidance for station selection by delimiting different regions (based on circulation, salinity, and geomorphology) such as tidal fresh, oligohaline, and mesohaline. Several primary goals were considered in selecting station locations. Selecting a suite of stations such that each segment would be characterized was the foremost goal. Another important criterion was the location of boundaries between segments (e.g. mouths of major tributaries and the upper boundary of the deep trough region). Boundary areas are important because of their influence on a particular region of the Bay or their relevance to problem areas. In large systems, i.e., the Potomac and Patuxent Rivers and the mainstem, multiple stations were located in some of the major salinity zones due to the large size of these systems and their importance to

management concerns. Existing water quality monitoring stations in the Potomac River and Patuxent River were incorporated into the Bay-wide network because of the wealth of historical data at these stations.

Secondary Selection Criteria. Locations of documented water quality problems in certain areas served as secondary considerations in locating stations. For example, additional stations were included in the lateral dimension of the deep trough region of the mainstem to characterize the deepwater anoxic/hypoxic conditions. Another example was the siting of stations in some of the smaller tributary segments in areas that were profoundly impacted by point-sources. Stations sited in these affected areas provide excellent opportunities to assess the effectiveness of control strategies targeted at reducing these major impacts.

Tertiary Selection Criteria. Another consideration in siting stations was their proximity to important living resource habitats and living resource monitoring sites. This criterion was accommodated only if the primary and secondary criteria above were also satisfied. These stations provide valuable data to correlate with living resources monitoring and thereby help to resolve the link between water quality and recent living resource declines.

Final Selection Criteria. The fourth and final consideration in locating stations was the historical record of water quality sampling. If a station already had a record of previous water quality data *and* it satisfied the three sets of criteria stated above, the station was adopted for this program to permit comparisons with historical data bases. In selecting stations for the Patuxent and Potomac Rivers, this criterion was elevated to a primary criterion. Additional historical stations in the Patuxent and Potomac were adopted into the Chesapeake Bay Program sampling program even if they did not fulfill all three sets of criteria above, because of the very long-term data sets associated with these stations.

Establishing Mid-Channel and Near-shore Stations. In both the mainstem and tributaries, stations were selected in mid-channel locations to provide a characterization of the entire water column in that region and to capture the lowered oxygen levels in the deeper layers. The water column at mid-channel also provides a more stable environment than shallow locations, which are subject to ephemeral influences such as wind-driven resuspension of bottom sediments and periodic advection of deep-channel water masses; thus, mid-channel stations provide data with less short-term variability. Minimizing short-term variability is desirable in order to detect long-term trends. As mentioned above, in the mainstem's deep trough region, lateral stations were established to track a particular concern. Two near-shore stations were located beside each of the four mid-channel stations. These near-shore stations were located at the 30-foot depth contour or at the boundary of adjacent embayments. Stations also were located at the boundary between the mainstem and the two largest tributaries in Maryland—the Susquehanna and Potomac Rivers—to assess the water quality interactions occurring across these critical regions.

Updating the Segmentation Scheme. During 1997, a workgroup was established to re-evaluate the segmentation scheme using the data generated by the program from 1985-1996. DNR uses the current segmentation scheme established by the EPA Chesapeake Bay Program (CBP) to classify stations and analyze data (see Table 1). Under the new segmentation scheme, four segments (CHOTF, NANOH, HNGMH, and POCOH) do not include long-term stations. The *Analytical Segmentation Scheme for the 1997 Re-evaluation and Beyond* (EPA 1997) provides a detailed description of CBP's segmentation and its development.

1.3.3 ***Temporal Aspects***

Water column samples are collected at least once a month at most stations, for a minimum of 12 samplings per year. In the Chesapeake mainstem and smaller tributaries sampling is conducted twice monthly in April, May, July, and August and once monthly during the remaining months for a total of 16 samplings per year. However, at the east and west transect stations, samples are not collected from November through February, resulting in only 12 samplings a year. On the Potomac and Patuxent, 20 samplings are conducted per year. Sampling frequency for each station is shown in Table 1. This frequency of sampling permits assessments to be made on a seasonal basis, which is a time scale consistent with many of the natural intra-annual changes in water quality indicators.

Because of the relatively small sample sizes resulting from only two to four sampling events per season, it is more difficult to detect seasonal trends in data from stations sampled only once per month. Nevertheless, with a long-term program, sufficient data can be collected to determine seasonal patterns in most water quality parameters at each site with high statistical confidence.

In 1994, *An Assessment of the Power and Robustness of the Chesapeake Bay Program Water Quality Monitoring Program: Phase II - Refinement Evaluations* (Alden et al. 1994) concluded that although the 12-cruise scenario was less statistically powerful than the 20-cruise scenario, the 12-cruise scenario was none-the-less adequate for the Chesapeake Bay Mainstem monitoring to capture long-term annual trends; the Chesapeake Bay Program decided on a 14-cruise scenario for the monitoring program. Based on these recommendations, in January 1996, Maryland dropped its Chesapeake Bay mainstem January and February cruises and reduced its cruises in March, June, September, and October to once per month. Experience has since shown that this reduced sampling frequency can miss some extremely important climatic and biological events (e.g., the 100-year flood of January 1996). Therefore, CBP has restored funding in Maryland for its January and February monitoring cruises beginning in January 1999, for a total of 16 cruises. When funding is available, second June mainstem cruise is also added to the sample schedule to better characterize the onset of summer hypoxia/anoxia conditions in deep water.

The higher frequency sampling in larger tributaries and the mainstem allows trends to be detected with high statistical confidence more easily at these locations. This two-tiered level of sampling frequency was judged to be the optimal allocation of effort given the limited level of resources. It provided for wide spatial coverage of almost every major tributary in Maryland as well as for more statistically rigorous information in the major systems that were the focus of major management strategies.

Figure 1 Map of Maryland Department of Natural Resources Chesapeake Bay Mainstem and Bay Tributary Water Quality Monitoring Stations. Red squares indicate the stations monitored since 1985 (or earlier). Orange circles indicate stations monitored since 1998, originally as part of Maryland's Pfiesteria sampling and added to the routine monitoring program in 2003.

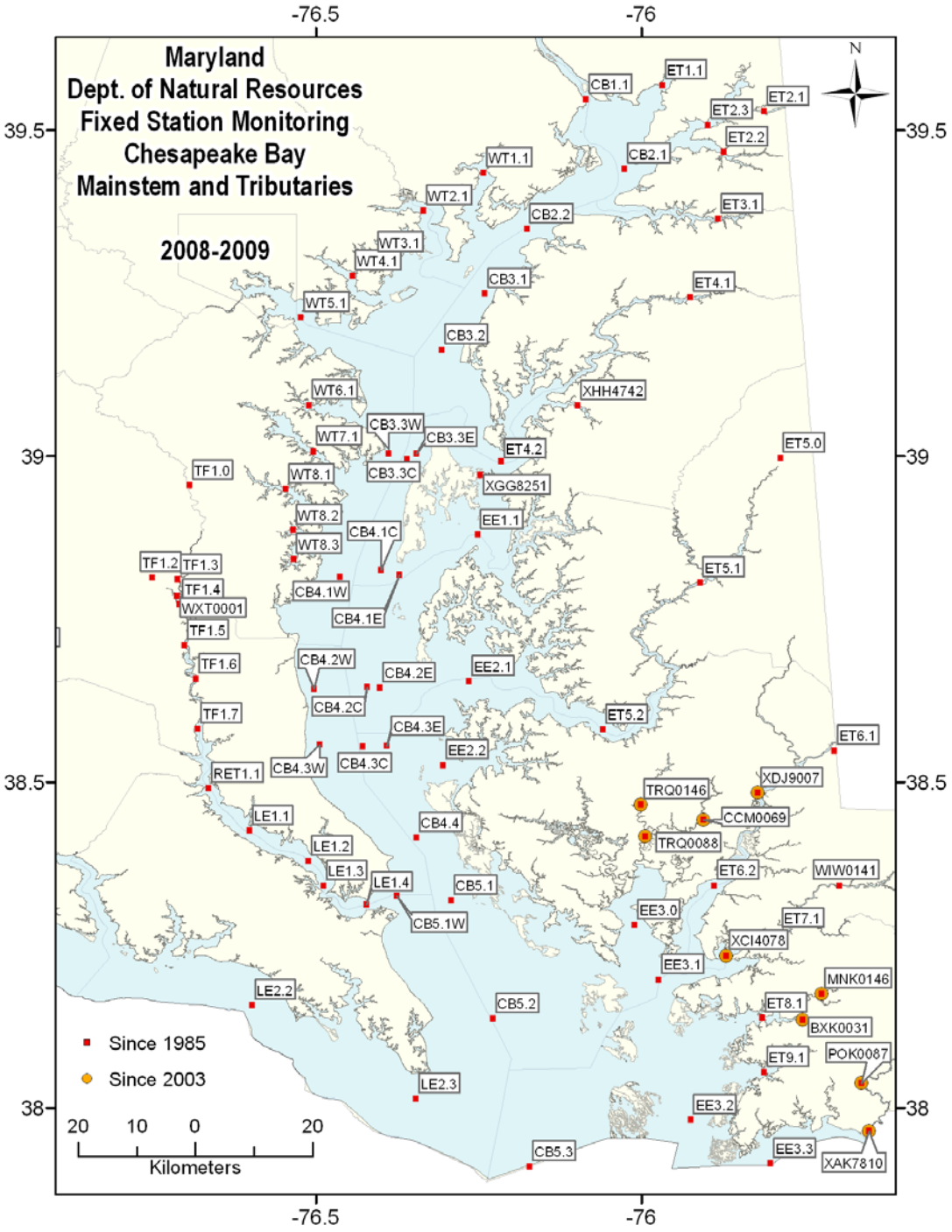

Table 1 Mainstem and Tributary sample locations and descriptions.

Component	Station Name	Location/Depth	Ches. Bay Program Segment	Sampling Coordination	Historical Station names	Latitude/ Longitude (NAD83 DMS)	Annual. Sample. Freq. x No. Of Depths
Mainstem	CB1.1	Mouth of Susquehanna River (700 yds from abandoned Light House on Hdg 040, 400 yds NNW of N 18 on line with N 20); 5.7 m.	CBTF1	PAR; VSS; plankton	OEP XKH3147	39 32.8762738 76 05.0886767	16x2
Mainstem	CB2.1	SW of Turkey Point (1 nm from Turkey Pt Light on Hdg 240, 800 yds SE of RG A); 6.1m.	CBTF1	PAR DNR Phytoplankton (live), plankton	CBI 927SS OEP XJH6680	39 26.4894865 76 01.5594740	16x2
Mainstem	CB2.2	W of Still Pond (500 yds W of G 49, 1.75 nm S of Taylor Island Pt off Still Pond); 11.5m.	CB2OH	PAR; VSS; plankton	CBI 92OU, 921W, 922Y OEP XJG0999	39 20.9239078 76 10.5472401	16x4
Mainstem	CB3.1	SE of Gunpowder Neck (2.1nm from south tip of Pooles Island Hdg 146, halfway between buoys 31 and 33); 11.2 m.	CB2OH	PAR	CBI 913R, 914S	39 14.9699926 76 14.4301009	16x4
Mainstem	CB3.2	NW of Swan Pt (400 yds NW of Tolchester Channel 13,1.9 nm from Swam Point on Hdg 328); 11.5 m.	CB3MH	PAR	CBI 909 OEP XHG4953 OEP XHG9915	39 09.8215856 76 18.3788588	16x4
Mainstem	CB3.3C	N of Bay Bridge (1.6 nm, from Sandy Pt Light on Hdg 145, 0.4 nm NNE of bridge at edge f cable cross); 20.7 m.	CB3MH	PAR, VSS , DNR Phytoplankton (live), plankton	CBI 858C, 859B OEP XFH1373 OEP XGF9784 EPA D2	38 59.7574462 76 21.5802267	16x4
Mainstem	CB3.3E	NE of Bay Bridge (1.9nm from Sandy Pt Light on Hdg 260, 1 nm NNE of Bridge in East Channel); 8.2 m.	CB3MH	PAR	CBI 859A OEP XFH0293 EPA D3	39 00.2468723 76 20.7101544	12x2
Mainstem	CB3.3W	NW of Bay Bridge (0.7 nm from Sandy Pt Light on Hdg 210, 0.7 nm SE Sandy Pt Water Tank); 9.1m.	CB3MH	PAR	CBI859D OEP XHF0366 EPA D1	39 00.2772786 76 23.2858222	12x2
Mainstem	CB4.1C	SW of Kent Pt (0.5nm from Bloody Pt Light just West of line from Bloody Pt to G 83); 31.0 m.	CM4MH	PAR	CBI 845G, 848E OEP XFF9178 EPA ‘83DO	38 49.5557571 76 23.9670797	16x4
Mainstem	CB4.1E	S of Kent Pt (1.4 nm SE Bloody Pt Light, 300 yds SW buoy 1 for Eastern Bay); 23.7 m.	CB4MH	PAR	CBI 851N EPA ‘83DO OEP XFF9178	38 49.0856140 76 22.2862891	12x4
Mainstem	CB4.1W	SE of Horseshoe Pt (3.5nm from Bloody Pt. Light on Hdg 260, 1.6 nm E of Franklin Manor); 9.1 m.	CB4MH	PAR , DNR Phytoplankton (live)	CBI 848G, H, I OEP XFF1844 OEP XFF8922	38 48.8987701 76 27.7630455	12x2
Mainstem	CB4.2C	SW of Tilghman Island (2nm from Sharps Island Light on Hdg 290, 300 yds NE of CR buoy) 26.2 m.	CB4MH	PAR	EPA ‘83DO OEP XEF8648	38 38.7710773 76 25.2758161	16x4
Mainstem	CB4.2E	SW of Tilghman Island (1.3nm from Sharps Island Light on Hdg 305, 0.9 nm E of CR buoy); 9.1 m.	CB4MH	PAR	OEP XEF8859	38 38.6991249 76 24.0789313	12x2

Component	Station Name	Location/Depth	Ches. Bay Program Segment	Sampling Coordination	Historical Station names	Latitude/ Longitude (NAD83 DMS)	Annual. Sample. Freq. x No. Of Depths
Mainstem	CB4.2W	NW of Plum Pt (6nm from Sharps Island. Light on Hdg 280, 1.0 nm E of Camp Roosevelt); 9.1 m.	CB4MH	PAR	OEP XEF8699 EPA '83DO	38 38.6122822 76 30.1298742	12x2
Mainstem	CB4.3C	E of Dares Beach (0.5 nm W of R 78, 5.7 nm from Sharps Island Light, Hdg 220); 25.6 m.	CB4MH	PAR; VSS Plankton	OEP XEF3343	38 33.3031773 76 25.6765828	16x4
Mainstem	CB4.3E	Mouth of Choptank River (1.7 nm. East of R78, 5 nm. from Sharps Island Light on Hdg 195); 21.6 m.	CB4MH	PAR	OEP XEF3465	38 33.3743630 76 23.4728861	12x4
Mainstem	CB4.3W	E of Dares Beach (1nm. East of Dares Beach, 3nm. West of R78); 9.7 m.	CB4MH	PAR	CBI 834H, J OEP XEF3405	38 33.4368892 76 29.6411944	12x2
Mainstem	CB4.4	NE of Cove Pt (2.4 nm from Cove Pt on Hdg 055); 28.6 m.	CB4MH	PAR, Quarterly Split Sample Location	OEP XDF4693	38 24.8744293 76 20.7387902	16x4
Mainstem	CB5.1	E of Cedar Pt (1 nm. ENE of mid-channel buoy HI, 4nm. from Cedar Pt. on Hdg 070); 33.2 m.	CB5MH	PAR, DNR Phytoplankton (live)	CBI 818N, P CBI 819N, O OEP XCG9223	38 19.1218979 76 17.5286492	16x4
Mainstem	CB5.2	Mid Bay E of Pt No Point (3 nm. From Point No Point Light on Hdg 080); 29.0 m.	CB5MH	PAR; VSS, Plankton	Benthos #58 (Versar) OEP XBG8262	38 08.2229854 76 13.6720392	16x4
Mainstem	CB5.3	NE of Smith Point (2nm. from Smith Point Light toward on Hdg 020, intersect MD/VA line and transect from Smith Pt to Holland bar Light); 25.3 m.	CB5MH	PAR	USGS 37524807 USGS 6094200 OEP XAG4699	37 54.6067961 76 10.2821397	16x4
Patuxent	TF1.0	At bridge on US Rt. 50 (upstream side of bridge; USGS Gage No 59440); 3.0 m.	PAXTF		OEP PXT0603 USGS 01594440 EPA E	38 57.3343692 76 41.6465749	20x1
Patuxent	TF1.2	Midstream of Western Branch at Water Street crossing in Upper Marlboro, MD; 3.0 m.	WBRTF		OEP WXT0045	38 48.8580017 76 45.0520702	20x1
Patuxent	WXT0001	Western Brach from pier at Mt Calvert House in Upper Marlboro, 0.1 miles above mouth; 1.0 m.	WBRTF			38 47.1230110 76 42.8058027	20x1
Patuxent	TF1.3	Mid-channel from MD Rt. 4 bridge near Waysons Corner; 3.7 m.	PAXTF		OEP PXT0494 EPA E5,5	38 48.6550025 76 42.7364996	20x1
Patuxent	TF1.4	West Shore from main pier at Jackson Landing; just below confluence with Western Branch; 3.0 m.	PAXTF		OEP PXT0456 EPA E6A	38 46.3810259 76 42.5562188	20x1
Patuxent	TF1.5	Mid-channel at Nottingham, 11.1m.	PAXTF	VSS , DNR phytoplankton (live); plankton	OEP PXT0402 EPA E8	38 42.6070912 76 42.0878729	20x4
Patuxent	TF1.6	Mid-channel off the wharf at Lower Marlboro, 6.0 m.	PAXOH	plankton	OEP XED9490 EPA E9 J.H. 5945	38 39.5070054 76 41.0291236	20x3
Patuxent	TF1.7	Mid-channel on a transect heading of approx. 115 degrees from Jack's Creek; 3.1 m.	PAXOH	VSS, plankton	OEP XED4892 J.H. 5946	38 34.9264583 76 40.8603554	20x2

Component	Station Name	Location/Depth	Ches. Bay Program Segment	Sampling Coordination	Historical Station names	Latitude/ Longitude (NAD83 DMS)	Annual. Sample. Freq. x No. Of Depths
Patuxent	RET1.1	Mid channel, 0.5 km ENE of Long Point, 11.1 m.	PAXMH	DNR phytoplankton (live); DNA probe	OEP XDE9401 EPA E14, 4 CB 1	38 29.4538477 76 39.8574145	20x4
Patuxent	LE1.1	Mid-channel SSW of Jack Bay sand-spit. NE of Sandgates; 12.5 m.	PAXMH	VSS, DNR phytoplankton (live), plankton, DNA probe,	OEP XDE5339 EPA E15	38 25.5208238 76 36.1058647	20x4
Patuxent	LE1.2	Mid-channel,1.6 km SW of Petersons Pt.; 17.8 m.	PAXMH	DNR phytoplankton (live)	OEP XDE2792	38 22.7321491 76 30.6793504	20x4
Patuxent	LE1.3	Mid-channel 1200 m due N of Pt. Patience, ESE of Half Pone Pt; 23.1 m.	PAXMH		OEP XDF0407	38 20.4525027 76 29.2932417	20x4
Patuxent	LE1.4	Mid-channel on a transect between Drum Pt. and Fishing Pt; 16.5 m.	PAXMH		OEP XCF8747	38 18.7201675 76 25.2906509	20x4
Patuxent	CB5.1W	Mid-channel on a transect between Cedar Pt and Cove Pt; 8.9m	PAXMH		OEP XCF9575	38 19.5132231 76 22.5444132	20x4
Potomac	PIS0033	Piscataway Creek at Maryland Rt 210 crossing; 1.0 m.	PISTF	Sampled in coordination with mainstem;		38 41.9049785 76 59.2039713	20x1
Potomac	XFB1986	Piscataway Creek off Ft. Washington Marina between DM4 and DM6, SW of dredged channel; 2.0 m.	PISTF	Sampled in coordination with mainstem; DNR phytoplankton (live)		38 41.8719243 77 01.3904020	20x1
Potomac	MAT0078	Mattawoman Creek at MD. Rt 225 crossing; 1.0 m.	MATTF	Sampled in coordination with mainstem		38 35.3113741 77 07.1188127	20x1
Potomac	MAT0016	Mattawoman Creek at green day beacon 5 off Sweden Pt; 2.0 m.	MATTF	Sampled in coordination with mainstem; DNR phytoplankton (live)		38 33.9049510 77 11.6071105	20x1
Potomac	TF2.1	At Fl buoy 77 off mouth of Piscataway Creek; 19.0 m.	POTTF	Sampled in coordination with mainstem; DNR phytoplankton (live)	OEP XFB2470 EPA – several	38 42.3986386 77 02.9254704	20x3
Potomac	TF2.2	Buoy 67 off mouth of Dogue Creek; 8.0 m.	POTTF	Sampled in coordination with mainstem; DNR phytoplankton (live)	OEP XFB1433 USGS 384136077054600 EPA – Several	38 41.4405123 77 06.6665873	20x3
Potomac	TF2.3	Buoy N54 mid-channel off Indian Head; 15.0 m.	POTTF	Sampled in coordination with mainstem; DNR phytoplankton (live); VSS, plankton	OEP XEA6596	38 36.4933884 77 10.4383095	20x3
Potomac	TF2.4	Buoy 44 between Possum Pt. And Moss Point; 9.0 m.	POTTF	Sampled in coordination with mainstem; DNR phytoplankton (live)	OEP XEA1840 USGS 06158710 EPA- Several	38 31.8040859 77 15.9220599	20x3
Potomac	RET2.1	Buoy 27 SW of Smith Point; 8.0 m.	POTOH	Sampled in coordination with mainstem; DNR phytoplankton (live)	OEP XDA4238 EPA – Several	38 24.2084721 77 16.1451661	20x3
Potomac	RET2.2	Buoy 19 mid-channel off Maryland Point; 11.0 m.	POTOH	Sampled in coordination with mainstem; DNR phytoplankton (live), VSS, plankton	OEP XDA1177 EPA - Several	38 21.1519219 77 12.3049300	20x3

Component	Station Name	Location/Depth	Ches. Bay Program Segment	Sampling Coordination	Historical Station names	Latitude/ Longitude (NAD83 DMS)	Annual. Sample. Freq. x No. Of Depths
Potomac	RET2.4	Mid-channel at Morgantown bridge (US Rt. 301); 19.0 m.	POTMH	Sampled in coordination with mainstem; DNR phytoplankton (live), VSS, DNA probe	OEP XDC1706 USGS 01660800 EPA - Several	38 21.7559638 76 59.4376865	20x4
Potomac	LE2.2	Potomac River off Ragged Point at Buoy 51B; 10.0 m.	POTMH	Sampled in coordination with mainstem; DNR phytoplankton (live), PAR, VSS, plankton	OEP XBE9541	38 09.4563269 76 35.8815408	20x4
Potomac	LE2.3	Mouth of Potomac River (1.6 nm from Pt Lookout on Hdg 240, 0.5 nm NW of Whistle A); 19.8 m	POTMH	Sampled on mainstem cruise	OEP XBF0893	38 00.8526905 76 20.7688597	20x4
Tributary	ET1.1	Northeast River at Daymarker 12 off Hance Pt, mid-channel; 3.0 m.	NORTF	Striped bass spawning	OEP XKI4220 OEP XKI3717 OEP XKI4523 OEP XKI5025	39 34.1856660 75 58.0692371	12x2
Tributary	ET2.1	C&D Canal E of Rt 213 Bridge at Chesapeake City; 13.0 m.	C&DOH	DNR spawn habitat, striped bass spawning, C& D Canal	OEP XKJ1810 OEP XKJ1811	39 31.7580105 75 48.6809202	12x2
Tributary	ET2.2	Bohemia River off Hack Pt, 75 yds ENE of daymarker R 4, midchannel; 3.0 m.	BOHOH	DNR juvenile striped bass spawning	OEP XJI8076 OEP XJI7678 EPA U9	39 28.0223692 75 52.4205850	12x2
Tributary	ET2.3	Elk River SE of Old Cornfield Pt at G 21, mid-channel; 12.0 m.	ELKOH	Striped bass spawning, DNR juvenile	OEP XKI0661 EPA U10	39 30.5236131 75 53.8694267	12x2
Tributary	ET3.1	Sassafras R from end of pier at Georgetown Yacht Basin, NW side of MD. Rt. 213 bridge; 5.0 m.	SASOH	DNR phytoplankton (live); DNA probe, Striped bass spawning, DNR juvenile	OEP XJI1970 EPA U1	39 21.8493407 75 52.9218567	12x2
Tributary	ET4.1	Chester River at Rt 290 bridge near Crumpton; 6.0 m.	CHSOH	Striped bass spawning	OEP CHE0367	39 14.6244548 75 55.4932080	16x2
Tributary	ET4.2	Lower Chester River South of Easter Neck Island 200 yds SW of buoy FL G 9; 16.0 m.	CHSMH	DNA probe , DNR oyster spat	OEP XGG9572 CBI CHO9C	38 59.5398961 76 12.9055791	16x4
Tributary	EE1.1	Eastern Bay between Tilghman Pt and Parsons Island, N of buoy R4; 13.0 m.	EASMH	DNR oyster spat	OEP XGG2649 CBI 851N	38 52.7994975 76 15.0873372	12x4
Tributary	ET5.1	Upper Choptank River 200 yds upriver from Ganey's Wharf, downstream of confluence with Tuckahoe Creek; 6.0 m.	CHOOH	Plankton, , DNR spawning habitat, DNR juvenile, striped bass spawning	OEP CHO0429	38 48.3872185 75 54.5823145	16x2
Tributary	ET5.2	Lower Choptank River, midriver 50yds NNE of G I, W of Rt 50 bridge at Cambridge; 11.0 m.	CHOMH2	DNR phytoplankton (live), Plankton, DNR juvenile, DNR spawning habitat	OEP XEH4766	38 34.8394323 76 03.5202530	16x4
Tributary	EE2.1	Choptank embayment between Todds Point and Nelson Pt; 8.0 m.	CHOMH1	DNA probe, Near DNR oyster spat	OEP XEG9440 OEP XEG9652	38 39.2945815 76 15.8585779	12x4
Tributary	EE2.2	Little Choptank River mid-channel West of Ragged Point, W of Buoy Fl g 3; 14.0 m.	LCHMH	DNA probe, DNR oyster spat	OEP XEG1617	38 31.5656341 76 18.2448493	12x2

Component	Station Name	Location/Depth	Ches. Bay Program Segment	Sampling Coordination	Historical Station names	Latitude/ Longitude (NAD83 DMS)	Annual. Sample. Freq. x No. Of Depths
Tributary	TRQ0146	Transquaking River at Decoursey Rd. bridge crossing; 2.2 m.	N/A	DNA probe *Pfiesteria* sampling 1998-2002		38 27.9394360 76 00.0061642	12x1
Tributary	TRQ0088	Transquaking River at bridge on Bestpitch Ferry Rd; 2.7 m.	N/A	DNA probe *Pfiesteria* sampling 1998-2002 Continuous monitoring		38 25.0361348 75 59.6067549	12x1
Tributary	CCM0069	Chicamacomico River at Drawbridge road crossing. 2.4 m.	N/A	DNA probe *Pfiesteria* sampling 1998-2002 Continuous monitoring 2000-2003		38 26.5366979 75 54.2854815	12x1
Tributary	EE3.0	Fishing Bay at daymarker 3, W of Roasting Ear Pt; 7.0 m.	FSBMH	VSS, DNR Phytoplankton (live), DNA Probe	OEP XCH6994 OEP XCH5991	38 16.8560762 76 00.6198796	12x2
Tributary	ET6.1	Upper Nanticoke River at old Rt. 313 bridge (fishing pier,1987) in Sharptown; 5.0 m.	NANTF	VSS, DNR juvenile, DNR oyster spat	OEP NAN03021	38 32.9039050 75 42.1870554	12x2
Tributary	XDJ9007	Nanticoke River at old Rt 50 bridge in Vienna; 1.8 m.	NANOH	DNA probe *Pfiesteria* sampling 1998-2002		38 29.0248218 75 49.2586029	12x1
Tributary	ET6.2	Lower Nanticoke River mid-channel near Fl G 11; 3.5 m.	NANMH	DNR Phytoplankton (live), DNA probe, VSS, DNR juvenile, DNR oyster spat	Near OEP XDI0567 Near OEP XDI0567	38 20.4963799 75 53.2476826	12x2
Tributary	EE3.1	North Tangier Sound, NW of Haines Pt, 100 yds N of buoy R16; 13.0 m.	TANMH		OEP XCI1717	38 11.8113063 75 58.3925178	12x4
Tributary	WIW0141	Wicomico River at upper ferry crossing on Upper Ferry Road; 3.9 m.	WICMH	DNA probe *Pfiesteria* sampling 1998-2002		38 20.4916929 75 41.7412721	12x1
Tributary	ET7.1	Lower Wicomico River at Whitehaven, 150 yds downriver of Ferry Road, mid-channel; 7.0 m.	WICMH	DNR Phytoplankton (live), DNA probe, VSS	OEP WIW0050	38 16.0701067 75 47.2761282	12x2
Tributary	XCI4078	Wicomico River at Island Pt. in channel at buoy Fl14; 4.4 m.	WICMH	DNA probe *Pfiesteria* sampling 1998-2002		38 14.0271564 75 52.1777364	12x1
Tributary	MNK0146	Manokin River on unnamed Rd. Off Stewart Neck Rd. below unnamed tributary; 4.7 m.	N/A	DNR phytoplankton (live), DNA probe *Pfiesteria* sampling 1998-2002		38 10.5079461 75 43.3412509	12x1
Tributary	BXK0031	Back Creek (tributary to Manokin) at Milliard Long Rd; 2.6 m.	MANMH	DNA probe *Pfiesteria* sampling 1998-2002		38 08.1379293 75 45.0938889	12x1
Tributary	ET8.1	Manokin River at upper extent of channel; approx 100 yds NNE of buoy R 8, mid-channel; 6.0 m.	MANMH	VSS, DNR oyster spat	OEP XBJ8215	38 08.2763096 75 48.8466000	12x2
Tributary	ET9.1	Big Annemessex River, NW of Long Pt in channel S of daymarker G5; 5.0 m.	BIGMH	VSS	OEP XBJ3312	38 03.2985616 75 48.6783371	12x2
Tributary	EE3.2	South Tangier Sound, mid-channel East of Smith Island, 500 yds NNW of buoy R8; 28.0 m.	TANMH	DNR oyster spat	OEP XAI8845 Near OEP XBI3003	37 58.8833649 75 55.4537786	12x4

Component	Station Name	Location/Depth	Ches. Bay Program Segment	Sampling Coordination	Historical Station names	Latitude/ Longitude (NAD83 DMS)	Annual. Sample. Freq. x No. Of Depths
Tributary	ET10.1	Pocomoke River on Alt US Rt. 13 (Market Street) on old drawbridge in Pocomoke City; 5.0 m.	POCTF	Striped Bass spawning	OEP POK0170	38 04.5687574 75 34.2749080	12x2
Tributary	POK0087	Pocomoke River off Rehobeth Rd in town of Rehobeth 1.3 m.	POCOH	DNA probe *Pfiesteria* sampling 1998-2002		38 02.3102060 75 39.6738628	12x1
Tributary	XAK7810	Pocomoke Sound at middle of mouth of river; 3.4 m.	POCOH	DNR Phytoplankton (live), DNA probe, *Pfiesteria* sampling 1998-2002		37 57.8374074 75 39.0283151	12x1
Tributary	EE3.3	Pocomoke Sound, near buoy W “A” Pa, state line; 4.0 m.	POCMH	DNR oyster spat	Near OEP XAJ4719 Near VA EE3.1	37 54.8729048 75 48.0889235	12x2
Tributary	WT1.1	Bush River E of Gum Point, E of Fl G9 on power line support; 2.0 m.	BSHOH		OEP XJG6254	39 26.1067440 76 14.5231684	12x2
Tributary	WT2.1	Gunpowder River, 200 yds E of Oliver Point at buoy G15; 2.5 m.	GUNOH	DNA probe	OEP XJF2798	39 22.6479745 76 20.0788035	12x2
Tributary	WT3.1	Middle River East of Wilson Point at channel junction daymarker WP; 3.0 m.	MIDOH	DNR phytoplankton (live), DNA probe	OEP XIF5484 EPA M2	39 18.3229657 76 24.5721377	12x2
Tributary	WT4.1	Back River, East of Stansbury Point, East of daymarker R12; 2.0 m.	BACOH	DNA probe	OEP XIF6633 Near OEP XIF6732	39 16.6526474 76 26.6207773	12x2
Tributary	WT5.1	Patapsco River East of Hawkins Point at Buoy G3; 14.0 m.	PATMH	DNR phytoplankton (live), DNA probe, plankton	OEP XIE2885	39 12.7856735 76 31.3521434	16x4
Tributary	WT6.1	Magothy River N of South Ferry Pt, mid-channel at buoy R12 and daymarker G11; 5.0 m.	MAGMH		OEP XHE4794	39 04.7103548 76 30.6031007	12x2
Tributary	WT7.1	Severn River, 200 yds upstream of Rt 50/301 bridge and 150 yds off NE shore; 9.0 m.	SEVMH		OEP XHE0497	39 00.4583154 76 30.2100203	12x2
Tributary	WT8.1	South River South of Poplar Point at daymarker R16; 9.0 m.	SOUMH		OEP XGE6972	38 56.9761002 76 32.7659239	12x2
Tributary	WT8.2	Rhode River between Flat Island and Big Island; 3.0 m.	RHDMH		OEP XGE3279	38 53.2172578 76 32.0941083	12x2
Tributary	WT8.3	West River just upstream of daymarker R6; 4.0 m.	WSTMH		OEP XGE0579	38 50.5481653 76 32.0482195	12x2
Tributary	XGG8251	Chester River at Kent Narrows; 5.9 m.	CHSMH			38 58.2675736 76 14.8401240	12x1
Tributary	XHH4742	Corsica River; 2.4 m.	CHSMH			39 04.684 76 05.382	12x1
Tributary	ET5.0	Choptank River at Red Bridges; non-tidal station; sampled from bank.	CHOTF		CHO0626	38 59.8311087 75 47.1864631	12x1

KEY FOR Historical Stations:

Abbreviation	Description
CBI	Chesapeake Bay Institute, Johns Hopkins University, 1949-1980
EPA/AFP	EPA, Annapolis Field Office studies, 1969-1970
EPA	EPA, Water Quality Office, Chesapeake Technical Support Laboratory, 1967-1969
USDI	U.S. Department of the Interior, Federal Water Pollution Control Administration, Chesapeake Technical Support Laboratory, 1965-1968
U.S.G.S	U.S. Geological Survey Water Quality of the Potomac River and Estuary Hydrologic Data Report, 1978-1981
OEP	Office of Environmental Programs, Maryland Department of Health and Mental Hygiene, 1984-1987; this program was moved to Maryland Department of the Environment 1987-1996 and to the Maryland Department of Natural Resources 1996-present; the current sample names were adopted in 2000 to conform to EPA Chesapeake Bay Program station names.

NOTE: Refer to Appendix 1 for details on the physical/chemical parameter sampling. Refer to the following work plans/scopes of work for details on the plankton monitoring components:

Butler, W.L. 2008. *Quality Assurance Quality Control Work Plan Monitoring Program: Phytoplankton Monitoring*. Department of Natural Resources, Field Office: Annapolis, MD. January, 2008.

Lacouture, R.V. 2008. *Quality Assurance Documentation Plan for the Phytoplankton Component of the Chesapeake Bay Water Quality Monitoring Program*. Morgan State University Estuarine Research Center, 10545 Mackall Road, St. Leonard, MD. May 2008.

2. MEASURED PARAMETERS

The Chemical and Physical Properties Component of the Chesapeake Bay Water Quality Monitoring Program measures a broad suite of physical and chemical parameters that are indicative of the Bay's eutrophication problem. Several "natural" properties such as salinity and temperature in the water column provide important information for interpretation of water quality indicators.

Some parameters–conductivity, temperature, dissolved oxygen, pH, Secchi depth–are measured *in situ*. Salinity is calculated from conductivity and temperature.

Beginning mid-to-late 2008, YSI Series 6000 instruments will be added to the field equipment inventory. Water quality parameters currently measured using HydroLab equipment will be measured using YSI equipment in the future.

The YSI instruments will be equipped with an optical dissolved oxygen sensor instead of the Standard Clark Polarographic Sensor. Temperature, pH, specific conductance and depth sensors will be similar to respective HydroLab sensors.

This document will be amended when the YSI instruments are fully incorporated into the inventory, the deployment of the new equipment has been implemented and procedures have been evaluated and documented

The other measured parameters–including nitrogen, phosphorus, carbon and silicon species, total suspended solids, volatile suspended solids and chlorophyll *a*–are determined in the laboratory. Table 2 lists the parameters measured, their detection limits, methods references, and holding times and conditions. Details of sample collection, sample processing and storage, and analytical procedures are described in Appendices I, VII, VIII and VIII B.

The Chesapeake Biological Laboratory Nutrient Analytical Services Laboratory (NASL) is updating the 2004 Standard Operating Procedures (SOP) to reflect changes in procedures and instrumentation and will be working with the EPA Quality Assurance Officer and DNR Quality Assurance Officer to develop a timeline for delivery of the updated and revised SOP to the EPA Chesapeake Bay Program. The revised CBL SOP will also include recommendations from the GAP Analysis.

Appendix VII is a work in progress. Documents included in Appendix VII are: the 2004 NASL SOP; AquaKem 250 overview methods for Ammonium, Nitrite, Orthophosphate, Particulate Phosphorous and Particulate Inorganic Phosphorous, and Silicate; “Determination of Dissolved Organic Carbon (NPOC), Total Organic Carbon, and Dissolved Inorganic Carbon in waters of Fresh/Estuarine/Coastal Waters using High Temperature Combustion and Infrared Detection”; “Determination of Dissolved Inorganic Nitrate plus Nitrite (NO3+NO2) in Fresh/Estuarine/Coastal Waters Using Enzyme Catalyzed Reduction”; and “Determination of Carbon and Nitrogen in Particulates and Sediments of Fresh/Estuarine/Coastal Waters, Plant and Animal Tissue, and Soils Using Elemental Analysis”.

Table 2 Water Column Parameters, Detection Limits, Methods References, Holding Times and Conditions.

Parameter (Units)	Instrument	Detection Limit (or Range)	Method Reference	Holding Time and Condition
IN SITU				
Temperature (° C)	HydroLab	-5 to +45 ° C	Linear thermistor network: HydroLab System Water Quality Instrumentation Manual (HSWQIM 1984)	Not applicable *in situ*
	YSI	-5 to +45 ° C	Sintered metallic oxide thermistor: 6-Series Multiparameter Water Quality Sondes User Manual Rev D, 2006	
Depth (m)	HydroLab	0-100 m	Strain-gage pressure transducer (HSWQIM 1984)	
	YSI	0-61 m	Differential strain gauge transducer: 6-Series Multiparameter Water Quality Sondes User Manual Rev D, 2006	
Dissolved Oxygen (mg/L)	HydroLab	0-20 mg/L	Au/Ag polarographic cell (Clark; HSWQIM 1984)	
	YSI	0–50 mg/L	RapidPulse Clark-type or ROX Optical Dissolved Oxygen: 6-Series Multiparameter Water Quality Sondes User Manual Rev D, 2006	
Conductance, Specific	HydroLab	0-200 mmhos/cm	Temperature conductivity reported at 25 C, Six electrode cell (HSWQIM 1984)	
	YSI	0 – 100 mS/cm	Specific Conductance (mS/cm = micromhos/cm) Temperature compensated four electrode; 6-Series Multiparameter Water Quality Sondes User Manual Rev D, 2006	
pH	HydroLab	0-14 pH	Glass electrode: Surveyor II uses pair of Ag/AgCl probes; Scout 2 uses Ag/Cl probe and cylindrical block of silver (HSWQIM 1984)	
	YSI		Glass electrode; Ag/AgCl reference electrode pair; 6-Series Multiparameter Water Quality Sondes User Manual Rev D, 2006	
Secchi Depth (m)		0.1 - 7.0 m	Welch (1948)	
Light Attenuation* (Photosynthetic Active Radiation) (two measurements - one from boat and one taken at depth with an up sensor)		400–700 nm	Parsons (1977); Smith (1969), CBP F01	

* Light Attenuation is not measured in Tributaries except the Patuxent River.

GRAB SAMPLES			
Parameter (Units)	**Detection Limit (or Range)**	**Method Reference**	**Holding Time and Condition**
Orthophosphate (mg/L as P)	0.0006 mg/L	EPA method 365.1 (EPA 1993) Aquakem 250	Freezing-28 d
Total Diss. Phosphorus (mg/L as P)	0.0015 mg/L	Valderrama 1981, Alkaline persulfate digestion	Freezing-28 d
Particulate Phosphorus (mg/L as P)	0.0021 mg/L	Aspila et al. 1976 Aquakem 250	Freezing-28 d
Nitrite (mg/L as N)	0.0006 mg/L	EPA method 353.2 (EPA 1993) Aquakem 250	Freezing-28 d
Nitrite + Nitrate (mg/L as N)	0.0007 mg/L	EPA method 353.2 (EPA 1993) and enzymatic nitrate method. Aquakem 250	Freezing-28 d
Ammonium (mg/L as N)	0.003 mg/L	EPA method 350.1 (EPA 1993) Aquakem 250	Freezing-28 d
Total Dissolved Nitrogen (mg/L as N)	0.05 mg/L	D'Elia et al. 1977; Valderrama 1981, Alkaline persulfate digestion	Freezing-28 d
Particulate Nitrogen (mg/L as N)	0.0105 mg/L	EPA method 440.0 (EPA 1997)	Freezing-28 d
Dissolved Organic Carbon (mg/L as C)	0.24 mg/L	Sugimura and Suzuki (1988)	Freezing-28 d
Particulate Carbon (mg/L as C)	0.0633 mg/L	EPA method 440.0 (EPA 1997)	Freezing-28 d
Silicic Acid (mg/L as Si)	0.01 mg/L	EPA method 366.6 (EPA 1997) Aquakem 250	4 ºC - 28 d
Total Suspended Solids (mg/L)	2.4 mg/L	Standard Method (APHA 19th or 20th edition) Method 2540 D	Freezing-28 d
Volatile Suspended Solids (mg/L)	0.90 mg/L	Standard Method (APHA 19th or 20th edition) Method 2540 D	Freezing-28 d
Chlorophyll *a* (µg/L)	0.1 µg/L	APHA (2005)	Freezing-28 d
Pheophytin *a* (µg/L)	0.1 µg/L	APHA (2005)	Freezing-28 d

REFERENCES for Table 2:

American Public Health Association (APHA), *Standard Methods for the Examination of Water and Wastewater, Method Number 10200H*, 21st Edition, 2005.

American Public Health Association (APHA), *Standard Methods for the Examination of Water and Wastewater, Method Number 2540 D*, 20th Edition, 1998.

American Public Health Association (APHA). 1975. Method 208D, total non-filterable residue dried at 103-105ºC (total suspended matter), in *Standard Methods for the Examination of Water and Wastewater*, 14th Edition. APHA: Washington, D.C. 1193 p.

Aspila, I., H. Agemian and A. S. Y. Chau. 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. *Analyst* 101:187-197.

D'Elia, C. F., P. A. Steudler and N. Corwin. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnol. Oceanogr*. 22:760-764.

Hydrolab System Water Quality Instrumentation Manual (HSWQIM). 1984-1998 (multiple editions). Published by Hydrolab Corporation, P.O. Box 50116, Austin, Texas.

Parsons, T.R., Takahashi, M. and B. Hargrave. 1977. *Biological Oceanographic Processes*. Pergammon Press. Oxford. 332 p. (pages 71-85).

Patton, C..J., A.E. Fischer, W.H. Campbell and E.R. Campbell. 2002. Corn leaf nitrate reductase- A nontoxic alternative to cadmium for photometric nitrate determinations in water samples by air-segmented continuous-flow analysis. Env Sci. and Technology 36(4):729-735

Smith, R. C. 1969. An underwater spectral irradiance collector. *J. Mar. Res*. Vol. 27: 341-351.

Sugimura, Y. and Y. Suzuki. 1988. A high temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. *Mar. Chem*. 24:105 - 131.

US Environmental Protection Agency (EPA). 1997. *US EP A Method 440.0. Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis.* Revision 1.4. National Exposure Research Laboratory, Office of Research and Development, US Environmental Protection Agency: Cincinnati, OH.

US Environmental Protection Agency (EPA). 1993. *Methods for the Determination of Inorganic Substances in Environmental Samples* EPA-600/R-93/100.

US Environmental Protection Agency (EPA). 1979. *Methods for Chemical Analysis of Water and Wastes*. EPA-600/4-79-020. 460 p.

Valderrama, J. C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar. Chem*. 10:109-122.

Welch, P.S. 1948. Chapter 11 in *Limnological Methods*. Blakiston: Philadelphia, PA. pp. 159-167.

3. FIELD MEASUREMENTS AND SAMPLING

Sampling procedures have been formulated for each part of the Maryland's Chemical and Physical Properties Component of the Chesapeake Bay Water Quality Monitoring Program to take measurements that meet the program objectives in an efficient, cost-effective, and logistically practical manner.

As defined in the Scope of Work, a total of 21 mainstem stations and 72 tributary stations are included in the Chemical and Physical Properties Component of the monitoring program (see Figure 1 and Table 1 above in Section 1). Water column samples are collected at least once a month, for a minimum total of 12 times per year. In major tributaries, sampling is conducted twice monthly March through October, for a total of 20 sampling events per year. In the Chesapeake Bay mainstem, sampling is conducted 16 times per year, except at east and west transect stations, where it is conducted 12 times per year (but not conducted in November through February). The current frequency of sampling for each station is shown in Table 1 (provided above in Section 1).

The water column is profiled for temperature, conductivity, dissolved oxygen, and pH using an *in situ* probe that transmits data to a shipboard readout via cable. Profiling is conducted at a minimum of 2 m sampling intervals. In strata where there is appreciable change in conductivity or dissolved oxygen (i.e., at the pycnocline), 1 m intervals are sampled. The protocols for determining profiling depths are detailed in Appendix I.

Water column grab samples collected for subsequent analysis in the laboratory are taken by submersible pump or water bottle. The number of depths sampled per station is listed in the last column of Table 1.

One or two depths are sampled at stations that do not normally exhibit vertical density stratification. For stations where samples are collected at a single depth, the grab is collected from depth of either 0.0 m or 0.5 m depending on the site, with one exception. Samples are collected from a depth of 0.3 m at station XGG8251. The depths of 0.5 m and l m above bottom are sampled at sites where grabs are made at two depths.

Four depths—0.5 m below the surface, l.5 m above the upper limit of the pycnocline, l.5 m below the lower limit of the pycnocline, and l m above the bottom—are sampled at stations that are normally density stratified. Grab sampling depths relative to the pycnocline are determined according to the protocols of Appendix I.

Details on filtration, containers, and storage techniques can also be found in Appendix I. This sampling protocol provides one or two measurements of the water column in well-mixed non-stratified regions and two additional measurements—one in the surface mixed layer and one in the bottom mixed layer—where the estuary is stratified into the typical two-layered flow pattern.

For the mainstem stations only, when there is an odor of hydrogen sulfide present in the bottom sample or the below pycnocline sample, a Hach Kit test for hydrogen sulfide presence on the bottom and/or below pycnocline sample(s) is performed.

Water transparency is measured by Secchi depth, determined in meters using a 20 cm standard Secchi disc lowered into the water column with a calibrated rope. Observations are made on the shady side of the boat.

4. LABORATORY ANALYSIS

Active chlorophyll *a* and pheophytin *a* are analyzed by the Maryland Department of Health and Mental Hygiene's (DHMH) Environmental Chemistry Division. See Appendices VIII and VIII B for DHMH's chlorophyll analysis Standard Operating Procedures.

All other laboratory-measured parameters are analyzed at the University of Maryland's Chesapeake Biological Laboratory (CBL), Nutrient Analytical Services Laboratory. See Appendix VII for CBL's Standard Operating Procedures, KoneLabs AquaKem 250 Methods, PCPN methods, Nitrate methods and DOC methods.

5. DATA MANAGEMENT, VERIFICATION AND DOCUMENTATION

Data collection for the Chemical and Physical Properties Component of the Chesapeake Bay Water Quality Monitoring Program begins when measurements from field recording instruments are entered onto field data sheets. A field log book is used to document any problems encountered in the field that might affect the field parameters or samples brought back to laboratory. The senior scientist, on board each cruise, ensures that all measurements are taken properly. All data acquisition processes in the field and laboratory measurements are recorded in the Cruise Report to ensure data quality. After field personnel complete data sheets for a given calendar month, they make photocopies of the sheets to keep in the Field Office, and send the original field sheets to data management staff at the DNR Tawes Building. The Field Office also generates a Cross Reference Sheet for each set of field sheets, which is sent to the DNR data management personnel along with the field data sheets. The Cross Reference Sheet allows the data management personnel to know what field, nutrient, lab, and chlorophyll lab sheets to expect. See Appendix II for field sheets and associated documentation, Appendix III for a Cross Reference Sheet and documentation, and Appendix IV for Cruise Report Documentation and Procedures.

Nutrient laboratory data sheets (nutrient volume sheets) and chlorophyll laboratory data sheets also are initiated in the field. The nutrient lab sheets and chlorophyll lab sheets are used to record basic information about samples, such as station, date, depth, and volume filtered. The sheets serve as sample transfer sheets, traveling with the samples to Chesapeake Biological Laboratory (CBL) for nutrient analysis or to the Maryland Department of Health and Human Services laboratory (DHMH) for chlorophyll analysis. Both the sheets and the samples are logged in at the respective laboratories.

At CBL, data generated from nutrient analyses are either (a) recorded directly to an electronic file; or, (b) handwritten onto a lab sheet and then keypunched into an electronic file by laboratory personnel. CBL does not keep active control charts. Instead, each instrument has an operator dedicated to that instrument. The dedicated operator is responsible for keeping track of the slopes of the regression analysis for that instrument to determine if the analyses are "in control." The analyst reviews the data and, if the data exceed their control limits, the entire run is re-analyzed. Re-analysis can occur for any number of reasons, such as, a poor r-squared (R^2) on the standard curve, the wrong set of pump tubes (which would provide abnormally low peaks), or high blank values (in the case of DOC). See Appendix VII for CBL's Standard Operating Procedures.

At DHMH, data generated from chlorophyll analyses are recorded from the spectrophotometer both to a chlorophyll lab sheet and directly into an electronic file. See Appendices VIII and VIII B for DHMH's standard operating procedures for chlorophyll analysis.

When laboratory staff complete the nutrient lab sheets and chlorophyll lab sheets, the sheets are sent to the DNR Tawes Building along with any electronic files that have been generated. See Appendix II for nutrient lab sheets, chlorophyll lab sheets, and associated documentation. See Appendix XI for a list of codes used on the sheets.

Data review and verification are conducted at four levels by DNR data management personnel. At the first level, DNR data management personnel review cross reference sheets and field data sheets: (1) comparing field sheets to cross reference sheets to ensure that all field sheets have been received; (2) reviewing all field sheets to check that they are filled out completely and legibly, and; (3) sending the sheets to a data entry service for keypunch (see Appendix XII for procedures). At the data entry service, the field sheet data are double-entered to minimize errors at the keypunch stage. The entered field data are sent back to DNR as an electronic file on a diskette for further processing.

At the second level, a programmer trainee generates reports and plots for data verification using the Water Quality Import v3 software. The WQ Import v3 software was designed in late 1998 and completely developed in 2000 in Microsoft Access. The WQ Import v3 software is used to conduct data management activities, such as performing an initial data check, conducting major key field checks, performing a parameter range check (including measured and calculated parameters), conducting combination checks for specific parameters, generating an error report and verification plots, generating a "data verified list," reforming data, creating a database, and submitting data. Data checks are listed in Exhibit 1.

Exhibit 1. Data Verification Conducted on Water Quality Data

(1) Individual Data Parameter Checks:
- (a) Range check for numeric data parameters (reports error if data are outside the normal range for that parameter).
- (b) Character validation check for character data parameters (reports error if the character data are not appropriate for that parameter).

(2) Parameter Combination Checks:
- (a) Field Data:
  - -- Sample layer depth check (checks to make sure layer depths are appropriate, e.g., reports error if surface layer depth is greater than 1.0 m, surface depth is greater than bottom depth, etc.).
  - -- Upper and lower pycnocline check (reports error if pycnocline depths are outside expected range).
  - -- Maximum and minimum wind parameter check (reports error if minimum wind exceeds maximum wind).
- (b) Laboratory Data:
  - -- APC code check for all laboratory related parameters (reports if APC code has been reported).
  - -- G code (greater than or less than detection limit flag) check for all laboratory related parameters (reports if lab has flagged values as greater or less than the detection limit).
  - -- Parameter combination check for the following parameters:
    - Parameters PO4 and TDP (reports error if PO4 > TDP).
    - Parameters NO23, NH4, and TDN (reports error if NO23 + NH4 > TDN).
    - Parameters NO2 and NO23 (reports error if NO2 > NO23).
- (c) Chlorophyll Data: APC code check with light path, extraction volume, and/or optical density parameters (reports error if values are outside expected range.

(3) Verification Plots for Review: Sampling dates and times and values for all chemical and physical parameters are plotted by station for review by biologists and the Quality Assurance Officer. Biologists and the QAO look at patterns and identify any outliers or unusual values to be checked for errors.

Third, system printouts or PDF files of each data set are sent to a biologist and the Quality Assurance Officer for verification and editing. The Quality Assurance Officer and DNR biologists ensure that measured or calculated information for all types of data are correct and that the codes associated with parameters are properly established. In addition, the Quality Assurance Officer identifies data problems, provides data correction instructions, and coordinates data correction activities. Possible errors are identified, and sent to the laboratory or field office for verification or verified over the phone. Any

necessary corrections are written on an edit form, which is given to a programmer. The programmer makes changes to correct the electronic data set, re-runs the verification programs, and updates the verification reports and plots. This procedure is repeated until a clean data set is produced. Sample verification reports and plots and a sample edit form are provided in Appendix XIII.

The fourth step is for data management staff to ensure that the overall data verification processes are completed, all data errors are corrected, and that the finalized data sets are created and formatted to be consistent with historical data sets. The final data set combining the field, lab, and chlorophyll data is created as an "MDB file" after the completion of data verification processes. This final data set is stored in the designated DNR data base directory on the \Tawesdata2\data_library server for data user access. A formatted submission data set and associated data documentation is also transferred to the Chesapeake Bay Program Data Center on a monthly basis. The data management process is diagramed in Figure 2.

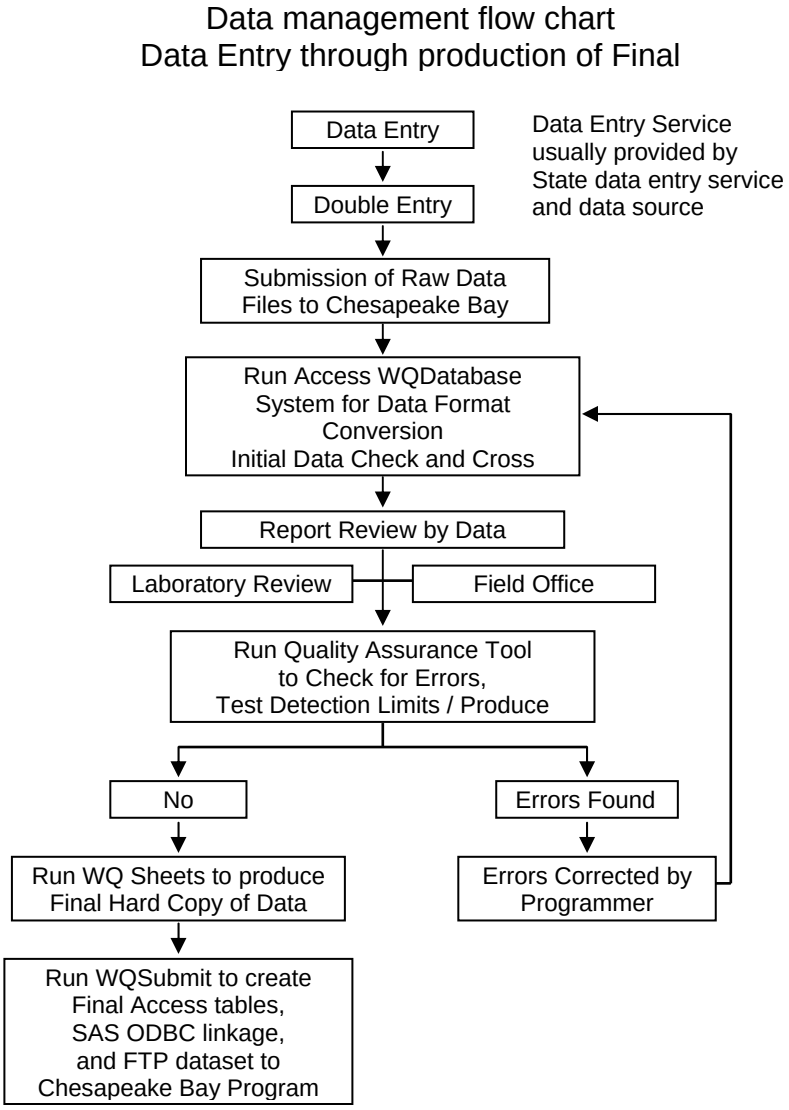

Figure 2 Data Management Flow Chart

A data tracking system has been designed and implemented to track the progress of data through the data management system. Data Status Forms are assigned to all data files received (see Appendix X for example sheet and documentation). Data sheets and tracking sheets used in data management are stored at the DNR Tawes Building for seven years. The data tracking system is diagramed in Figure 3.

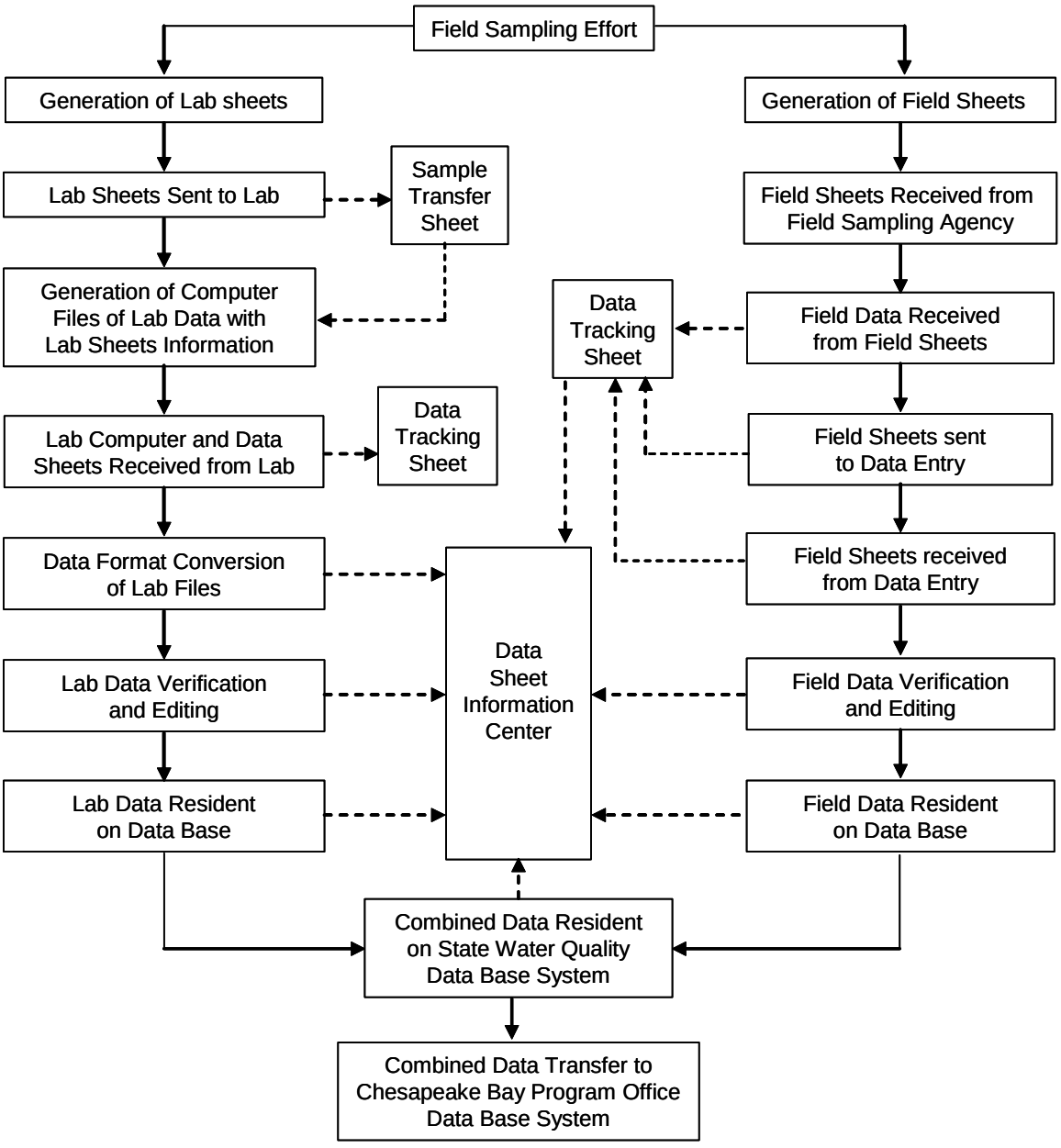

Figure 3 Data Tracking Flow Chart

Additionally, data from duplicate field samples are reviewed and analyzed by a scientist. Precision charts are created comparing the duplicates and identifying cases where the coefficient of variability (CV) exceeds 20 percent. The Chesapeake Bay Program Quality Assurance Officer is working with Maryland

Department of Natural Resources to formalize this process, the timing of this process, and the appropriate responses to CVs greater than 20 percent.

6. PROJECT QUALITY ASSURANCE/QUALITY CONTROL

The data collected as part of the Chemical and Physical Properties Component of the Chesapeake Bay Water Quality Monitoring Program are used in making management decisions regarding Chesapeake Bay water quality as described in the Introduction. DNR follows specific procedures to ensure that the design is properly implemented and that monitoring measurements are made and managed with sufficient accuracy, precision, and detection limits. General discussions of quality assurance and quality control aspects associated with accuracy, precision, data management, reporting, and audits are provided in the subsections below. For detailed descriptions of quality assurance and control procedures used in the field, the laboratories, and data management, see the attached appendices.

6.1 **Accuracy**

The accuracy (closeness to the true value) of the collected data is controlled and assured by the proper use, calibration, and maintenance of both field and laboratory equipment for the measurement of physical and chemical parameters. All instruments are identified by a unique number, used as an index for documentation of calibration, repair, and preventive maintenance. Where possible, standards used for calibration purposes are validated against a primary standard such as those available from the National Institute of Standards and Technology (NIST).

Daily quality control checks (including the running of blanks and standards) are used to control and assure laboratory accuracy. See Appendices VII, VIII and VIII B for details on the frequency of running blanks and standards and for additional procedures for laboratory quality assurance and control.

Accuracy of laboratory results is also assessed through DNR's participation in the Chesapeake Bay Coordinated Split Sample Program (CSSP), a split sampling program in which the coordinated split samples are analyzed by five laboratories involved in Chesapeake Bay monitoring. CSSP was established in June 1989 to establish a measure of comparability between sampling and analytical operations for water quality monitoring throughout the Chesapeake Bay and its tributaries. DNR follows the protocols in the *Chesapeake Bay Coordinated Split Sample Program Implementation Guidelines* (EPA 1991) and its revisions. Split samples are collected quarterly. Results are analyzed by appropriate statistical methods to determine if results differ significantly among labs. When a difference occurs, discussion begins regarding techniques and potential methods changes to resolve discrepancies. A summary of the coordinated split sample program and a copy of the split sample custody log are provided in Appendix IX.

Additionally, CBL and DHMH will participate two times per year in the United States Geologic Survey (USGS) reference sample program and will permit USGS to release the results to the Chesapeake Bay Program Quality Assurance Officer.

Procedures to control and assure the accuracy of field measurements involve the calibration of field instruments, the verification of these calibrations, equipment maintenance, and collection of filter blanks. These procedures are detailed in Appendices V and VI.

When field replicate control limits are exceeded, or when field blank values exceed lowest calibration standards, information about the issue is presented to the Analytical Methods and Quality Assurance Work Group (AMQAW). AMQAW may suggest corrective actions to field and laboratory procedures.

6.2 **Precision**

Precision (repeatability) of the chemical analytical methods is determined and documented from duplicate analyses. Precision of the entire measurement system for laboratory-analyzed parameters, including field processing, storage, and chemical analysis, can be assessed from duplicate field samples. Duplicate field samples are routinely collected approximately every 20 samples, as described in Appendix I. The protocols for duplicate analyses in the laboratory are described in the Standard Operating Procedures for CBL and DHMH, Appendices VII and VIII and VIII B.

6.3 **Data Review and Data Verification**

Data review and data verification ensure the quality assurance and quality control of data. Corrective actions routinely taken when data checks fail are detailed above in Section V, DATA MANAGEMENT, VERIFICATION AND DOCUMENTATION.

6.4 **Audits**

Performance audits for chemical analyses conducted at the University of Maryland's Chesapeake Biological Laboratory are based on the results of samples distributed by the EPA Chesapeake Bay Program Blind Audit Program. These samples must fall within the 95% confidence interval for acceptance. If results fall outside this range, corrective actions for each parameter and measurement are taken. CBL prepares the blind audit samples for all CBP participating laboratories and also analyzes some of those samples. For dissolved nitrogen and dissolved phosphorus, a laboratory quality assurance officer determines the concentrations in the ampules, prepares the concentrates, and seals the ampules. A different person then analyzes the sample blindly. For the particulate fractions (particulate carbon/particulate nitrogen and particulate phosphorus), samples are filtered and then placed in pouches in the freezer until they are ready to be sent to the other CBP participating laboratories: Delaware Department of Natural Resources and Environmental Control, Hampton Roads Sanitation District, Maryland Department of Health and Mental Hygiene, Massachusetts Water Resource Authority, Morgan State University, University of Maryland's Horn Point Laboratory, University of Delaware College of Marine Science, Old Dominion University, the Pennsylvania Academy of Science, Pennsylvania Department of Environmental Resources, Virginia Institute of Marine Science, Virginia Division of Consolidated Laboratory Services, Virginia Polytechnic Institute's Occoquan Laboratory, and, finally, Chesapeake Biological Laboratory, itself.

Once annually, the EPA Chesapeake Bay Program quality assurance officer conducts an onsite audit of the mainstem laboratory and field programs. The DNR Quality Assurance Officer communicates on a weekly basis with the field program staff and confers with the laboratory quality assurance officers to ensure that all aspects of the program are being conducted properly.

Internal Audits of field sampling are regularly conducted annually by the Field Quality Assurance Officer. Field sampling audit results are then communicated to the Quality Assurance Officer.

6.5 **Reporting**

Quality assurance information for field duplicate samples in the mainstem and tributaries is stored on the routine computerized water quality data sets as replicate observations that can be used to assess precision. For both the tributary and mainstem chemistry, laboratory quality assurance/control information on duplicates and spikes are stored on a computerized data set as a companion to the regular data sets and submitted to the CBPO quarterly. The DNR Quality Assurance Officer provides a summary of any relevant quality assurance/control information in quarterly progress reports for the mainstem program. The EPA Chesapeake Bay Program quality assurance officer reports on results of field and laboratory audits for the mainstem program.

6.6 **Data Quality Indicators**

To ensure that data are of the quality required to support Chesapeake Bay Program management decisions, Maryland's Chesapeake Bay Water Quality Monitoring Program strives to provide monitoring data of known and consistent quality to the CBPO by generally following the guidelines outlined in Chapter II, Section E of the *Recommended Guidelines for Sampling and Analysis in the Chesapeake Bay Monitoring Program, August 1996* (EPA 1996). These guidelines recommend precision goals of field and lab measurements of <20 percent of the coefficient of variation; accuracy goals within 80 to 120 percent, and the completeness goals of 100 percent. Detection limit ranges are provided in Table 2 above. Field measurement minimum detection limits are listed in Table 3.

7. DATA ANALYSIS AND REPORTING

As noted above, the key objectives of the Chesapeake Bay water quality monitoring program are to accurately describe the current state of the Bay mainstem and tidal tributaries and to detect long-term trends. Trends are analyzed using techniques recommended by the Chesapeake Bay Program's Tidal Monitoring and Analysis Work Group (TMAW, formerly the Data Analysis Work Group–DAWG), including the *Guidance for the Analysis of Water Quality Trends in Chesapeake Bay* (Eskin et al. 1993) developed by the DAWG in 1993. This published guidance provides general discussion on developing analytical objectives, reviewing and assembling data, analyzing data, and interpreting results. Data analysis topics covered in the document include:

PARAMETER	MINIMUM DETECTION LIMIT
Water Temperature	0.1 ºC
Depth	0.5 m
Dissolved Oxygen	0.0 mg/L
Conductance, Specific	Down to 1 micromhos/cm at low levels (accurate to 3 significant digits)
pH	0.1 pH units
Secchi Depth	0.1 m
Salinity	0.1 ppt
Light Attenuation (PAR)	0.05% at 100% light

Table 3 Minimum Detection Limits for Field Measurements

- Selecting appropriate spatial and temporal scales;
- Exploring data characteristics such as distribution, censoring, trend characteristics (step versus monotonic), variances, seasonality, persistence, and missing data;
- Adjusting for flow variability; and,
- Considering the power and robustness of the tests.

The document also briefly discusses specific statistical tests, such as the seasonal Kendall test, Van Belle and Hughes intrablock tests, and Mann-Kendall tests, and corrections for serial dependence. TMAW makes recommendations and is working to update the *Guidance for the Analysis of Water Quality Trends in Chesapeake Bay* to help analysts reach technically sound conclusions and interpretations and to foster a consistent approach to trend analysis among the various investigators and multiple jurisdictions involved in the monitoring and analysis of Chesapeake Bay water and habitat quality.

Beyond analysis of the Maryland monitoring data, DNR staff participates in Chesapeake Bay Program Monitoring and Analysis Subcommittee (MASC) activities to produce Bay-wide analyses and reports with cooperating state, federal and local agencies. This activity leads to a better Bay-wide understanding of water and habitat quality and addresses the linkage between water quality and living resources. The Bay Agreement of 1987 also called for a re-evaluation of the nutrient strategies in 1991 and in 1997. Annual updates of water and habitat quality status and trends also were analyzed and summarized in *The State of the Chesapeake Bay 2004*, the *Chesapeake Bay Nutrient Reduction Progress & Future Directions Nutrient Reduction Reevaluation Summary Report* (CBP 1997), *Bay 2007 Health and Restoration Assessment*, Tributary Strategy Team annual reports, and Basin Summary Reports.

The monitoring data also are used extensively in mathematical modeling efforts to project the water quality response of Chesapeake Bay to various management alternatives. A three-dimensional Chesapeake Bay and Tributary Water Quality time-variable model is presently under review. Results for earlier versions of the model have already been used to set nutrient reduction goals agreed to in the 1987 Bay Agreement and affirmed by the 1991 and 1997 Re-evaluations.

Other components of the DNR Chesapeake Bay Water Quality Monitoring Program are required to produce cumulative "Level I" data reports annually that describe the results of that component from the inception of the programs. These components include the Phytoplankton, Benthic, Ecosystem Processes, and River Input Programs. In addition to documenting the results of the individual monitoring components, these cumulative reports are intended to serve as "building blocks" for more integrated levels of analysis among the coordinated components.

8. PROJECT ORGANIZATION AND RESPONSIBILITY

This section lists the individuals responsible for the major aspects of the Chemical and Physical Properties Component of Maryland's Chesapeake Bay Water Quality Monitoring Program.

Director and Principal Investigator: Thomas Parham, Tidewater Ecosystem Assessment, DNR.

> RESPONSIBILITIES: The director and principal investigator is responsible for overseeing the administrative aspects of the program including fiscal management, coordination among other DNR managers and coordination with cooperating agencies and institutions. This individual is also responsible for the technical design, conduct and data analysis of the program.

Quality Assurance Officer: Bruce Michael, Resource Assessment Service, DNR.

RESPONSIBILITIES: The quality assurance officer is responsible for documenting and assuring the conduct of field, laboratory, and data management procedures that comprise this study.

Field Sampling Operations: Sally Bowen, Project Chief, Monitoring Field Office, DNR

RESPONSIBILITIES: This individual is responsible for administration of the field sampling activities including sample collection, sample storage and sample delivery to laboratories.

Field Sampling Quality Assurance Officer: Greg Gruber, Natural Resources Biologist IV, Monitoring Field Office, DNR.

RESPONSIBILITIES: This individual is responsible for assuring the quality of field procedures and equipment used in this study.

Laboratory Analyses/Water Column Chemistry: Carl Zimmerman, Chesapeake Biological Lab, University of Maryland

RESPONSIBILITIES: This individual is responsible for analysis of water samples collected in the mainstem and tidal tributaries.

Chlorophyll Analyses/Water Column Chemistry: Asoka Katumuluwa, Chief, Division of Environmental Chemistry, Laboratory Administration, DHMH

RESPONSIBILITIES: This individual is responsible for administration of the chlorophyll analysis of water samples.

Communications - Field: Bruce Michael, Resource Assessment Service, DNR

RESPONSIBILITIES: This individual is responsible for communications with Field Supervisors.

Communications - Laboratory: Renee Karrh, Thomas Parham, Tidewater Ecosystem Assessment, DNR

RESPONSIBILITIES: This individual is responsible for communications with Laboratory Supervisors.

Data Management: Tony Allred, Tidewater Ecosystem Assessment, DNR

RESPONSIBILITIES: This individual is responsible for overseeing the management of field and laboratory data collected under this program; managing historical field and laboratory data collected under this program; and maintaining existing data management software.

9. PROCEDURAL CHANGE PROTOCOL

Any permanent changes to field, laboratory or data management procedures must be approved by the Chesapeake Bay Program Office Quality Assurance Officer. Proposed changes are to be documented and submitted within 30 days using the Chesapeake Bay Program Procedure Modification Tracking Form. (See Appendix 14 for example Chesapeake Bay Program Procedure Modification Tracking Form).

10. LOG OF SIGNIFIGANT CHANGES

Procedural changes have been made over the years to address evolving water quality sampling program requirements, goals, budgetary changes, recommendations of the Analytical Methods and Quality Assurance Work Group and other issues. (See Appendix 15, LOG OF SIGNIFIGANT CHANGES).

A preliminary draft of the chronological list of changes to the monitoring program has been created and will be updated annually. The list is comprised of change implementation-dates and brief descriptive summaries of modified procedures. Additionally, changes in measured parameter analytical-detection-limits are summarized in tabular form.

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APPENDIX I

MARYLAND DEPARTMENT OF NATURAL RESOURCES
CHESAPEAKE BAY WATER QUALITY MONITORING PROGRAM

WATER COLUMN SAMPLING AND SAMPLE PROCESSING PROCEDURES

I. DEPTH SAMPLING PROTOCOLS

A. HydroLab/YSI Depth Sampling Protocols (Mainstem and Tributary)

1. Take readings of temperature, specific conductance, dissolved oxygen, and pH at 0.5 m, 1.0 m, 2.0 m and 3.0 m. Thereafter, take readings at a minimum of 2.0 m intervals (subject to conditions specified in A 2. below) and at the bottom. Mainstem bottom equals total depth minus one meter, rounded up to a whole meter. Tributaries bottom equals total depth minus one meter (not rounded). Tributaries hydrographic readings are also taken at 2.0 m.

2. If the change in DO exceeds 1.0 mg/l OR if the change in specific conductance equals or exceeds 1,000 micromhos/cm over any 2.0 m interval, take readings at the 1.0 m interval between these two readings. For total depths less than or equal to 10.0 m, take readings at 1.0 m intervals.

3. If a grab sampling depth above or below the pycnocline has not been sampled for *in situ* parameters, obtain readings at this depth.

4. At a minimum, take readings at 0.5, 1.0, 3.0 m, bottom, and every odd-numbered whole meter depth.

B. Grab Sampling Depth Protocols

1. At stations where two depths are sampled, take collections at:
   a. 0.5 m below surface.
   b. 1.0 m above bottom to nearest 1.0 m that is at least one full m from bottom (mainstem).
   c. 1.0 m above bottom (trib).

   NOTES: If total station depth is <1.5 m, take bottom sample also at 0.5 m. Exercise caution when taking bottom samples; if disturbed bottom sediments appear to have been included in a sample, resample after sediment has settled or take sample slightly higher in the water column.

2. ***Pycnocline Exists***: At stations where 4 depths are sampled and a pycnocline exists (see Section C, below), take collections at:

a. 0.5 m below surface.
b. 1.5 m above upper boundary of pycnocline.
c. 1.5 m below lower boundary of pycnocline.
d. 1.0 m above bottom to nearest 1.0 m that is at least one full m from bottom (mainstem).
e. 1.0 m above bottom (trib).

3. ***No Discernable Pycnocline***: At stations where 4 depths are sampled and there is no discernable pycnocline (see Section C, below), take collections at:

a. 0.5 m below surface.
b. at closest profile depth one third the distance from the surface to the bottom.
c. at closest profile depth two thirds the distance from the surface to the bottom.
d. 1.0 m above bottom to nearest 1.0 m that is at least one full m from bottom (mainstem).
e. 1.0 m above bottom (tributary).

C. Pycnocline Determination (Only for Stations Sampled at four depths)

The pycnocline is a region in which the water density changes appreciably with increasing depth and thus forms a layer of much greater stability than is provided by overlying surface waters.

1. The pycnocline Calculated Threshold Value (CTV) is used to determine the boundaries of the pycnocline and to calculate the depths at which grab samples should be collected.

The pycnocline Calculated Threshold Value (CTV) is derived using the equation below.

$$CTV = \frac{C_b - C_s}{D_b - D_s} \times 2$$

Where:

C_b = bottom conductivity (micromhos/cm),
C_s = surface conductivity (micromhos/cm),
D_b = depth of bottom conductivity measurement (m),
D_s = depth of surface conductivity measurement (m),
CTV = calculated threshold value (micromhos/cm)

ex. bottom conductivity: 15800 micromhos/cm
surface conductivity: 9500 micromh0s/cm
depth of bottom conductivity measurement: 14.6 m
depth of surface conductivity measurement: 0.5 m

$$CTV = \frac{15800 - 6300}{14.6 - 0.5} \times 2 = 893.6 \text{ micromhos/cm}$$

2. If the Calculated Threshold Value is greater than 500 micromhos/cm, a pycnocline exists with boundaries at the first and last depths where the change in conductivity is greater than the CTV. For example, continuing with the CTV value: 893 derived in the example calculation above, and evaluating conductivity readings moving up in the water column from the bottom, the lower boundary of the pycnocline occurs at first depth where the change in conductivity from that measured at the preceding depth exceeds 893. Moving upward in the water column, the upper boundary of the pycnocline occurs at last depth where the change in conductivity from that measured at the preceding depth exceeds 893. Samples will be taken as described in section B. 2., above.

   NOTE: In the rare cases when the sample is theoretically 'below the bottom' or 'above the surface', the follow these procedures. If the below pycnocline (BP) sample is determined to be below the bottom sample, collect the BP sample at the bottom sample depth. If the above pycnocline (AP) sample is determined to be above the surface sample, collect the AP sample at 0.5 m.

3. Take samples as described in section B. 3. (No Discernable Pycnocline), above, if either of the following two conditions are present:
   a. the CTV is less than 500 micromhos/cm.
   b. the CTV is equal to or greater than 500 micromhos/cm BUT no depth interval exceeds that CTV.

NOTES: Upper and lower boundaries of the pycnocline may be the same point. If this is the case, collect the Above Pycnocline sample 1.5 m above the upper pycnocline limit and collect the Below Pycnocline sample 1.5 m below the lower pycnocline limit.

D. Hydrogen Sulfide Protocols

1. For the mainstem only, when there is an odor of hydrogen sulfide present in the bottom sample or the below pycnocline sample, perform a Hach Kit test for hydrogen sulfide presence on the bottom and/or below pycnocline sample(s).

2. Immediately upon collection of the sample that meets the requirements in D. 1. above, transfer a portion of sample from the plastic sample container to the 25 ml Hach Test

glass container (from the Hach Hydrogen Sulfide Test Kit, Model HS-6), for hydrogen sulfide determination.

3. Immediately perform test for H_2S presence following instructions in Hach Hydrogen Sulfide Test Kit. Record results on the Cruise Report.

E. Secchi Depth

Measure water transparency using Secchi depth. Determine Secchi depth in meters to the nearest 0.1 meter using a 20-cm standard Secchi disc lowered into the water column with a calibrated rope. Make observations on the shady side of the boat.

F. Photosynthetic Active Radiation (PAR)

PAR readings (in μMoles/square meter/second) are taken in the field in order to calculate a light attenuation coefficient. Take PAR measurements with a LICOR quantum meter (Model LI-1000 Data Logger) with an attached underwater probe (Model LI-192SA). The probe is a flat, upwardly-directed probe.

Begin a vertical profile of light penetration by taking an initial reading with the sensor just below the surface of the water (0.1 m). Take subsequent measurements at either 0.25-m or 0.50-m intervals depending on the turbidity of the water column, (taking shallower measurements in more turbid water). Continue to take readings until a value less than ten percent (10 %) of the surface reading (0.1 m) is attained. Once the readings stabilize, allow at least five readings to flash on the display before recording the data reading for a specific depth. Record in the data logger the mean of the previous five readings that appear on the instrument display. Alternatively, the mean value may be recorded on the field datasheet.

The light measurements made for each profile are log-scale regressed against depth to determine the compensation depth, i.e., the depth of penetration of one percent (1 %) of the surface PAR. The compensation depth is used in computing the integrated carbon production for that water column. When light profiles are not available, the secchi disk depth is used to calculate the compensation depth. Over the study period, 1984-1996, a regression has been made between the secchi depth and the compensation depth for the same water column (for those stations where both secchi data and LICOR data are taken). By using this regression, a compensation depth can be estimated from a secchi depth.

The following table lists the parameters measured and the associated qualifiers to be

recorded for light attenuation:

FIELD	DESCRIPTION
SOURCE (PK, FK)	Code identifying agency or contractor that measured the data
PROJECT (PK, FK)	Agency monitoring project code
STATION (PK, FK)	CBP station name
SAMPLE_DATE (PK)	Date on which the PAR readings were taken
SAMPLE_TIME (PK)	Time at which the PAR readings were taken
DEPTH (PK)	Depth at which the PAR readings were taken (meters)
EPAR_S	PAR reading ($\mu M/m^2/s$) taken at the boat just before or during the measurement of PAR readings at depth
EPARU_Z	PAR reading ($\mu M/m^2/s$) taken at depth (up sensor)
UNITS	Units for PAR ($\mu M/m^2/s$)
METHOD	Method code identifying the field measurement procedure
COMMENTS	Comments related to the collection of PAR readings

II. SAMPLE COLLECTION

A. Lower submersible pump to desired depth.

B. Allow hose to flush completely before taking sample (flush time is pump dependent).

C. Rinse premarked sample container (plastic container) and cap three times with sample water.

D. Collect sample, cap the plastic container, and begin water sample processing and appropriate storage/preservation.

E. Any time a field duplicate is required (whenever indicated on the station lab data sheet), follow the procedures in the section "Split-sample collection method for field duplicates".

F. Enter on lab and field sheets all identifying information pertinent to samples collected.

III. SPLIT-SAMPLE COLLECTION METHOD FOR FIELD DUPLICATES

A. Samples for field duplicates are generated approximately one for every 20 samples collected.

B. Collect sample as in section II. A and II. B above.

C. Rinse duplicate collection container three times and fill with sample water.

NOTE: Collection container must be large enough to generate two complete samples. If more than one gallon of sample is needed for samples, fill a plastic bucket (2.5 to 5 gallon) with sample water and draw all samples from the bucket, taking care to maintain a homogeneous mixture as water is drawn from the duplicate container.

D. Begin water sample processing and appropriate storage/preservation.

E. Enter all identifying information pertinent to samples collected on lab and field sheets.

NOTE: Lab and field sheets must have a replicate number entered for each duplicate generated.

IV. FILTRATION, PROCESSING AND STORAGE OF CHLOROPHYLL SAMPLES

A. For every depth sampled, clean bell and frit with deionized water (DI-H_2O; stored in a high density polyethylene container) generated at the Field Office. Set up bell and frit for filtering. Ensure that there is a trap in line between the manifold and the vacuum source.

B. Place a Whatman GF/F glass fiber filter pad (pore size = 0.7 μm) on the filter frit. When handling the pad, use clean forceps.

C. Mix sample thoroughly by agitating plastic sample container vigorously, then rinse graduated cylinder three times with sample.

D. Fill graduated cylinder with sample and filter desired volume through filtration unit. Keep the vacuum below 10 inches of Hg. Filter sufficient volume of sample (100 - 1500 ml) to solidly color the filter pad. Do not suck the filter dry. In order to avoid cell damage, decrease the amount of vacuum as the final volume approaches the level of the filter and release the vacuum as the last of the water is pulled through the pad. Record the total volume filtered.

E. Add approximately 1 ml of $MgCO_3$ suspension (Laboratory grade from Fisher Scientific prepared in a 1.0 g $MgCO_3$ to 100 ml of DI-H_2O ratio) to the last 25 ml of sample in the filtration bell. This is equivalent to less than 1 mg of $MgCO_3$ per 15 ml extract.

NOTE: Filtrate for nutrient analysis should not be saved from this filtration.

F. Remove filter pad with forceps, fold filter in half with sample inside, place in pre-marked foil square, and carefully fold square in thirds, horizontally and vertically, to seal filter inside.

Be sure forceps do not touch sample residue on the filter pads, because the sample will adhere to the forceps.

G. Be sure that foil square is marked with date, station, sample layer code, volume of sample filtered, sample number, and "CHLA".

H. Place sample FOIL into premarked zip-lock plastic bag. Store bag of chlorophyll samples in Research Vessel freezer for mainstem samples or an ice chest for tributary samples. If samples are stored on ice, place in freezer on return to Field Office.

I. Record sample identifier, date, volume filtered (L), depth (m), layer, start time, end time, study code, submitter code, data category code, field scientist sign off, and replicate number, if necessary, on chlorophyll calculation sheet. This sheet is submitted to the laboratory with the samples.

NOTE: Filter pad with chlorophyll sample should be exposed to as little direct sunlight as possible. Store in foil as soon as possible.

V. FILTRATION, PROCESSING AND STORAGE FOR PARTICULATE FRACTIONS (PARTICULATE P, C, N AND TOTAL SUSPENDED SOLIDS)

A. Processing and storage - PC, PN:
For each depth sampled, thoroughly clean all bells and frits with DI-H_2O, set up filter apparatus, filters (two pre-combusted 25 mm GF/F filters, pore size = 0.7 μm), and bells for filtering. Filter 10-200 ml through each filter. Filter enough of the sample to leave noticeable color on the filter pad. Make sure filter is sucked dry. Using forceps, fold each filter in half. Place both filters in a foil square labeled with date, PC/PN-CBL sample number, station, sample layer, and volume filtered. Fold as described in IV.F. and then place folded foil in zip-lock bag, and put in freezer (large boats) or on ice (small boats).

B. Processing and storage - PP, TSS:
For each depth sampled, thoroughly clean all glassware with DI-H_2O. Set up two flasks, filters (two pre-weighed and numbered 47 mm GF/F filters placed with the pad number facing down), and bells for filtering. After rinsing a graduated cylinder three times with sample water, measure 50 - 200 ml of sample into each filter bell. Use the filtrate as an equipment rinse and discard. Note amount filtered through each filter. Then filter enough additional (another 50 -300 ml) to leave a noticeable color on the filter pad. Use this filtrate as required for filtered parameter analysis.
After collecting filtrate, make sure filter is sucked dry, and rinse three times with 10 ml rinses of water, sucking dry after each rinse. Using forceps, fold each filter in half. Place both filters in a foil square labeled with date, TSS/PP-CBL sample number, station, sample layer, and volume filtered. Fold as described in IV.F. and write sample number on foil with Sharpie permanent marker to facilitate post-combustion sample

identification. Place foil square in zip-lock bag, and put in freezer (large boats) or on ice in (small boats).

NOTES: Volume filtered is the total sample volume passed through one filter pad. The second pad must filter the same sample volume.

Ten percent of the filters that CBL supplies for field filtering TSS must be pre-rinsed 3 times with deionized water, dried at 103-105 °C for 1 hour, then weighed, re-dried and reweighed until a constant weight is obtained. (Alternatively, purchase certified, pre-weighed filters.)

C. Processing and storage - VSS:
VSS samples are collected from the surface sample at pre-determined stations. Thoroughly clean all glassware with DI-H_2O. Set up one flask, filter (1 pre-weighed, pre-combusted and numbered 47 mm GF/F filters, placed wrinkled side up), and bell for filtering. The number for the pad is written on the individual Petri dish that the pad came in. You must write this number on the foil square label. After rinsing a graduated cylinder three times with sample water, measure 50 - 200 ml of sample into each filter bell. Use the filtrate as an equipment rinse and discard. Note amount filtered through the filter. Then filter enough additional (another 50 -300 ml) to leave a noticeable color on the filter pad. You may use this filtrate as required for filtered parameter analysis. After collecting filtrate, make sure filter is sucked dry, and rinse three times with 10 ml rinses of water, sucking dry after each rinse. Using forceps, fold each filter in half. Place the filter in a foil square labeled with date, VSS-CBL sample number, pad number, station, sample layer, and volume filtered. Fold as described in IV.F. and write sample number on foil with Sharpie permanent marker to facilitate post-combustion sample identification. Place foil square in the TSS/PP zip-lock bag, and put in freezer (large boats) or on ice in (small boats).

VI. FILTRATION, PROCESSING AND STORAGE FOR "DISSOLVED" FRACTIONS (NH_4, NO_2, NO_3, PO_4, Si, TDN, TDP, DOC)

A. This filtrate always comes from particulate phosphorus/TSS filters, section V, above. Use GF/F filters, and pre-rinse the filter and flask with at least 50 ml of sample water. If sample is taken from particulate filtration, take it prior to rinsing with DI-H_2O.

B. Processing and storage - NH_4, NO23, NO_3, PO_4, Si:
Triple rinse, with filtrate, four like-numbered autoanalyzer (AA) vials and caps. Fill approximately 7/8 full, allowing for sample expansion upon freezing. Place three of the AA vials in a rack in the freezer; the fourth vial (silica) should be stored at 4 °C in the R/V refrigerator. On small boats, keep all samples iced in a cooler, then freeze all but silica upon return to Field Office.

NOTE: The number on all vials and tubes is the CBL sample number and should match the number on TSS/PP and PC/PN foil pouches for each particular sample.

C. Processing and storage - TDN, TDP:

Triple rinse test tube, cap, and 10 ml graduated cylinder with filtrate. Be sure number on test tube corresponds to the number on the vials and sample number. Use 10 ml graduated cylinder to measure EXACTLY 10.0 ml of filtrate. (The 10 ml graduated cylinders will be calibrated annually by the Chesapeake Biological Laboratory.) Shake any remaining rinse water out of the test tube. Pour into pre-rinsed test tube and cap sample, then freeze sample in test tube rack on large boats. On small boats, keep sample on ice in cooler, then freeze upon return to Field Office.

D. Processing and storage - DOC (Bay Tributary stations only; not measured for Mainstem stations): Triple rinse 50 ml test tube and cap with filtrate. Fill the 50 ml test tube 3/4 full with filtrate and cap sample, then freeze in test tube rack. On small boats, keep sample on ice, then freeze at Field Office.

VII. ROUTINE MAINTENANCE OF FILTRATION UNITS AND CONTAINERS FOR MAINSTEM CRUISES AND AFTER RETURNING FROM FIELD

A. After each day's sampling on mainstem cruises, filtration units, flasks, frits and graduated cylinders should be cleaned with a non-phosphorus liquid soap, rinsed with tap water three times, then rinsed with 10% HCL (prepared from concentrated HCL from Fisher Scientific diluted with DI-H_2O), tap rinsed, and finally rinsed three times with DI-H_2O. All open flasks, filtration units and graduated cylinders should then be covered to prevent contamination if filtering is not to begin immediately. The filtration unit used for chlorophyll *a* filters should be washed with soap and rinsed with tap and DI water and not be rinsed with 10 percent HCl.

B. Big boat units are cleaned at the end of each day's sampling. Small boat or land run units are rinsed with DI-H_2O at end of each day's use and cleaned weekly, or after processing 20 to 30 samples.

APPENDIX II

MARYLAND DEPARTMENT OF NATURAL RESOURCES
CHESAPEAKE BAY WATER QUALITY MONITORING PROGRAM

FIELD SHEET, LAB SHEET, AND CHLOROPHYLL LAB SHEET DOCUMENTATION AND PROCEDURES

The following information reviews the conventions for filling out the Field Sheets, Lab Sheets (nutrient volume sheets), and Chlorophyll Lab Sheets used for the Chesapeake Bay Monitoring Program.

Refer to the examples of Field Sheets A and B, Lab Sheets (nutrient volume sheets), and Chlorophyll Lab Sheets at the end of this appendix. The codes used for this program are listed in Appendix XI.

NOTE: Leave blank any boxes on the Field Sheet for which data are not collected.

Field Sheet A:

The Field Sheets are sent along with a Cross Reference Sheet from the Field Office to the Data Management Unit at the DNR Tawes Building. (See Appendix III for information on the Cross Reference Sheet.) The Field Office must provide the following information on the Field Sheet.

1. Sequence Number (boxes 3-9, upper right hand corner)

The following convention has been used to designate the 7-digit sequence number for the mainstem, where YY is last two digits of year, NNN is the cruise number (that year), and SS is the station order for that week's cruise:

MAINSTEM Convention YYNNNSS

For example, sequence number 8401204 is the 12th cruise in 1984 at station 4 for that weeks cruise.

The following convention has been used to designate the 7-digit sequence number for the tributary sampling, where YY is last two digits of year, MM is month, T is for tributary, P is for Patuxent, M is for Potomac, C is for CORE, and XX is arbitrary ordering number:

TRIB Convention	YYMMTXX
PXT Convention	YYMMPXX
POT Convention	YYMMMXX
CORE Convention	YYMMCXX

For example, 9603P05 is the fifth field sheet for a March Patuxent cruise in 1996.

NOTE: The ordering numbers continue to increase throughout the month. For example, the first Patuxent cruise in March may have sheets numbered 01-14 and the second Patuxent cruise for that same month would have sheets numbered 15-28.

2. Sampling Station Number (boxes 10-18)

   Enter the appropriate Chesapeake Bay Program station location (e.g.MWT5.1, MET5.2) beginning with the box numbered 10. Put only one character (including decimal points) per box.

3. Start Date (boxes 20-25)

   Enter the start date beginning with year, month, day.

4. Start Time (boxes 27-30)

   Enter the start time of the sampling effort at a station location in military time.

5. End Date (boxes 32-37)

   If the end date for a particular station is the same as the start date, the end date boxes can be left blank.

6. End Time (boxes 39-42)

   Enter the end time of the sampling collection effort at a station location in military time. The end time is the time at the end of *in situ* data collection (meter readings).

7. Number of Samples (boxes 44-45)

   Enter the number of samples taken (including duplicates) at the station location. Routinely, there are two to five samples collected at stations for the Chesapeake Bay Monitoring program.

8. Submitter Code (boxes 47-48)

   The submitter codes specify the collection group and the lab that will perform the analyses.

9. Data Category Code (boxes 50-51)

   The data category codes, which are listed in Appendix XI, specify the code for the type of sample being collected. For example, for the Chesapeake Bay Program Main Bay Sampling, the code is 'MB' - Chesapeake Bay Monitoring Sample- MD. Main Bay".

10. Total Depth (M) (boxes 53-55)

    Enter the total depth at the station in meters to the nearest 0.1 m for tributary stations and to the nearest 0.5 m for mainstem stations.

11. Study Code (boxes 57-58)

    The study codes, which are listed in Appendix XI, indicate the type of monitoring program. For example, '01' is the study code for the "Chesapeake Bay Monitoring Program - Main Bay".

12. Sample Method (line #2, box 10)

The sample method codes, which are listed in Appendix XI, indicate the sample method used for the sampling effort. For example, '1' is the code for 'Grab Samples'.

Note: If no water samples are collected (not even bacti samples), the code is '7'.

13. Air Temperature degrees Celsius (line #2, boxes 11-14)

Air temperature is reported in degrees Celsius to the nearest 0.5 degrees. The value is recorded in boxes 12-14. There is a box (#11) to indicate a plus (+) or minus (-) value. Leave this blank if temperature is greater than or equal to zero; write in a minus (-) sign if it is below zero.

14. Tide State (line #2, box 18)

Tide state codes are listed in Appendix XI. For example, the code 'E' specifies an ebb tide.

15. Weather Code Yesterday (line #2, boxes 20-21)

Enter the code for yesterday's weather in these boxes. The weather codes with their corresponding descriptions are listed in Appendix XI. Additional weather information can be included in the comments section if appropriate.

16. Weather Code Today (line #2, boxes 23-24)

Enter the code for current weather (while at station) in these boxes. Additional weather information can be included in the comments section if appropriate.

17. Percent Cloud Cover (line #2, boxes 25-27)

Enter the amount of cloud cover in these boxes. Percent Cloud Cover is reported as values from 00 to 100 percent. Numbers must be **right** justified, e.g., _ _ 5 (not 5 _ _).

18. Wind Direction (line #2, boxes 28-30)

Record wind direction using the codes:

N - Northerly direction
S - Southerly direction
E - Easterly direction
W - Westerly direction

Record wind direction in boxes 28-30 using up to three letters to designate the prevailing conditions. An example of wind direction would be 'north by north east' and the codes in boxes 28-30 would be 'NNE'. If only one or two letters are needed to designate the conditions, use the boxes beginning with box #28 for the codes. Letters must be **left** justified, e.g., S W _ (**not** _ S W)

19. Wind Velocity (knots) (line #2, boxes 31-32, 33-34)

Record wind velocity in knots in boxes 31-32, 33-34. Record the minimum (or lower range) velocity in boxes 31-32; record the maximum (or upper range) velocity in boxes 33-34. For example, if the wind is blowing from10 to 20 knots, the minimum wind velocity is '10,' and the maximum wind velocity is '20.' If only one number is needed to designate the wind velocity conditions, enter the identical numbers in both the boxes for minimum velocity as well as in the boxes for maximum velocity. Beaufort wind force scale values may be used when recording wind velocity. 01-03, 04-06, 07-10, 11-16, 17-21, 22-27, and 28-33.

number	Wind speed				Mean wind speed (kt / km/h / mph)	Description	Wave height		Sea conditions	Land conditions
	kt	km/h	mph	m/s			m	ft		
0	0	0	0	0-0.2	0 / 0 / 0	Calm	0	0	Flat.	Calm. Smoke rises vertically.
1	1-3	1-6	1-3	0.3-1.5	2 / 4/ 2	Light air	0.1	0.33	Ripples without crests.	Wind motion visible in smoke.
2	4-6	7-11	4-7	1.6-3.3	5 / 9 / 6	Light breeze	0.2	0.66	Small wavelets. Crests of glassy appearance, not breaking	Wind felt on exposed skin. Leaves rustle.
3	7-10	12-19	8-12	3.4-5.4	9 / 17 /11	Gentle breeze	0.6	2	Large wavelets. Crests begin to break; scattered whitecaps	Leaves and smaller twigs in constant motion.
4	11-16	20-29	13-18	5.5-7.9	13 / 24 / 15	Moderate breeze	1	3.3	Small waves.	Dust and loose paper raised. Small branches begin to move.
5	17-21	30-39	19-24	8.0-10.7	19 / 35 / 22	Fresh breeze	2	6.6	Moderate (1.2 m) longer waves. Some foam and spray.	Smaller trees sway.
6	22-27	40-50	25-31	10.8-13.8	24 / 44 / 27	Strong breeze	3	9.9	Large waves with foam crests and some spray.	Large branches in motion. Whistling heard in overhead wires. Umbrella use becomes difficult.
7	28-33	51-62	32-38	13.9-17.1	30 / 56 / 35	Near gale	4	13.1	Sea heaps up and foam begins to streak.	Whole trees in motion. Effort needed to walk against the wind.

20. Secchi (M) (line #2, boxes 35-38)

Record Secchi depth in meters to the nearest 0.1 meter.

21. Flow Value (line#2, boxes 39-46)

Note that flow is not recorded in regular scientific notation, but is recorded as follows. Box #39 is the flow basis code, where:

1 = measured in cubic feet per second (CFS)
2 = estimated in cubic feet per second (CFS)
3 = measured in million gallons per second (MGS)
4 = estimated in million gallons per second (MGS)
5 = measured in gallons per day (GPD)
6 = estimated in gallons per day (GPD)

Boxes 40-44 are for the five-digit mantissa and box 45 is for the exponential value in base 10. These boxes are to be left blank at boat or other stations where flow is not recorded.

For example, estimated flow 4.5_{cfs} = 2.450001, where "2" indicates that the flow is estimated in cubic feet per second, "45000" indicates that the mantissa is 4.5000, and "1" indicates multiply the mantissa by 10^1.

The final box, #46, is for greater or less than (G or L).

Note: Flow value is not a required parameter and is seldom measured.

22. Senior Scientist (line #2, boxes 47-49)

The three initials of the senior scientist (the scientist in charge of the sampling effort for that day) are entered in these boxes.

23. DO Method (line #3, box 50)

The codes for the dissolved oxygen (DO) methods are listed in Appendix XI. For example, the code 'H' is used for a DO value collected with a Hydrolab.

24. Equipment Set Unit # (line #3, boxes 51-52)

The numbers assigned to equipment packages is recorded in these boxes.

25. Probe Number (line #3, boxes 53-54)

Enter the Hydrolab probe number in these boxes. If using spares, enter the same equipment letter in probe number box and record spare number in comments boxes.

The text of the label over boxes 53-54 on the field sheets used on Patuxent River project is “LiCor Number” instead of “Probe Number”. (See Patuxent field sheet example at the end of this appendix).

26. Flow/Tide Unit Number (line #3, boxes 55-56)

Enter in boxes 55-56 the number of the meter used to measure the flow value. These boxes should be left blank if flow was not recorded for the station.

The text of the label over boxes 55-56 on the field sheets used on Patuxent River project is "LiCor Method" instead of "Flow/Tide Unit Number". (See Patuxent field sheet example at the end of this appendix).

27. Wave Height (M) (line #3, boxes 57-59)

Wave height is recorded in meters.

28. Upper Pycnocline Limit (M) (line #3, boxes 60-62)

The calculated value for the upper pycnocline limit is recorded in meters and is entered in these boxes. If no pycnocline exists, leave these boxes blank.

29. Lower Pycnocline Limit (M) (line #3, boxes 63-65)
The calculated value for the lower pycnocline limit is recorded in meters and is entered in these boxes. If no pycnocline exists, leave these boxes blank.

30. Scientist Signoff (line #3, boxes 66-68)

A DATA SHEET WITH NO SCIENTIST SIGNOFF WILL NOT BE SENT TO THE DATA ENTRY SERVICE.

The scientist who checked over the field sheet for:

- the correct codes
- the correct date
- the correct start time and end time
- the correct sampling station number
- reasonable values for the parameters
- the values for the parameters are entered on the sheet properly

enters his/her initials in these boxes.

Ideally, the individual who initiates the signoff is a separate individual from the one who enters the values on the data sheet. This process of using two separate individuals whenever possible, one to enter the values onto the sheet and one to check over the values that are entered, can help minimize transcription errors and correct aberrations in protocol. However, when a scientist works alone, the same scientist who enters the values checks the sheets before leaving the station.

31. Comments (beginning on line #3 - #5)

Any comments that are necessary to fully describe the sampling effort should be entered in the Comment section. Use one box for each character, decimal point, or period.

31. Replicate Number (line #6, box 11)

If the values for specific conductance, water temperature, DO, etc. are repeated for a single depth and are entered on the field sheet, indicate this by entering the replicate number (from 2 to 9) in these boxes. A blank in box #11 defaults to 01.

32. Depth (M) (line #6, columns 13-15)

Enter the depth at which the suite of parameters is measured (in meters).

33. Water Temperature degrees C (line #6, columns 17-20)

The water temperature is recorded in degrees Celsius. The value is recorded in columns 18-20; column #17 is to indicate a minus (-) value. Leave this column blank if temperature is greater than or equal to zero; write in a minus (-) sign if it is below zero.

34. Field pH (line #6, columns 22-25)

Enter values for field pH in these columns (round pH to the nearest tenth).

35. Value Corrected (line #6, column 27)

Use one of the three codes for DO correction in Appendix XI.

36. DO (mg/l) (line #6, columns 28-32)

Enter the DO value in columns 29-32. Column #28 is used to indicate greater than (G) or less than (L) values. A less than (L) in column #28 indicates that the value for DO in columns 29-32 is the detection limit for the DO probe. The code "A" may be used in the column designated for G/L when an adjustment has been made for drift found in the post calibration of the meter. The code "E" may be used in the column designated for G/L when the value in columns 29-32 is considered an "estimate" rather than a value that falls within the stated error range. Estimated values may reflect variability at that water depth (i.e., in the mixing zone) or they may reflect an estimate that appears reasonable to the analyst but they have some reservations; see the comments section for notes explaining the "E" value.

37. Specific Conductance (micromhos/cm at 25°C) (line #6, columns 34-39)

Enter the values for specific conductance in columns 35-39. Use column #34 to indicate greater than (G) or less than (L) values. The code "A" may be used in the column designated for G/L when an adjustment has been made for drift in post-calibrating the meter. The code "E" may be used in the column designated for G/L when the analyst considers the value in columns 35-39 to be credible, but outside normal measurement variability.

NOTE: Hydrolab reports microsiemens/cm. Since specific conductance is reported at 25°C, microsiemens/cm=micromhos/cm at 25°C.

38. Salinity (ppt) (line #6, columns 40-43)

Enter a value for salinity in columns 40-43.

39. Lab Login Section (line # 6, columns 49-63)

This section is used to record the number of replicate water samples which were collected, the depth at which the samples were collected, the layer from which the samples were collected, and the bottle numbers that the samples were assigned. (Note the designation AP and BP indicate above and below pycnocline only if a pycnocline actually was present. If no pycnocline they indicate below surface and above bottom at 1/3, 2/3 depths.)

A. Replicate (line #6, column 49)

If more than one sample is collected for analysis at an identical depth, indicate this by entering a 1, 2, 3, etc. to differentiate the replicates. Leaving this column blank results in a default to 1.

B. Sample Depth (M) (line #6, columns 50-52)

Record the depth in meters at which the samples were collected. Meter readings are required for this depth.

C. Layer Code (line #6, columns 53-54)

Indicate at which layer the samples were collected. The layer codes are listed in Appendix XI. Enter layer code (S=surface, B=bottom, AP=above pycnocline, BP=below pycnocline). Left justify single-character codes (i.e., codes with only one letter).

D. Bottle Numbers (line #6, columns 55-63)

Enter the bottle numbers assigned to the samples. Up to nine alphanumeric characters can be used. If less than nine characters are used, left justify. These bottle numbers are the same as those indicated on lab sheets.

40. Pycnocline Threshold Calculations

This section is used as a worksheet to calculate the pycnocline. The following symbols are used in the formula.

Δ = Delta (used to indicate change)

$\bar{X}$ = Mean

$\bar{X}\,\Delta\,M$ = indicates mean change (Delta) per meter

41. Date entered (entered by keypunch at bottom left of sheet)

Date returned from keypunching (entered by keypunch at bottom of sheet).

42. Page____of_____ (bottom right of sheet)

If only one sheet is generated at a station, leave this blank; the default value is 'page 1 of 1.' When two sheets are generated at one station, enter in this area 'page 1 of 2' for the first sheet generated, and 'page 2 of 2' for the second sheet generated. The second sheet generated at a sampling location is Field Sheet B, discussed next.

Field Sheet B:

Use Field Sheet B when two field sheets are generated at one sampling location.

1. Sequence Number

   Use the same convention (described above) for sequence number for this field sheet. The second sheet generated at one location must have the identical sequence number as the first sheet. The two sheets should not be stapled together.

2. Top Half of Form

   The top of this form only has lines for Sampling Station Number, Date, Start Time, and End Time (the boxes have been replaced with lines). Enter this information to alleviate the problem of mismatched or unidentifiable sheets.

3. Bottom Half of Form

   The bottom half of this form is the same as the field sheet previously discussed. There is no need to enter information on the second sheet for the Lab Login or pycnocline calculation.

Nutrient Lab Sheet (also called nutrient volume sheet; for nutrient analysis)

When nutrient samples are collected, a nutrient lab sheet is generated, and serves as a Sample Transfer Sheet. The nutrient lab sheet lists multiple stations that contain information for several samples on one sheet. Information on the sheet includes the sample number, layer, depth, time, salinity, and volume sampled for each set of nutrient parameters (e.g., TSS/PP, PC/PN). This sheet is filled out by field personnel and must accompany the samples to CBL. CBL produces electronic files on a data diskette for uploading onto the Chesapeake Bay Program mainframe and keypunch is not necessary.

1. Cruise Identification Number (Mainstem stations only)

   Enter the cruise identification number in the space provided (year and cruise number, e.g., 97018 for 1997, Cruise Number 18).

2. Date

   Enter the date in the space provided. It does not need to be in any specific format.

3. Scientist Signoff

   The scientist must check the sheet for completeness and accuracy, and then initial in the signoff space.

4. Station, Sample Number, Layer

   Enter the station, sample number, and layer code (S=surface, B=bottom, AP=above pycnocline, BP=below pycnocline), if not preprinted.

5. Sampling Time (column 5)

   Enter the sampling time in military time in column 5.

6. Salinity (column 6)

   Enter the salinity in parts per thousand (ppt) in column 6.

7. Vol. (ml) (final 2 or 3 columns)

   In the final 2 or 3 columns, enter the volume sampled for each set of sample parameters (e.g., TSS/PP, PC/PN) in milliliters.

Chlorophyll Lab Sheet: (for chlorophyll analysis)

When chlorophyll samples are collected, a chlorophyll lab sheet is generated, and serves as a Sample Transfer Sheet. The chlorophyll lab sheet is very similar to the nutrient lab sheet. Like the nutrient lab sheet it is a multiple station sheet that contains information for several samples on one sheet. Information on the chlorophyll lab sheet completed by the field personnel include the survey name, submitter agency and field scientist name, study code, submitter code, data category code, field scientist signoff, and sample number, bottle number (usually the same as the sample number), billing tracking category (CT), sampling station number, replicate number (RP), depth, layer code, start date, start time, end time, and volume filtered. This sheet is filled out by field personnel and accompanies the samples to DHMH.

When the samples and chlorophyll lab sheet arrive at DHMH, lab personnel stamp the date received and time received in the appropriate boxes (upper right of chlorophyll lab sheet). DHMH produces a hard copy of the data (a "chlorophyll sheet") as well as an electronic file on a data diskette for uploading onto the Chesapeake Bay Program mainframe so keypunch is not necessary.

The sheet must be filled out with the following information by the field personnel before it arrives at the lab:

1. Survey (top left)

   Write in the survey name that indicates the appropriate monitoring program. For example, 'Main Bay' indicates the "Chesapeake Bay Monitoring Program - Main Bay".

2. Submitter (top left, below Survey)

   The submitter specifies the agency (DNR) and the field scientist conducting the collection and submitting the samples to the laboratory for analysis.

3. Sequence Number (top right, boxes 3-9)

   For the Mainstem stations, field personnel assign the sequence number by the using the last two digits of the year, the cruise number, and the sheet number (e.g., 8701701 for sheet number 1 of cruise number 17 in 1987) . For Bay Tributary stations, however, laboratory personnel assign the sequence number on the chlorophyll lab sheet.

4. Study Code (line #4, boxes 17-18)

   The study codes, listed in Appendix XI, indicate the type of monitoring program. For example, '01' is the study code for the "Chesapeake Bay Monitoring Program - Main Bay".

5. Submitter Code (line #4, boxes 19-20)

   The submitter codes, located in Appendix XI, specify the collection group and the lab that will perform the analyses.

6. Data Category Code (line 4, boxes 21-22)

   The data category code indicates the type of data being collected. See Appendix XI for a list of codes.

7. Field Scientist Signoff (line #4, boxes 23-25)

   A DATA SHEET WITH NO SCIENTIST SIGNOFF WILL NOT BE SENT TO THE DATA ENTRY SERVICE.

   The scientist who checked over the chlorophyll lab sheet for:
   the correct codes
   the correct date
   the correct start time
   the correct sampling station number
   the correct values for the volume (amount filtered)
   the values are entered on the sheet properly

   enters her/his initials in the signoff boxes.

8. Sampling Station Number (line #5, boxes 10-18)

   Enter the appropriate station location beginning with the box numbered 14. Put only one character (decimal point, period) per box.

9. Replicate Number (line #5, box 19)

   If the sample is a replicate sample, (if more than one sample is taken at this depth) indicate this by entering the replicate number (2 through 9). If this field is left blank, it defaults to 1.

10. Depth (M) (line #5, boxes 20-22)

Enter the depth in meters at which the sample was collected.

11. Layer Code (line #5, boxes 23-24)

    Enter the layer code for the sample (S=surface, B=bottom, AP=above pycnocline, BP=below pycnocline). If the layer code is only one character, left justify.

12. Start Date (line #5, boxes 25-30)

    Enter the Start Date (from the field sheet) in these boxes beginning with year, month, day.

13. Start Time (line #5, boxes 31-34)

    Enter the start time (from the field sheet) in military time.

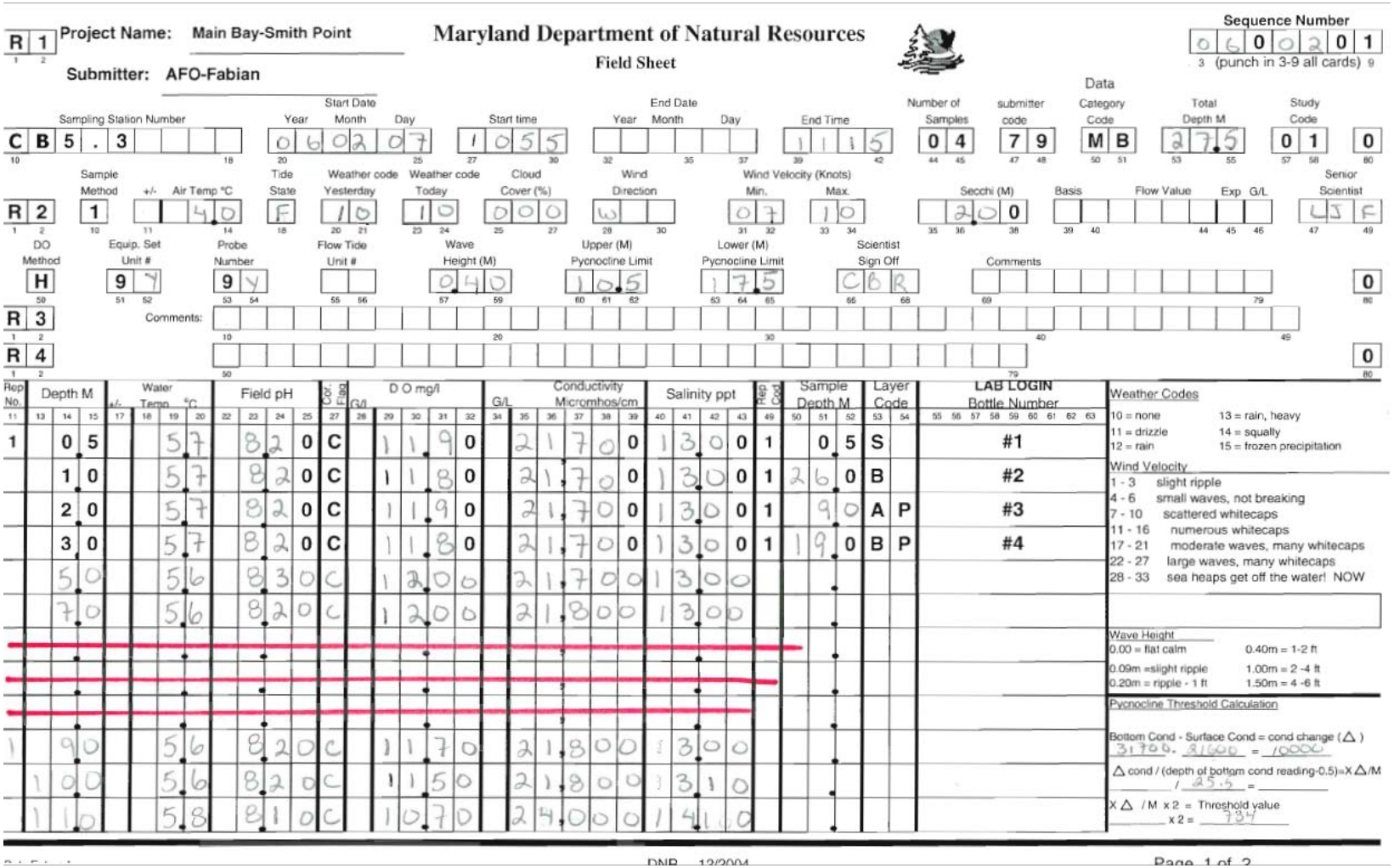
14. End Date (line #5, boxes 35-40)
    Enter the end date (from the field sheet) in these boxes beginning with year, month, day.

15. End Time (line #5, boxes 41-44)

    Enter the end time (from the field sheet) in military time.

16. Amount Filtered (Liters) (line #5, boxes 45-47)

    Enter the amount of sample filtered in liters in these boxes.



R 1 Project Name: Main Bay-Smith Point
Submitter: AFO-Fabian

Maryland Department of Natural Resources
Field Sheet

Sequence Number: 0 6 0 0 2 0 1 (punch in 3-9 all cards)

Sampling Station Number	Start Date Year	Month	Day	Start time	End Date Year	Month	Day	End Time	Number of Samples	submitter code	Data Category Code	Total Depth M	Study Code
C B 5 . 3	0 6	0 2	0 7	1 0 5 5				1 1 1 5	0 4	7 9	M B	2 7.5	0 1 0

Sample Method	+/-	Air Temp °C	Tide State	Weather code Yesterday	Weather code Today	Cloud Cover (%)	Wind Direction	Wind Velocity (Knots) Min.	Max.	Secchi (M)	Basis	Flow Value	Exp	G/L	Senior Scientist
R 2	1	4 0	F	1 0	1 0	0 0 0	W	0 7	1 0	2 0 0					U J F

DO Method	Equip. Set Unit #	Probe Number	Flow Tide Unit #	Wave Height (M)	Upper (M) Pycnocline Limit	Lower (M) Pycnocline Limit	Scientist Sign Off	Comments
H	9 7	9 4		0 4 0	1 0 5	1 7 5	C B R	0

R 3 Comments:

R 4 0

Rep No.	Depth M	+/-	Water Temp °C	Field pH	Col Filter	DO mg/l	G/L	Conductivity Micromhos/cm	Salinity ppt	Rep Code	Sample Depth M	Layer Code	LAB LOGIN Bottle Number
1	0.5		5.7	8.2 0	C	11.90		21,700	13.0 0	1	0.5	S	#1
	1.0		5.7	8.2 0	C	11.80		21,700	13.0 0	1	2.6 0	B	#2
	2.0		5.7	8.2 0	C	11.90		21,700	13.0 0	1	9.0	A	P #3
	3.0		5.7	8.2 0	C	11.80		21,700	13.0 0	1	19.0	B	P #4
	5.0		5.6	8.3 0	C	12.00		21,700	13.00				
	7.0		5.6	8.2 0	C	12.00		21,800	13.00				
1	9.0		5.6	8.2 0	C	11.70		21,800	13.00				
1	0.0		5.6	8.2 0	C	11.50		21,800	13.10				
1	1.0		5.8	8.1 0	C	10.70		24,000	14.10				

Weather Codes
10 = none; 11 = drizzle; 12 = rain; 13 = rain, heavy; 14 = squally; 15 = frozen precipitation

Wind Velocity
1 - 3 slight ripple
4 - 6 small waves, not breaking
7 - 10 scattered whitecaps
11 - 16 numerous whitecaps
17 - 21 moderate waves, many whitecaps
22 - 27 large waves, many whitecaps
28 - 33 sea heaps get off the water! NOW

Wave Height
0.00 = flat calm; 0.09m = slight ripple; 0.20m = ripple - 1 ft; 0.40m = 1-2 ft; 1.00m = 2 -4 ft; 1.50m = 4 -6 ft

Pycnocline Threshold Calculation
Bottom Cond - Surface Cond = cond change (△)
31700 - 21600 = 10000
△ cond / (depth of bottom cond reading-0.5)=X △/M
10000 / 25.5 = _____
X △ / M x 2 = Threshold value
_____ x 2 = 784

DNR 12/2004 Page 1 of 2

R 1 Project Name: Main Bay:Smith Point

Submitter: AFO-Fabian

Maryland Department of Natural Resources

Field Sheet

Sequence Number: 0 6 0 0 2 0 1 (punch in 3-9 all cards)

Sample Station Number: CB5.3 DATE: 2-7-06 Start Time: 1055 End Time: 1115

Rep No.	Depth M	+/-	Water Temp °C	Field pH	Cor: F Bot	G/L	D O mg/l	G/L	Conductivity Micromhos/cm	Salinity ppt	Rep Code	Sample Depth M	Layer Code	LAB LOGIN Bottle Number
	120		5.9	8.00	C		9.60		29,900	18.50				
	130		5.9	7.90	C		9.60		30,000	18.60				
	140		5.9	8.00	C		9.60		30,100	18.60				
	150		5.9	7.90	C		9.50		30,200	18.70				
	160		5.9	7.90	C		9.50		30,300	18.70				
	170		5.9	7.90	C		9.50		30,500	18.90				
	180		6.0	7.90	C		9.50		31,300	19.40				
1	190		6.0	8.00	C		9.50		31,400	19.50				
	210		6.1	8.00	C		9.60		31,600	19.70				
	230		6.1	8.00	C		9.50		31,700	19.70				
	250		6.1	8.00	C		9.60		31,700	19.70				
	260		6.1	8.00	C		9.60		31,700	19.70				

Date Entered DNR 1/2005 Page 2 of 2

RECEIVED 2/10/06

CRUISE # 06002

DATE 2-7-06

DNR-MANTA

MAIN BAY-WINTER

SCIENTIST SIGNOFF LJF

STATION	SAMPLE #	LAYER CODE	DEPTH (M)	TIME (MLTY)	SALINITY (ppt)	TSS/PP FILT.(ml)	PC/PN (ml)	VSS (ml)
CB5.3	1	S	0.5	1055	13.0	500	200	
	2	B	26.0		19.7	400	150	
	3	AP	9.0		13.0	500	150	
	4	BP	19.0	↓	19.5	400	150	
LE2.3	5	S	0.5	1006	11.6	500	150	
	6	B	19.0		13.5	400	150	
	7	AP	7.0		11.9	400	150	
	8	BP	13.0	↓	12	400	150	
CB5.2	9	S	0.5	1201	11.0	500	150	500
	10	S DUP	0.5		11.0	500	150	500
	11	B	30.0		18.00	150	80	
	12	AP	7.0		11.2	400	150	
	13	BP	21.0	↓	17.3	250	100	
CB5.1	14	S	0.5	1312	10.5	500	150	
	15	B	33.0		16.8	200	150	
	16	AP	8		11.7	400	150	
	17	BP	18.0	↓	15.3	250	150	
CB4.4	18	S	0.5	1358	9.6	400	150	
	19	B	31.0		16.6	250	150	
	20	B DUP	~~31.0~~		16.6	250	150	
	21	AP	5.0		9.9	400	150	
	22	BP	19.0	↓	16.5	250	150	

R 1

Survey: Main Bay page 1 of 4

Maryland Department of Natural Resources 000469

Sequence Number: 060201 (3 (punch in 3-9 all cards) 9)

Submitter: AFO-L. Fabian (410)990-4524

Batch Number:

Chlorophyll Sheet

Date Received by Lab: Year Month Day — FEB 10 2006

Time Received by Lab: Hour Minute

Study Code: 01 | Code: 79 | Data Category Code: MB | Field Scientist Sign Off: LJF

17 are 1C

R 2

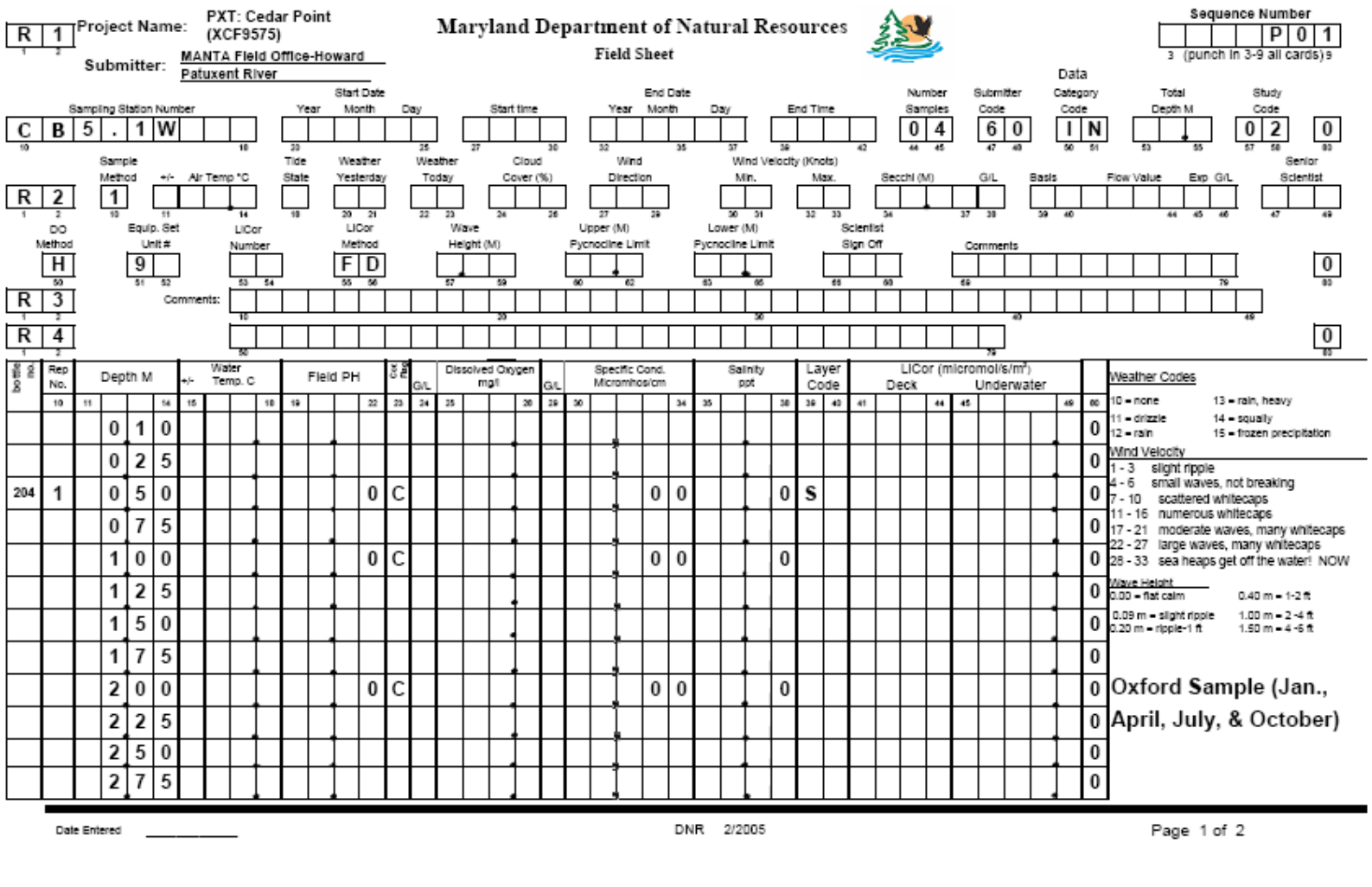
#	Bottle Number	CT	Sampling Station	REP	Depth M	Layer Code	Start Date Year Month Day	Start Time	End Date Year Month Day	End Time	Amt. Filtered Liters
1	1	1C	CB5.3	1	05	S	060207	1055		1115	050
2	2	1C		1	26.0	B					040
3	3	1C		1	9.0	AP					050
4	4	1C		1	19.0	BP					040
5	5	1C	LE2.3	1	05	S	060207	1006		1019	050
6	6	1C		1	19.0	B					040
7	7	1C		1	7.0	AP					050
8	8	1C		1	13.0	BP					046
9	9	1C	CB5.2	1	05	S	060207	1201		1228	050
10	10	1C		2	05	S					050
11	11	1C		1	30.0	B					035
12	12	1C		1	7.0	AP					040
13	13	1C		1	21.0	BP					035
14	14	1C	CB5.1	1	05	S	060207	1312		1337	050
15	15	1C		1	33.0	B					048
16	16	1C		1	8.0	AP					050
17	17	1C		1	18.0	BP					035

R 3

Date Reported: Year Month Day

Final Lab Sign Off

Please Send Report to:
DNR - TEA Tawes
ATTN: Bruce Michael
580 Taylor Avenue
Annapolis, MD 21401
PH: 410-260-8627 Fax: 410-260-8640



Project Name: PXT: Cedar Point (XCF9575)

Submitter: MANTA Field Office-Howard / Patuxent River

Maryland Department of Natural Resources

Field Sheet

Sequence Number: P01 (punch in 3-9 all cards)

Sampling Station Number	Start Date (Year Month Day)	Start time	End Date (Year Month Day)	End Time	Number Samples	Submitter Code	Data Category Code	Total Depth M	Study Code
CB5.1W					04	60	IN		02 0

Sample Method	+/-	Air Temp °C	Tide State	Weather Yesterday	Weather Today	Cloud Cover (%)	Wind Direction	Wind Velocity (Knots) Min.	Max.	Secchi (M)	G/L	Basis	Flow Value	Exp	G/L	Senior Scientist
1																

DO Method	Equip. Set Unit #	LICor Number	LICor Method	Wave Height (M)	Upper (M) Pycnocline Limit	Lower (M) Pycnocline Limit	Scientist Sign Off	Comments
H	9		FD					0

Comments:

Effort No.	Rep No.	Depth M	+/-	Water Temp. C	Field PH	Cod Bot	G/L	Dissolved Oxygen mg/l	G/L	Specific Cond. Micromhos/cm	Salinity ppt	Layer Code	LICor (micromol/s/m²) Deck	LICor (micromol/s/m²) Underwater	
		010													0
		025													0
204	1	050			0	C				00	0	S			0
		075													0
		100			0	C				00	0				0
		125													0
		150													0
		175													0
		200			0	C				00	0				0
		225													0
		250													0
		275													0

Weather Codes

10 = none; 11 = drizzle; 12 = rain; 13 = rain, heavy; 14 = squally; 15 = frozen precipitation

Wind Velocity

1 - 3 slight ripple
4 - 6 small waves, not breaking
7 - 10 scattered whitecaps
11 - 16 numerous whitecaps
17 - 21 moderate waves, many whitecaps
22 - 27 large waves, many whitecaps
28 - 33 sea heaps get off the water! NOW

Wave Height

0.00 m = flat calm; 0.09 m = slight ripple; 0.20 m = ripple-1 ft; 0.40 m = 1-2 ft; 1.00 m = 2 -4 ft; 1.50 m = 4 -6 ft

Oxford Sample (Jan., April, July, & October)

Date Entered _____

DNR 2/2005

Page 1 of 2

APPENDIX III

MARYLAND DEPARTMENT OF NATURAL RESOURCES
CHESAPEAKE BAY WATER QUALITY MONITORING PROGRAM

CROSS REFERENCE SHEET
DOCUMENTATION AND PROCEDURES

The following documentation outlines the conventions for filling out the Cross Reference Sheet. (Note: Although this sheet is labeled "Progress Report," it is actually the Cross Reference Sheet, not the "Quarterly Progress Report" discussed in Appendix IV.) The Cross Reference Sheet is sent along with Field Sheets from the Field Office to the DNR Tawes Building, so that the DNR data management staff knows what data to expect in the form of field sheets and lab data.

The cross reference sheet includes the name of the program, the sampling month and year, the name of the Field Office representative who originates the sheet, the station name, the sampling day, the sampling depth, the sequence number, and columns for tracking lab sheets, chlorophyll sheets. There is also a comment line to explain missing samples, stations, field abnormalities, or potential data problems.

1. The name of the program

2. The sampling month and year

3. The name of the Field Office representative who originates the sheet

4. The station name

5. The sampling day

6. The sampling depth

7. The sequence number

8. Column for tracking receipt of laboratory data

9. Column for tracking receipt of chlorophyll data

10. Column for tracking receipt of bacti data

11. Column for comments

An example Cross Reference Sheet (labeled "Progress Report/Cross Reference Sheet") follows.

Maryland Department of Natural Resources
RAS/MANTA
Chesapeake Bay Mainstem
Progress Report / Cross Reference Sheet

Month/ Year: February / 2006 **Submitted by: Laura Fabian**

Station	Day	Depth (M)	Sequence #	Sample #	Nutrients (CBL)	Chloro. (DHMH)	Plankton (Butler)	Comments
CB5.3 Smith Point	7	0.5	0600201	1		3-460	N/S	
		26.0		2			N/S	
		9.0		3			N/S	
		19.0		4			N/S	
LE2.3 Point Lookout	7	0.5	0600202	5			N/S	
		19.0		6			N/S	
		7.0		7			N/S	
		13.0		8			N/S	
CB5.2 Point No Point	7	0.5/1	0600203	9			N/S	Extra plankton samples @ S & B for Horn Point.
		0.5/2		10		↓	N/S	
		30.0		11		3-460A	N/S	
		7.0		12			N/S	
		21.0		13			N/S	
CB5.1 Cedar Point	7	0.5	0600204	14				17.0 Meter Plankton for W. Butler.
		33.0		15				
		8.0		16			N/S	
		18.0		17		↓	N/S	

APPENDIX IV

MARYLAND DEPARTMENT OF THE NATURAL RESOURCES
CHESAPEAKE BAY WATER QUALITY MONITORING PROGRAM

CRUISE REPORTS/QUARTERLY PROGRESS REPORT DOCUMENTATION AND PROCEDURES

The Cruise Report is filled out by Field Office personnel for each cruise and provided to the Quality Assurance Officer at DNR. Every three months, the Quality Assurance Officer combines and summarizes the Cruise Reports, creating a Quarterly Progress Report to submit to the CBPO.

The Cruise Report includes the cruise identification number, name of the program, the scheduled sampling date, the name of the Field Office representative who submits the sheet, additional sampling activities, the station names, the sampling dates and times, the time that filtering is finished, the time the station is left, the presence or absence of hydrogen sulfide odor and results of any Hach tests conducted, the research vessel name, the names of the captain, crew, and scientific party, the departure time and location, the return time and location, weather conditions, the temperature, the barometric pressure, the estimated wind speed and direction, the equipment conditions, the morning dissolved oxygen check, sample status, and additional comments.

Information Filled out by Field Personnel:

Page 1

1. Cruise I.D. (top left of sheet)

   Provide the cruise identification number in the space provided at top left of sheet.

2. Page____of_____ (top right of page)

   When more than one sheet is generated and sent with samples, enter this information in the area provided, 'Page_____of_____'. If only one sheet is generated, indicate this by entering page 1 of 1.

3. Day # (top right of page, under page number)

   Provide the day number of the cruise (i.e., Day #1, Day #2, or Day #3)

4. Study Location (top of sheet)

   If not preprinted, provide the name of the study location (e.g., Mainstem Cruise Report) at the top center of the sheet.

5. Scheduled Sampling Date

Provide the scheduled sampling date in the space provided.

6. Submitted by

Provide the name of the field scientist who originates the sheet.

7. Station Sampled (1st column of sheet)

The station sampled should be preprinted in the first column of the sheet. If not preprinted, enter the station name. (For example, if the samples were collected from station MCB5.3, the station sampled would be "MCB5.3").

8. Date

Enter the actual date sampled in the space provided.

9. Time

Enter the time the samples are taken.

10. FF (finished filtering)

Enter the time that filtering is finished.

11. LS (left station)

Enter the time of leaving the station.

12. H_2S odor

For both below pycnocline (BP) and bottom (B) layer samples:

- If H_2S odor is present (rotten egg smell), enter "+" and perform a Hach test for hydrogen sulfide. Record the Hach reading.

- If no H_2S odor is present, enter "-".

13. Cruise I.D. (top left of sheet)
Provide the cruise identification number in the space provided at top left of sheet.
Cruise ID numbers consist of last 2 digits of year, 0, and cruise # of year. For example, the cruise ID for the third trip of 2008 would be 08003.

14. Date

Enter the actual sampling date in the space provided.

15. R/V Utilized

Enter the name of the research vessel in the space provided.

16. Captain, Crew and Scientific Party

Enter the names of the Captain, Mate, and scientists on board, identifying the agency/company that the scientists represent (e.g., Versar, ANS, DNR)

17. Departure Time and Location

Enter the departure time and location.

18. Return time and location

Enter the return time and location.

19. Weather conditions

- Enter the air temperature in degrees Celsius for the morning (AM) and afternoon (PM).
- Enter the barometric pressure in inches of mercury for the morning (AM) and afternoon (PM).
- Enter the estimated wind speed in knots and the direction from which the wind is blowing for the morning (AM) and afternoon (PM).

20. Equipment conditions

Enter the refrigerator (FRIDGE) temperature in degrees Celsius.
Enter the freezer temperature in degrees Celsius.

21. Morning Dissolved Oxygen (DO) Check

Enter the meter used, the calculated DO, meter reading, and whether or not it changed. Meter readings are logged in Cruise Report when a sonde is changed during a survey.

22. Sample Status

Enter the status of the sample in cases when unusual events might affect a sample. For example, a refrigerator/freezer failure, or samples transported at odd times.

23. Additional Comments

Enter additional comments as needed.

Pages 3 and 5 are the same as Page 1 (for additional stations).
Pages 4 and 6 are the same as Page 2 (for additional stations).

APPENDIX V

MARYLAND DEPARTMENT OF NATURAL RESOURCES
CHESAPEAKE BAY WATER QUALITY MONITORING PROGRAM

FIELD INSTRUMENT QUALITY ASSURANCE/QUALITY CONTROL

These procedures refer to Hydrolab Series 4041, Series 2, Series 3, Series 4a and Series 5 instruments. Detailed calibration procedures will be performed as described their respective operation manuals.

NOTE:

Beginning in mid-2008, YSI Series 6000 instruments will be added to the field equipment inventory. Water quality parameters currently measured using Hydrolab equipment will be measured using YSI equipment in the future.

The YSI instruments will be equipped with an optical dissolved oxygen sensor instead of the Standard Clark Polarographic Sensor. Temperature, pH, specific conductance and depth sensors will be similar to respective Hydrolab sensors.

This document will be amended when the YSI instruments are fully incorporated into the inventory and the use of the new equipment and procedures has received approval from the Chesapeake Bay Program Quality Assurance Officer.

I. Calibration

A. Set up a calibration log book for each instrument with make, model, serial numbers and first-in-service date. Assign a letter for DNR use as required.

B Calibrate instruments on Friday for use the next week. After one to four days of field deployment, instruments are post-calibrated to determine if any parameter has drifted (see below).

C. Specific conductance calibration shall be made using standards generated by the field office from dry KCl and deionized water. Standards used are 147, 294, 720, 1413, 2767, 6668, 12900, 24820 and 58640 microSiemens/cm (μS) (microSiemens/cm is equivalent to micromhos/cm at 25°C). Respective concentrations are 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2 and 0.5 molar KCl. Specific conductance calibration with Series 4041, Series 2 and Series 3 instruments is a one point calibration (slope of line only) with one of the above standards. The zero point is factory set and cannot be adjusted by user. Specific conductance calibration with Series 4a and 5 instruments is a two point calibration. The zero point is set with the probe dry and the slope is set with one of the

above standards.

D. A pH calibration shall be made using premixed standards of color coded pH 4.00, pH 7.00 and pH 10.00 purchased from Fisher Scientific. Standards are certified as accurate at 25°C (pH 4.00 ± 0.01, pH 7.00 ± 0.01, pH 10.00 ± 0.02), used before their labeled expiration dates and color coded as follows: red for pH 4.00, yellow for pH 7.00 and blue for pH 10.00.

E. Dissolved Oxygen (DO) calibration of a standard Clark Polarographic probe shall be done with the common standard of water-saturated air. All Hydrolab instruments listed above are equipped with this probe. Temperature from the instrument and local barometric pressure from a standard Fortner Mercury Barometer are used to determine the oxygen concentration in water-saturated air from theoretical DO tables. The DO membrane is visually inspected before an instrument is calibrated. If the membrane is damaged, the membrane and electrolyte are replaced. The DO probe is calibrated 24 hours later. Instruments used for Mainstem Cruises receive a morning-of-use DO check. See V.II.B.

F. Temperature is calibrated by the manufacturer and cannot be adjusted by the user.

G. Depth shall be calibrated on station and at a known depth.

H. Record all calibration and post-calibration information (e.g. barometric pressure, calibration values and instrument readings), maintenance procedures and repairs in the log book. An example of an instrument calibration log is included.

I. During calibration, post-calibration and field deployment, record in the log book any unusual circumstances which may affect the instrument readings.

II Field Verification of Instrument Performance

A. Teams carry two calibrated instruments in case of failure. Readings from the instrument in use are compared to those from the second instrument only when the field scientist recording measurements observes readings (a) that are outside reasonably expected values, (b) that are variable or erratic, or (c) if the instrument displays an error message. If these instruments do not agree within QA/QC guidelines and the field scientist reasonably believes that the primary instrument is not working correctly, the second instrument is used. This is noted on the field sheet, cruise report, and instrument calibration log. The instrument supervisor is also informed.

B. The instrument to be used each day of a Mainstem Cruise receives a dissolved oxygen validation check. The instrument is set up for a dissolved oxygen calibration, but the

calibration is only adjusted if the reading is greater than ± 0.2 mg/L from theoretical saturation value.

III. Post-Operational Maintenance

A. Post Field Deployment Maintenance:

1. Daily: At the end of each day of use, probes are rinsed with de-ionized or tap water and submerged in the storage cup filled with sufficient tap water to cover the pH and reference probes.

2. Weekly: At the end of each week, the entire instrument (sonde, cable and display) and basket carrier are rinsed with tap water. Probes are rinsed with de-ionized or tap water and submerged in the storage cup filled with sufficient tap water to cover the pH and reference probes.

3. Post-calibration of dissolved oxygen, pH and specific conductance is done weekly on Friday after one to four days of field sampling but may be done within 24 hours after last use. Instruments are post-calibrated after probes stabilize to room temperature. Instruments shall be post-calibrated using the same standards and procedures as were used in calibration except the calibration settings are not adjusted. The DO membrane is visually inspected before post-calibration. If the membrane or sensor is damaged, the damage is recorded in the calibration log and the sensor is post-calibrated as is. Readings of additional standards shall be done if pH and specific conductance readings during field deployment fall outside the zero and slope calibration standards. The post-calibration standards and instrument readings are recorded in the calibration log.

4. If a parameter's post-calibration readings are outside QA/QC guidelines, a note shall be made in the calibration log and the field quality assurance officer shall be informed. The QA officer will determine if the instrument is operating correctly and if data recorded that week are reliable.

   Instrument parameter readings that are outside QA/QC guidelines during post-calibration are flagged on field data sheets with the Analytical Problem Code (APC) value 'F'. The description of APC 'F' is: "Field data instrument post calibration failed but data within theoretical limits, (e.g. post cal failed but data kept)" see Appendix XI.

   All field data, including instrument data, are re-evaluated during quality assurance,

(see page 18, step 3 of DATA MANAGEMENT, VERIFICATION AND DOCUMENTATION). If the analyst/biologist and Quality Assurance Officer determine that the data are not usable, values are flagged with the APC code 'V'. The definition for APC code 'V' is: "Sample results rejected due to quality control criteria", see Appendix XI.

B. Routine Maintenance (conducted at least every ten weeks)

1. Dissolved oxygen sensor: All Series of Hydrolab instruments use the same Standard Clark Polarographic Sensor. Remove old membrane and electrolyte, polish gold cathode, fill sensor with fresh electrolyte and install new DO membrane.

2. Specific conductance sensor:

   a. Series 4041, 2 and 3 (six nickel electrode array): Remove cell block, clean with laboratory soap and rinse with de-ionized water. Polish all surfaces of the six electrodes with 400 grit wet/dry sandpaper. Wipe electrodes with cotton swab soaked with 100% methanol and then fresh cotton swab soaked with 100% acetone, rinsing with de-ionized water after each wiping. Reinstall cell block.
   b. Series 4a and 5 (graphite sensor): Wipe all surfaces of probe with cotton swab soaked with laboratory soap and then fresh cotton swab soaked with 100% methanol, rinsing with de-ionized water after each wiping.

3. pH sensor: each generation of instruments uses a two sensor system (*in situ* and reference sensors)

   a. Series 4041 and 2: *in situ* and reference sensors are Ag/AgCl glass sensors. Reference sensor is inside a sleeve capped with a porous Teflon™ junction and filled with electrolyte (aqueous pH 7 buffer saturated with KCl). Remove junction sleeve from reference sensor and rinse sensor with de-ionized water. Wipe both glass sensors with cotton swab soaked in 100% methanol and 100% acetone, rinsing with de-ionized water after each wiping. Soak sensors in 0.1 N HCl for no more than 30 minutes. Rinse with de-ionized water after soaking. Install new junction on sleeve, fill sleeve with fresh electrolyte and reinstall sleeve on reference sensor.
   b. Series 3, 4a and 5: *in situ* sensor is Ag/AgCl glass sensor. Reference sensor is a pellet of silver inside a sleeve capped with a porous Teflon™ junction and filled with electrolyte (4M KCl aqueous solution saturated with $AgCl_2$). Clean *in situ* glass sensor as above. Remove junction sleeve from reference sensor. Do not clean pellet of silver. Install new junction on sleeve, fill sleeve with fresh electrolyte and reinstall sleeve on reference sensor.

4. Depth sensor:

a. Series 4041: Does not have a depth sensor.

b. Series 2: Soak sensor in "Lime-Off" (Jungle Products) for no more than 30 minutes. Rinse sensor thoroughly with deionized water.

c. Series 3, 4a and 5: Inspect sensor port and remove any obstructions. No further maintenance is required.

5. Temperature thermistor: All Series of Hydrolab instruments use similar temperature thermistors. Wipe with cotton swab soaked in 100% methanol and 100% acetone, rinsing with de-ionized water after each wiping.

IV. Secchi disk: Each year the secchi disk line is calibrated by comparing its 0.2m marks to a metal meter stick. Each mark is a small piece of colored flat synthetic webbing pulled through the line and sewn for security. Marks are moved if the webbing does not line up with the corresponding line on the meter stick.

V. Annual audits of all field equipment log books, maintenance records and field procedures will be conducted by the field quality assurance officer provided there is time available. This information will be reported to the DNR Quality Assurance Officer. (See Quality Assurance Project Plan, Section 8: Project Organization and Responsibility).

APPENDIX VI

MARYLAND DEPARTMENT OF NATURAL RESOURCES
CHESAPEAKE BAY WATER QUALITY MONITORING PROGRAM

FIELD PROCEDURES QUALITY ASSURANCE / QUALITY CONTROL

I. Cleaning procedures:

A. Autoanalyzer cups and caps: Cups and caps are used only one time and then discarded.

B. DOC tubes: Place tubes in 10 % HCl bath for approximately 24 hours; follow by rinsing tubes several times in deionized water.

C. DOC caps: Place tubes in 10 % HCl bath for approximately 24 hours; follow by rinsing tubes several times in deionized water.

D. TDN/TDP tubes: New tubes are digested using potassium persulfate followed by multiple deionized water rinses. Tubes that are "in-the-cycle" are cleaned by emptying old contents, rinsing the tubes and caps with 3-4 tap water rinses followed by 6 rinses with deionized water.

II. Review of procedures for field and lab sheets in the field

A. "Scientist signoff" duties

The field scientist is responsible for recording values on the field data sheets and on the lab sheets. This includes entering all Hydrolab values, calculating the pycnocline, and ensuring that the field data sheet is complete. This individual is also responsible for transcribing necessary header information onto the lab sheets.

B. "Senior scientist" duties

The individual who enters their initials in the 'senior scientist' boxes is the scientist who is officially designated as being in charge of the cruise.

Mainstem Cruises:

1. The senior scientist as field quality assurance officer on cruise should ensure that:

a. Thermometers are placed in refrigerator and freezer to monitor daily temperatures (4

°C for refrigerator and -10 to -20 °C for freezer) and record data on cruise report. If temperatures are too high, they should be set lower if possible and if not possible, notify the Captain of the research vessel.

b. Check with Captain of the research vessel to ensure that weather and location instruments used on board ship (e.g., Raytheon factory calibrated barometer, anemometer, or GPS) are functioning properly and, if not, record it in the Cruise Report.

c. Check to make sure all equipment necessary to accomplish sampling is on board and functioning before leaving dock.

d. Document and report back to the field quality assurance officer any deviations from existing protocol or problems that have arisen during the cruise.

III. Dissolved Oxygen Calibrations and Checks

Dissolved Oxygen calibration shall be done every morning on the common standard of water-saturated air. After correcting for the barometric pressure and temperature the oxygen content of water-saturated air can be checked against standard D.O. tables. The DO membrane is also visually checked every time the meter is calibrated or post-calibrated. If the membrane appears damaged, the meter is posted as is. Then the membrane and electrolyte are replaced and the meter is calibrated after 24 hours.

IV. Spare Hydrolab Meter

As discussed in Appendix 5 (Field Instrument Quality Assurance/Quality Control), teams carry two calibrated Hydrolab meters in case of failure. The meter in use is compared to the reserve meter any time (a) the field scientist recording measurements observes values outside the "typically expected range"; (b) the meter generates variable or erratic values; or, (c) the meter in use displays an error message. If the meters do not agree within acceptable limits, the reserve meter is used. This is noted under the additional comments section.

V. Deionized water

The deionized water at the Field Office is generated from tap water using a Thermo Scientific Barnstead DIamond TII RO/DI system with a GE SmartWater external pre-filter. The RO/ DI system is linked to a Thermo Scientific Barnstead DIamond TII 60L storage reservoir. The system uses a thin film composite reverse osmosis membrane with pretreatment to produce RO water. This water is then put through a two-stage deionization process combined with UV oxidation and a 0.2 micron final filter. The reagent grade water provided by this system exceeds ASTM Type II and NCCLS/CAP Type I standards. All manufacturer recommendations are followed regarding cartridge replacement and system sanitation (Refer

to Thermo Scientific. 2007. Barnstead DIamond TII Type II Water System Operation Manual and Barnstead DIamond TII Type II Storage Reservoir Operation Manual). The GE SmartWater pre-filter was placed inline to improve the integrity of feed-water going into the Barnstead DIamond System. The pre-filter is changed at least every three (3) months or more frequently during periods of heavy use. A log is kept at the front of the DI System Manual to document all changes and updates made to the system.

VI. Transfer of nutrient samples/sheets to laboratory

A. Place volume sheets in CBL supply bin or deliver sheets to CBL.
B. Place samples in Research Vessel from CBL or pack samples in dewatered ice or refrigerator/freezer at Field Office for later delivery.

VII. Transfer of chlorophyll samples/sheets to laboratory

A. Send chlorophyll sheets along with samples to DHMH.
B. Pack samples in dewatered ice or refrigerator/freezer at Field Office for delivery to DHMH.

NUTRIENT ANALYTICAL SERVICES LABORATORY
STANDARD OPERATING PROCEDURES

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INTRODUCTION

The following pages document the analytical methodologies performed by the Nutrient Analytical Services Laboratory at the University of Maryland Chesapeake Biological Laboratory (CBL). This manual includes sections on dissolved inorganic nutrients, dissolved organic nutrients and particulate nutrients. Within each of these sections, sample collection, storage, preparation and analysis are discussed. A final section addresses data management and quality assurance/quality control (QA/QC).

Many of the procedures described are used for the Maryland Mainstem portion of the Chesapeake Bay Program.

Instrumentation includes:

- Technicon AutoAnalyzer II,
- Two channel Technicon TrAAcs-800 Nutrient Analyzer,
- Shimadzu TOC-5000 and 5000 A Total Organic Carbon Analyzers,
- Turner Designs TD-700 Fluorometer,
- Shimadzu UV-120-02 Spectrophotometer,
- Exeter Analytical, Inc. CE-440 Elemental Analyzer, and
- Dionex DX-120 Ion Chromatograph.

PC Computers with complete spreadsheet packages are used for data reduction and management.

High quality (18.3 megohm-cm) deionized water is provided via 2 Barnstead NANOpure II systems. The Barnstead system produces Type 1 Reagent Grade water equal to or exceeding standards established by the American Society for Testing and Materials. First, water is filtered through a reverse osmosis membrane. Final product water then passes through a series of five filters: one organic colloid, two mixed bed, one organic free, and one final 0.2 µm filter. Throughout this manual, the term "deionized water" refers to 18.3 megohm-cm water and "frozen" refers to temperature < -20°C.

Table 1. Parameters routinely analyzed at the Nutrient Analytical Services Laboratory at CBL

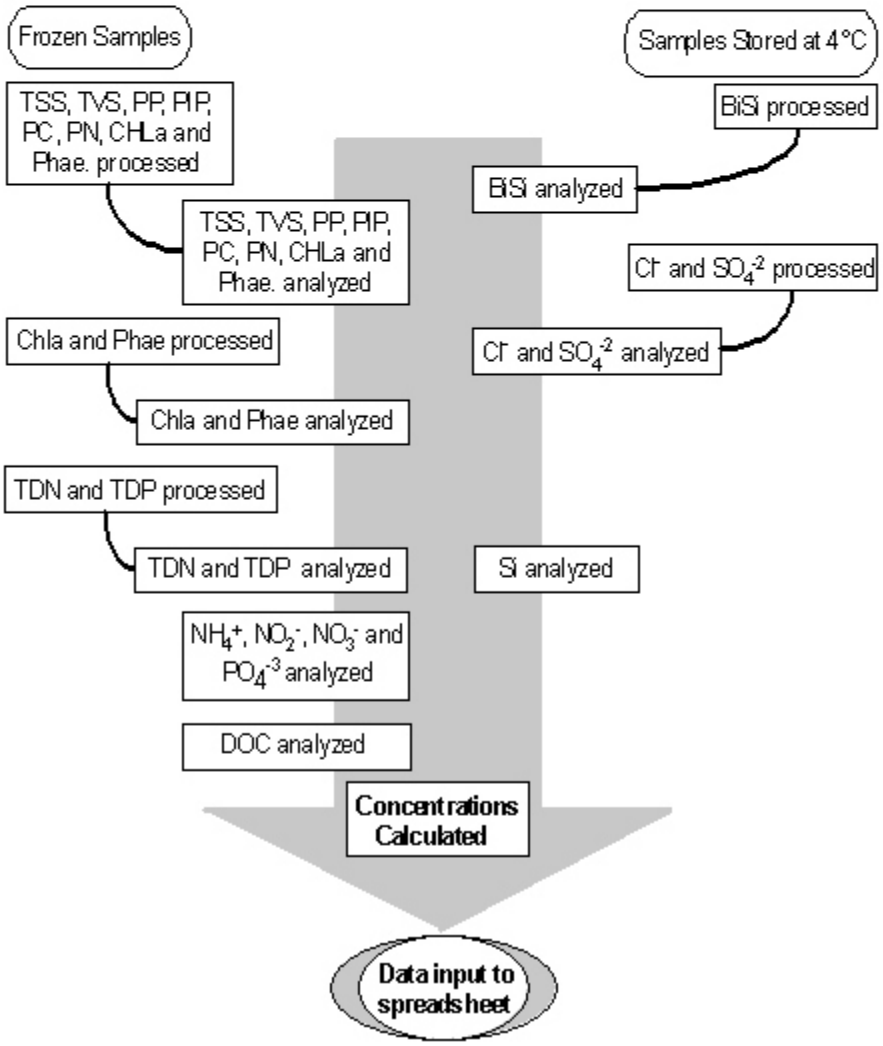
DISSOLVED INORGANIC	ORGANIC	PARTICULATE
Ammonium-Nitrogen	Persulfate Nitrogen	Carbon
Nitrite-Nitrogen	Persulfate Phosphorus	Nitrogen
Nitrite+Nitrate-Nitrogen	Dissolved Carbon	Phosphorus
Phosphate-Phosphorus		Total Suspended Solids
Silicate		Total Volatile Solids
Sulfate		Biogenic Silica
Chloride		Chlorophyll *a*
Nitrate-Nitrogen		Phaeopigments

Sample - Data Reduction Process

Samples arrive to NASL via overnight mail or courier.

- NH_4^+, NO_2^-, NO_3^- and PO_4^{-3} - filtered water, frozen, polystyrene AAI cups
- TDN and TDP - filtered water (measured volume), frozen, borosilicate glass test tubes
- Si - filtered water, refrigerated (4°C), polystyrene AAI cups
- DOC - filtered water, frozen, Teflon vials
- TSS, TVS, PP, PIP, PC, PN, CHLa and Phae - particulate matter retained on GF/F filter pad, frozen, foil pouches
- BiSi - particulate matter retained on membrane filter, refrigerated, polypropylene centrifuge tubes
- Cl^- and SO_4^{-2} - filtered water, refrigerated, polybottle

Received samples are inspected for damage, counted and verified against field data sheets (if available) and transferred to appropriate storage area (freezer or refrigerator). Field data sheets are filed appropriately and samples are thus entered into the analysis queue.



DISSOLVED INORGANIC ANALYTES

Instrumentation

All of the dissolved inorganic nutrient procedures, particulate phosphorus and biogenic silica procedures require the use of a segmented continuous flow analyzer such as the Technicon AutoAnalyzer II or TrAAcs-800. Samples and reagents are continuously added in a specific sequence along a path of glass tubing and mixing coils. Within the system of tubes, air bubbles injected at precise intervals sweep the walls of the tubing and prevent diffusion between successive samples. Reactions in the AutoAnalyzer or TrAAcs-800 do not develop to completion as in manual methods, but reach identical stages of development within each sample since every sample follows the same path, timing and exposure to specific reagents.

The basic function of each component of the segmented continuous flow analyzer is discussed briefly below. This explanation is similar to that of Sanborn and Larrance (1972).

Automatic Sampler

At a timed interval, the sampler probe alternately draws fluid from a tray of discrete samples and a wash fluid receptacle. After each sample is drawn, the sample tray advances to the next sample position. A bubble of air, which acts as a diffusion barrier, is aspirated into the sample stream between sample and wash. The ratio of sample-to-wash time and the number of samples analyzed per hour are controlled by the TrAAcs software, or by a cam or timer assembly located in the top well of the AutoAnalyzer II sampler. Cams and timer assemblies are adjusted easily to provide various sampling rates.

The wash solution separates successive samples in the sample stream as indicated on the graphical record by alternating minima (wash) and maxima (sample). The sample probe is connected to a stream divider that delivers identical samples simultaneously to each manifold via the pump.

Proportioning Pump

The proportioning pump is a peristaltic-type pump that continuously delivers air, reagents and samples to the manifold. Plastic pump tubes of various diameters are pressed between a series of moving rollers and a platen. The motion of the rollers along the tubes delivers a continuous flow of samples and reagents. The delivery rate is determined by the inside diameter of the tubes since the rollers move at a constant rate. These pump tubes are available in a large assortment of delivery rates. The pump holds a maximum of 28 tubes and has an air bar that mechanically measures and injects identically sized air bubbles into the analytical stream. The pump tubes delivering reagents, air and samples are connected to appropriate manifolds.

Manifold

Each analysis requires a manifold specifically designed for the chemical determination employed. The manifolds are composed of a series of glass coils, injection fittings and heating baths arranged for the proper sequence of reactions leading to color development. The glass coils permit mixing of the sample and the reagents; as two solutions with different densities travel around each turn of the mixing coil, the denser solution falls through the less dense one, causing mixing and creation of a homogeneous mixture

of the two solutions. The length of the coil determines the amount of time allowed for chemical reaction between the addition of successive reagents. Injection fittings for each of the reagents are placed between mixing coils; thus, a sample enters one end of the manifold, a reagent is added, and mixed; and then another reagent is added and mixed. After the addition of all reagents, and an adequate reaction time, the solution flows into a colorimeter.

Colorimeter

The colorimeter measures the absorption of monochromatic light by the solution in the flow cell. Light from a single source passes through two separate but identical interference filters that pass light within a narrow spectral band. The light then passes through the appropriate flow cell and is projected onto a phototube. The phototube generates an electrical signal in response to the intensity of the impinging light. The output from each phototube is a measure of transmittance and is converted electronically by the colorimeter to a signal proportional to absorbance. The relationship between transmittance and absorbance is given by the equation $A = \log 1/T$; where A = absorbance and T = transmittance. The resulting signal is linear in absorbance and directly proportional to concentration. As each sample passes through the flow cell, the signals are sent to a recorder.

Recorder

Results of the analyses are continuously recorded by strip chart recorders and/or by computer using an IBM compatible DP500 software system by Labtronics Inc., or AACE software system by Bran and Luebbe. Each recorder can simultaneously monitor two separate analyses. The DP500 and AACE systems can each collect and analyze data from up to four different detectors simultaneously. The output of the colorimeter is proportional to absorbance and standards of known concentration must be analyzed to relate absorbance to concentration. The analog signals can be converted to absorbance values by referring to the Technicon reference curve and the standard calibration control.

Sampling and Storage

Collected water samples are filtered through Whatman GF/F filters (nominal pore size 0.7 μm), placed in either polypropylene bottles or directly into 4 ml AutoAnalyzer cups and frozen. Samples for silicate are treated in the same manner but are refrigerated at 4°C. All samples are routinely analyzed within 28 days.

Operating Procedures

The following describes step-by-step operating procedures for the AutoAnalyzer II system.

1. *Colorimeter* - Turn power on and allow 10 minutes for warm-up. Set standard calibration setting for desired determination.

2. *Recorder (or Computer)* - Turn power on and allow 10 minutes for recorder warm-up. Check recorder paper supply. If using computer for data collection, load software and select appropriate sample method and sample table. Refer to Labtronics Inc. DP500 and Bran and Luebbe AACE users' manuals for descriptions of system operation.

3. *Sampler Water Reservoirs* - Check and fill the deionized water reservoirs.

4. *Pump* - Connect pump tubes and attach platen to pump. Start pump with deionized water flowing through the system. Check for leaks in tubes at connections and for a regular bubble pattern in the manifold.

5. *Recorder* - Start recorder. Paper should begin to move.

6. *Colorimeter* - Check ZERO and FULL SCALE knob. ZERO simulates a zero output so that ZERO adjustment (screwdriver) of the recorder can be made. Set knob to NORMAL and establish a baseline with deionized water using the BASELINE CONTROL adjustment knob and a standard calibration (STD CAL) setting of 1.0; i.e., wherein the full scale absorbance is 1.00.

7. Allow reagents to pump through the system and note any rise in baseline and readjust the baseline to zero. Refer to this rise as the REAGENT BLANK (at a STD CAL of 1.0).

8. An extremely wide range of nutrient concentrations found in Chesapeake Bay waters, both temporally and spatially, requires use of a standard curve covering a large range and that covers a few STD CAL control settings.

9. Reset zero baseline at the STD CAL control setting normally used for that determination (e.g., particulate phosphorus STD CAL of 4.0). Next, switch the STD CAL setting to 1.0. There should be no deflection of the pen at zero baseline. Note peak heights of standards at the various STD CAL settings along with the STD CAL settings. This allows the operator to use STD CAL settings in the range of 1 to 4 (for this example) in analyzing standards and samples that otherwise would have gone off scale. Intersperse standards in the run after approximately every 20 samples, including a standard analyzed at each STD CAL setting employed during the preceding 40 samples. A visual comparison with the day's initial standard curve should indicate no greater variance than 5% of the peak height (e.g., initial standard peak height 60.0; subsequent standards acceptable in the range of 57.0 to 63.0). If the variance exceeds 5%, identify the source of the problem, correct and re-analyze affected samples. Adjust baseline after approximately every 20 samples. If an adjustment of more than 1 unit is required, identify the source of the problem, correct and re-analyze affected samples.

10. At completion of the run, remove lines from reagents and place tubes in deionized water.

11. *Shut-down* - Turn off recorder. Wash system with 1 N hydrochloric acid for 15 minutes, followed by a 15 minute wash with deionized water. Turn off pump, release proportioning platen and loosen pump tubes. Turn off colorimeter.

Glassware

Glassware are cleaned appropriately for the determination. Most are acid-washed with 10% hydrochloric acid followed by numerous rinses with deionized water. Other cleaning protocols are listed in their specific sections.

Calibration and Standardization

Please refer to each specific determination for the appropriate STD CAL control setting and for the standard concentrations used.

The STD CAL control setting located on the colorimeter allows the operator to adjust the electrical output to the concentration range of the samples. Extremely low values (µg/L) require high STD CAL settings (high sensitivity) whereas high values (mg/L) require lower STD CAL settings (lower sensitivity).

Concentrations of nutrients are calculated from the linear regression of the standard concentration (independent variable) against the corresponding peak height (dependent variable). All standards analyzed at a particular STD CAL setting are included in the regression for that set of calculations. Only samples whose peak heights were measured at that individual STD CAL setting are calculated from that regression. If a broad range of sample concentrations requires that more than one STD CAL setting be used throughout the course of a run, then a separate regression must be employed for each STD CAL setting. For example, peak heights obtained from standards read at STD CAL 9.0 are used to obtain the linear regression for calculating only the concentrations of samples whose peak heights were read at

STD CAL 9.0. Likewise, peak heights obtained from standards read at STD CAL 2.0 are used to obtain the linear regression for calculating only the concentrations of samples whose peak heights were read at STD CAL 2.0.

Peak heights are read manually from the strip chart or automatically by the DP500 or AACE software systems. Operator vigilance is necessary throughout the run to ensure that all peaks indicate steady state conditions for the reaction for each sample. If steady state conditions are not obtained, the samples are re-analyzed.

Stock standards are prepared with primary standard grade chemicals of each nutrient in deionized water. As a general rule, stock solutions are made every 6 months and the preparation date logged. Secondary standards, where appropriate, are prepared with deionized water. Working standards are prepared daily with deionized water or the appropriate matrix as described by the specific determination method and should encompass the range of the samples.

All analysis documents are kept electronically and in notebooks. A copy is also given to the investigator or granting agency. Information provided includes:

- name of the method;
- collection date;
- source of samples;
- analyst;
- analysis date;
- sample number;
- sample concentration;
- results of duplicate analyses;
- results of spike analyses; and.
- results of analyses of commercially prepared standard reference materials.

Literature Cited

Sanborn, H. and J. Larrance. 1972. An operations manual of the AutoAnalyzer for seawater nutrient analysis. NOAA/NMFS. Seattle, Washington. 44pp.

ORTHOPHOSPHATE Revised 2/2004

Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex which is reduced to an intensely blue-colored complex by ascorbic acid. Color is proportional to phosphorus concentration.

Methodology

Technicon Industrial Method No. 155-71W/Tentative. 1973. Technicon Industrial Systems. Tarrytown, New York, 10591.

USEPA. 1979. Method No. 365.1 *in* Methods for chemical analysis of water and wastes. United States Environmental Protection Agency, Office of Research and Development. Cincinnati, Ohio. Report No. EPA-600/4-79-020 March 1979. 460pp.

Froelich, P.N. and M.E.Q. Pilson. 1978. Systematic absorbance error with Technicon AutoAnalyzer II colorimeter. Water Res. 12:599-603.

Instrumentation

Technicon AutoAnalyzer II

Manifold Assembly

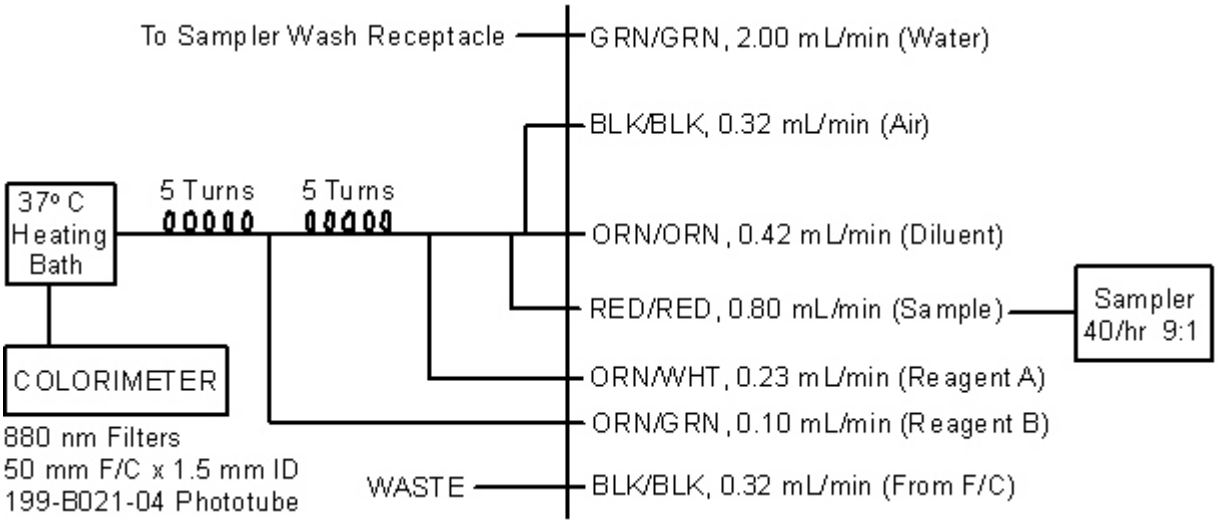


Figure 1. Manifold assembly for orthophosphate method.

Specifications

Standard Calibration Settings: 9.0, 6.0, 2.0, 1.0
Damp: Normal
Sampling Rate: 40/hour, 9:1 sample/wash ratio
Filter: 880 nm
Phototube: 199-B021-04
Flowcell: 50 mm
Relative Absorbance (18 µM P): ~.85

Interferences: Silicon at a level of 100 µM Si causes an interference equivalent to approximately 0.04 µM P. High iron may cause precipitation (i.e., loss) of phosphorus. High sample turbidity and color may interfere.

Reagents

Deionized Water Diluent

Sodium dodecyl sulfate, (SDS)[$CH_3(CH_2)_{11}OSO_3Na$; m.w.=288.38; phosphate ≤0.0001 %]	0.05 g
Deionized water	up to 500 mL

Add 0.05g of sodium dodecyl sulfate to approximately 500 mL of deionized water. Mix well.

Sulfuric Acid Solution, 4.9 N

Sulfuric acid (H_2SO_4), concentrated (sp. gr. 1.84)	136 mL
Deionized water	up to 1000 mL

To a 1000 mL volumetric flask, add 136 mL of concentrated sulfuric acid to approximately 800 mL of deionized water while cooling (cold water bath). After the solution is cooled, dilute to 1000 mL with deionized water.

Ammonium Molybdate Solution

Ammonium molybdate [$(NH_4)_6Mo_7O_{24}\cdot 4\ H_2O$]	40 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 40 g of ammonium molybdate in 800 mL of deionized water. Dilute to 1000 mL with deionized water. Store in plastic bottle away from direct sunlight.

Ascorbic Acid Solution

Ascorbic acid ($C_6H_8O_6$)	18 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 18 g of ascorbic acid in 800 mL of deionized water. Dilute to 1000 mL with deionized water and dispense ~40 mL aliquots into clean polybottles and freeze. Thaw overnight in the refrigerator before use.

Antimony Potassium Tartrate Solution

Antimony potassium tartrate [$K(SbO)C_4H_4O_6\cdot ½\ H_2O$]	3 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 3 g of antimony potassium tartrate in 800 mL deionized water. Dilute to 1000 mL with deionized water.

Working Reagents

Reagent A

Sulfuric Acid Solution, 4.9 N	50 mL
Ammonium Molybdate Solution	15 mL
Antimony Potassium Tartrate Solution	5 mL
Sodium dodecyl sulfate, (SDS) [$CH_3(CH_2)_{11}OSO_3Na$; m.w. 288.38; phosphate $\leq$0.0001%]	0.15 g

Reagent B

Ascorbic Acid Solution	30 mL
SDS	0.05 g

Standards

Stock Orthophosphate Standard, 12,000 µM

Potassium phosphate, monobasic (KH_2PO_4), dried at 45°C	1.632 g
Deionized water	up to 1000 mL
Chloroform ($CHCl_3$)	1 mL

In a 1000 mL volumetric flask, dissolve 1.632 g of potassium phosphate in ~800 mL deionized water. Dilute to 1000 mL with deionized water then add 1 mL of chloroform as a preservative (1 mL contains 12 µmoles P).

Secondary Orthophosphate Standard

Stock Orthophosphate Standard	1 mL
Deionized water	up to 100 mL

In a 100 mL volumetric flask, dilute 1 mL of Stock Orthophosphate Standard to 100 mL with deionized water (1 mL contains 0.12 µmoles P).

Working Orthophosphate Standards

Dilute 0.1, 0.25, 0.5, 1.0, 1.5, 2.5, 5 and 10 mL of Secondary Orthophosphate Standard to 100 mL with deionized water to yield concentrations of 0.12 µM P (0.00372 mg/L), 0.3 µM P (0.0093 mg/L), 0.6 µM P (0.0186 mg/L), 1.2 µM P (0.0372 mg/L), 1.8 µM P (0.0558 mg/L), 3.0 µM P (0.093 mg/L), 6.0 µM P (0.186 mg/L) and 12.0 µM P (0.372 mg/L), respectively.

Refractive Index Correction

Working Reagents

Refractive Reagent A

Sulfuric Acid Solution, 4.9 N	50 mL
Deionized water	20 mL
SDS	0.15 g

Refractive Reagent B

Ascorbic Acid Solution	30 mL
SDS	0.05 g

Procedure

1. Obtain peak heights for all samples and standards with Refractive Reagent A and Reagent B in place of Reagent A and Reagent B of the orthophosphate determination. Peak heights for the refractive index correction must be obtained at the same STD CAL setting and on the same colorimeter as the corresponding samples and standards (Froelich and Pilson 1978).

2. Subtract the refractive index peak heights from the heights obtained for the orthophosphate determination.

3. Calculate the regression equation using the corrected standard peak heights.

4. Calculate the concentration of samples from the regression equation using the corrected sample peak heights.

When a large data set has been amassed in which each sample's salinity is known, a regression for the refractive index correction on the colorimeter can be calculated. For each sample, the apparent orthophosphate concentration due to refractive index is calculated from its peak height obtained with Refractive Reagent A and Reagent B, and the regression of orthophosphate standards obtained with orthophosphate Reagent A and Reagent B. The salinity is entered as the independent variable and its apparent orthophosphate concentration due to its refractive index in that colorimeter is entered as the dependent variable. The resulting regression allows the operator to subtract an apparent orthophosphate concentration when the salinity is known, as long as other matrix effects are not present; thus, the operator is not required to obtain the refractive index peak heights for all samples when a large data set has been found to yield consistent orthophosphate concentrations due to salinity.

AMMONIUM Revised 2/2004

Determination of ammonium is by the Berthelot Reaction in which a blue-colored compound similar to indophenol forms when a solution of ammonium salt is added to sodium phenoxide, followed by the addition of sodium hypochlorite. The addition of a potassium sodium tartrate and sodium citrate solution prevents precipitation of hydroxides of calcium and magnesium.

Methodology

Technicon Industrial Method No. 804-86T. August 1986. Technicon Industrial Systems. Tarrytown, New York, 10591.

Kerouel, R. and A. Aminot. 1987. Procédure optimisée hors-contaminations pour l'analyze des éléments nutritifs dissous dans l'eau de mer. Mar. Environ. Res. 22:19-32.

Instrumentation

Technicon TrAAcs-800 Nutrient Analyzer

Manifold Assembly

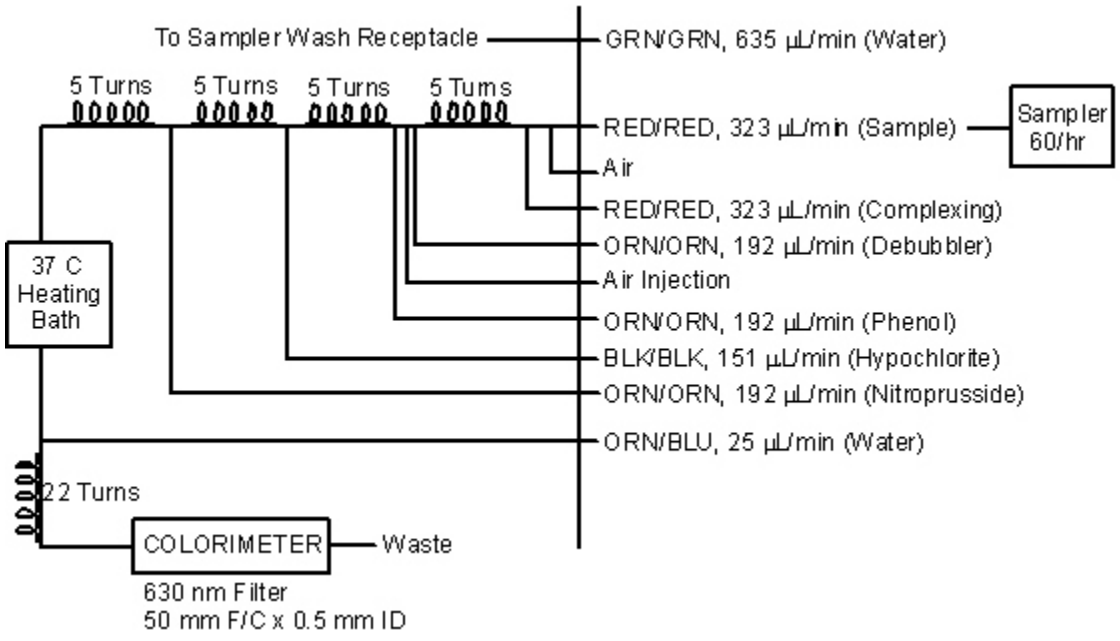


Figure 2. Manifold assembly for ammonium method.

Specifications

Gain: 40 - 45
Baseline: 32 - 38
Sampling Rate: 60/hour
Filter: 630 nm
Flowcell: 50 mm
Relative Absorbance (12 μM N): 0.22 - 0.24

Contamination: Ammonium determination is highly susceptible to skin and atmospheric contamination (Kerouel and Aminot 1987).

Reagents

Complexing Reagent

Sodium potassium tartrate ($KNaC_4H_4O_6 \cdot 4\ H_2O$)	39 g
Sodium citrate [$HOC(COONa)(CH_2OONa)_2 \cdot 2\ H_2O$]	28 g
Sulfuric acid (H_2SO_4), concentrated (sp. gr. 1.84)	as required
Deionized water	up to 1000 mL
Brij-35, 30%	1 mL

In a 1000 mL beaker, dissolve 39 g of potassium sodium tartrate and 28 g of sodium citrate in 950 mL of deionized water. Adjust the pH of the solution to 5.0 using concentrated sulfuric acid. Dilute to 1000 mL with deionized water in a volumetric flask. Add 1 mL of 30% Brij-35 and mix thoroughly. Store in a light resistant container and refrigerate. Make fresh weekly.

Alkaline Phenol Reagent

Phenol (C_6H_5OH), 88%	23.6 mL
Sodium hydroxide (NaOH), 50% (w/w)	18 g
Deionized water	up to 250 mL

In a 250 mL volumetric flask, slowly add 23.6 mL of 88% phenol to ~150 mL of deionized water. Weigh out exactly 18 g of 50% (w/w) sodium hydroxide, then while in an ice bath, slowly add the sodium hydroxide to the phenol/water solution. Dilute to 250 mL with deionized water. Store in a light resistant glass container and refrigerate. Make fresh weekly.

WARNING: Phenol is extremely dangerous and should be handled accordingly. Prepare reagent fresh weekly under a fume hood and wearing protective gloves.

Sodium Hypochlorite, 1%

Sodium hypochlorite (CLOROX Ultra - 6.0%)	44 mL
Deionized water	up to 200 mL

In a 250 mL volumetric flask, dilute 44 mL of any commercially available bleach containing 6.0% sodium hypochlorite to 250 mL with deionized water. Make fresh every other day.

Sodium Nitroprusside (Sodium Nitroferricyanide), 0.05%

Sodium nitroprusside [$Na_2Fe(CN)_5NO \cdot 2\ H_2O$]	0.5 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 0.5 g of sodium nitroprusside in 900 mL of deionized water. Dilute to 1000 mL with deionized water. Store in a light resistant container.

Standards

Stock Ammonium Standard, 1,500 µM

Ammonium sulfate [$(NH_4)_2SO_4$], dried at 45°C	0.100 g
Deionized water	up to 1000 mL
Chloroform ($CHCl_3$)	1 mL

In a 1000 mL volumetric flask, dissolve 0.1 g of ammonium sulfate in ~900 mL of deionized water. Dilute to 1000 mL with deionized water. Add 1 mL chloroform to act as a preservative (1 mL contains 1.5 µmoles N).

Working Ammonium Standards

Dilute 0.1, 0.2, 0.4, 0.6 and 0.8 mL of Stock Ammonium Standard to 100 mL with deionized water to yield concentrations of 1.5 µM N (0.021 mg/L), 3 µM N (0.042 mg/L), 6 µM N (0.084 mg/L), 9 µM N (0.126 mg/L) and 12 µM N (0.168 mg/L), respectively.

Procedure

1. Check the level of all reagents at start-up to ensure adequate supply.

2. Place all lines into their respective containers and start the proportioning pump. Allow ~10 minutes equilibration time.

3. Place a saline primer (artificial seawater) before the actual samples to avoid an abnormal peak for the first sample. Place a deionized water cup before an intersample calibrant.

   A typical tray protocol appears as follows:

   P, 5C, H@2, 2L@6, A@119, 20S>7, W@120, 2I@3, S@119, 20S>26,....

   where:
   P = primer (100% FS standard),
   C = calibrant,
   H = 100% FS standard,
   L = 20% FS standard,
   A = artificial seawater,
   S = samples,
   W = deionized water, and
   I = intersample calibrant.

4. At shut-down, remove all reagent lines from their respective containers excluding the sampler solution line and place them in deionized water containing 1 mL/L of 30% Brij-35.

5. After 5 minutes, allow all lines to pump dry. Stop the pump and release the platen.

NOTES:

1. The gain setting of 40 - 45 should yield peaks of ~70 - 80 lines with a 12 µM N standard.

2. Reagent blanks appear to be quite variable. If the reagent blank is >10 lines with the appropriate gain setting (40 - 45) and baseline setting (32 - 38), then prepare fresh reagent.

3. Close attention should be paid to prevent contamination of samples. Smoking and the use of ammonium-containing cleaning solutions should be prohibited within the air-handling limits surrounding the laboratory. Sample bottles should be stored in a clean environment and only opened when necessary. Samples should never be pipetted by mouth. Samples should be analyzed immediately upon opening.

NITRITE

Revised 2/2004

Nitrite reacts under acidic conditions with sulfanilamide to form a diazo compound that couples with N-1-naphthylethylenediamine dihydrochloride to form a reddish-purple azo dye measured at 520 nm..

Methodology

Technicon Industrial Method No. 818-87T. February 1987. Technicon Industrial Systems. Tarrytown, New York, 10591.

Instrumentation

Technicon TrAAcs-800 Nutrient Analyzer

Manifold Assembly

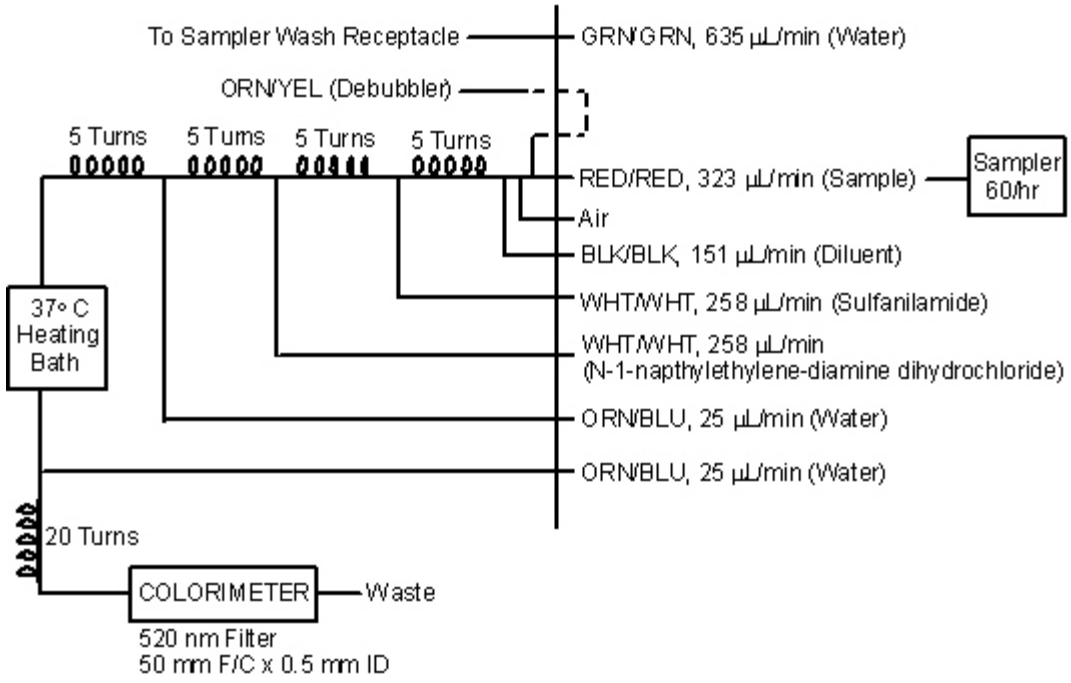


Figure 3. Manifold assembly for nitrite method.

Specifications

Gain: 53
Baseline: 36 - 37
Sampling Rate: 60/hour
Filter: 520 nm
Flowcell: 50 mm
Relative Absorbance (3 µM N): ~0.165

Reagents

Sulfanilamide Solution

Sulfanilamide (4 $NH_2C_6H_4SO_2NH_2$)	2.5 g
Hydrochloric acid (HCl), concentrated	25 mL
Deionized water	up to 500 mL
Brij-35, 30%	few drops

In a 500 mL volumetric flask, add 2.5 g of sulfanilamide and 25 mL of concentrated hydrochloric acid to ~300 mL of deionized water. Swirl to dissolve and dilute to 500 mL with deionized water. Add a few drops of Brij-35, 30% and mix thoroughly. Store in light resistant container. Refrigerate when not in use.

N-1-Naphthylethylenediamine Dihydrochloride Solution

N-1-naphthylethylenediamine dihydrochloride ($C_{12}H_{14}N_2 \cdot 2HCl$)	0.25 g
Deionized water	up to 500 mL
Brij-35, 30%	few drops

In a 500 mL volumetric flask, dissolve 0.25 g of N-1-naphthylethylenediamine dihydrochloride in ~300 mL of deionized water. Dilute to 500 mL with deionized water. Add a few drops of Brij-35, 30% and mix thoroughly. Store in a light resistant container. Refrigerate when not in use.

Standards

Stock Nitrite Standard, 5,000 µM

Sodium nitrite ($NaNO_2$), dried at 45°C	0.345 g
Deionized water	up to 1000 mL
Chloroform ($CHCl_3$)	1 mL

In a 1000 mL volumetric flask, dissolve 0.345 g of sodium nitrite in ~600 mL of deionized water. Dilute to 1000 mL with deionized water. Add 1 mL of chloroform as a preservative (1 mL contains 5 µmoles N).

Secondary Nitrite Standard

Stock Nitrite Standard	0.8 mL
Deionized water	up to 100 mL

In a 100 mL volumetric flask, dilute 0.8 mL of Stock Nitrite Standard to 100 mL with deionized water to yield a concentration of 40 µM N.

Working Nitrite Standards

Dilute 0.5, 1, 2.5, 5 and 7.5 mL of Secondary Nitrite Standard to 100 mL with deionized water to yield concentrations of 0.2 µM N (0.0028 mg/L), 0.4 µM N (0.0056 mg/L), 1 µM N (0.014 mg/L), 2 µM N (0.028 mg/L) and 3 µM N (0.042 mg/L), respectively.

Procedure

Run this analysis concurrently with ammonium, using a synthetic seawater cup prior to a sample set and a deionized water cup prior to an intersample calibrant (See Ammonium methodology). The 3-way stream divider (PT 2 connector, P/N 116-B000-01) used to split the sample is the appropriate size for the pump tubes used. Stream dividers with smaller ports create gas bubbles with the rapid movement of sample through the fitting.

NOTES:

1. A gain setting of 53 should yield peaks of ~80 lines using a 3 μM N standard.

2. This is a very steady analysis with a smooth baseline, well defined peaks, minimal baseline correction and minimal carry-over correction.

NITRITE + NITRATE

Revised 2/2004

Filtered samples are passed through a granulated copper-cadmium column to reduce nitrate to nitrite. The nitrite (originally present plus reduced nitrate) then is determined by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine dihydrochloride to form a colored azo dye. Nitrate concentration is obtained by subtracting the corresponding nitrite value from the nitrite + nitrate concentration.

Methodology

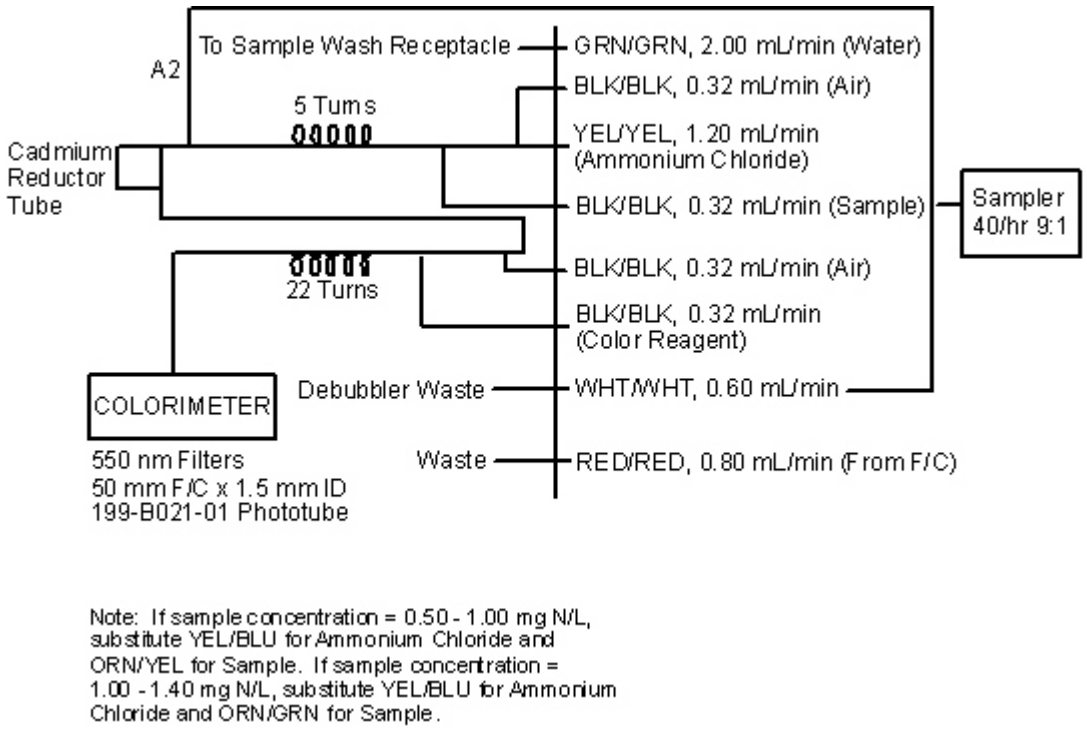
Technicon Industrial Method No. 158-71 W/A† Tentative. 1977. Technicon Industrial Systems. Tarrytown, New York, 10591.

USEPA. 1979. Method No. 353.2 *in* Methods for chemical analysis of water and wastes. United States Environmental Protection Agency, Office of Research and Development. Cincinnati, Ohio. Report No. EPA-600/4-79-020 March 1979. 460pp.

Instrumentation

Technicon AutoAnalyzer II

Manifold Assembly



Note: If sample concentration = 0.50 - 1.00 mg N/L, substitute YEL/BLU for Ammonium Chloride and ORN/YEL for Sample. If sample concentration = 1.00 - 1.40 mg N/L, substitute YEL/BLU for Ammonium Chloride and ORN/GRN for Sample.

Figure 4. Manifold assembly for nitrite + nitrate method.

Specifications

Standard Calibration Settings:
ORN/GRN sample tube: 3.0, 1.0
ORN/YEL sample tube: 2.0, 1.0
BLK/BLK sample tube: 9.0, 6.0, 2.0, 1.0
Dilution loop: 3.0, 1.0
Damp: Normal
Sampling Rate: 40/hour, 9:1 sample/wash ratio
Filter: 550 nm
Phototube: 199-B021-01
Flowcell: 50 mm
Relative Absorbance (20 µM NO_3^--N; BLK/BLK sample tube): ~.55

Interferences: Metal ions may interfere if present in sufficient concentrations. The presence of large concentrations of sulfide and/or sulfate greatly reduce the sensitivity of the copper-cadmium column.

Reagents

Alkaline Water

Sodium hydroxide (NaOH, pellets)	0.20 ± 0.02 g
Deionized water	up to 1000 mL

Add 0.20 g of sodium hydroxide pellets to 1000 mL of deionized water.

Copper Sulfate Reagent, 2%

Copper sulfate ($CuSO_4 \cdot 5\ H_2O$)	2 g
Deionized water	up to 100 mL

In a 100 mL volumetric flask, dissolve 2 g of copper sulfate in ~80 mL of deionized water. Dilute to 100 mL with deionized water.

Ammonium Chloride Reagent

Ammonium chloride (NH_4Cl)	10 g
Alkaline Water	up to 1000 mL
Copper Sulfate Reagent, 2%	6 drops

In a 1000 mL volumetric flask, dissolve 10 g of ammonium chloride in ~800 mL of Alkaline Water and dilute to 1000 mL with Alkaline Water. Attain a pH balance of 8.5. Add 6 drops of 2% Copper Sulfate Reagent.

Color Reagent

Sulfanilamide ($C_6H_8N_2O_2S$)	20 g
Phosphoric acid (H_3PO_4), concentrated (80%)	200 mL
N-1-naphthylethylenediamine dihydrochloride ($C_{12}H_{14}N_2 \cdot 2HCl$)	1 g
Deionized water	up to 2000 mL
Brij-35, 30%	1 mL

In a 2000 mL volumetric flask, add 200 mL concentrated phosphoric acid and 20 g of sulfanilamide to ~1500 mL deionized

water. Dissolve completely. Add 1 g of N-1-naphthylethylenediamine dihydrochloride and dissolve. Dilute to 2000 mL with deionized water and add 1 mL of 30% Brij-35. Store in refrigerator. Prepare every 6 weeks.

Preparation of Copper-Cadmium Column

1. Use good quality cadmium filings (25 - 60 mesh size).

2. Clean 10 g of cadmium with 20 mL of acetone. Rinse twice with 20 mL of deionized water. Clean cadmium next with 50 mL of 1 N hydrochloric acid for 1 minute. Cadmium turns silver in color. Decant the hydrochloric acid and wash the cadmium with another 50 mL of 1 N hydrochloric acid for 1 minute.

3. Decant the hydrochloric acid and wash the cadmium several times with deionized water.

4. Decant the deionized water and add 20 mL of 2% (w/v) copper sulfate ($CuSO_4 \cdot 5H_2O$). Wash the cadmium until no blue color remains in the solution.

5. Add another 20 mL of 2% (w/v) copper sulfate and wash the cadmium until no blue color remains in the solution. The cadmium will be dark brown in color.

6. Decant and wash thoroughly (~10 times) with deionized water.

7. Fill the reductor column (22 cm length of 0.110-inch ID tubing) with Ammonium Chloride Reagent and transfer the prepared cadmium particles to the column using a Pasteur pipette or some other method that prevents contact of the cadmium pellets with air. Do not allow any air bubbles to be trapped in the column. Pack the entire column uniformly with filings such that, visually, the packed filings have separation gaps $\leq$ ~1 mm.

8. When the entire column is well-packed with granules, insert glass wool plugs at the outlet end of the column. With reagents running through the system, attach the column. Remember to have no air bubbles in the valve and to attach the column to the intake side of the valve first.

9. Check for good flow characteristics (good bubble pattern) after the addition of air bubbles beyond the column. If the column is packed too tightly, an inconsistent flow pattern will result. Allow Ammonium Chloride Reagent to flow through the column for one hour.

10. Prior to sample analysis, condition the column with approximately 100 mg nitrate/L for 5 minutes followed by 100 mg nitrite/L for 10 minutes.

11. Analyze Working Standard A (40 µM NO_3^--N) and Working Nitrite Standard (40 µM NO_2^--N). If the peak height of Working Standard A is <90% of the peak height of Working Nitrite Standard, prepare a new cadmium reduction column.

Standards

Stock Nitrate Standard, 5,000 µM

Potassium nitrate (KNO_3), dried at 45°C	0.5055 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 0.5055 g of potassium nitrate in ~800 mL of deionized water. Dilute to 1000 mL with deionized water (1 mL contains 5 µmoles N).

Working Standard A

Stock Nitrate Standard	0.8 mL
Deionized water	up to 100 mL

In a 100 mL volumetric flask, dilute 0.8 mL of Stock Nitrate Standard to 100 mL with deionized water to yield a concentration of 40 µM N (0.56 mg N/L).

Working Nitrate Standards

Dilute 2.5, 5, 10, 15 and 25 mL of Working Standard A to 100 mL with deionized water to yield concentrations of 1 µM N (0.014 mg N/L), 2 µM N (0.028 mg N/L), 4 µM N (0.056 mg N/L), 6 µM N (0.084 mg N/L) and 10 µM N (0.140 mg N/L), respectively.

Dilute 0.8 mL of Stock Nitrate Standard to 200 mL with deionized water to yield concentrations of 20 µM N (0.28 mg N/L).

Dilute 1.0 mL and 1.5 mL of Stock Nitrate Standard to 100 mL with deionized water to yield concentrations of 50 and 75 µM N (0.70 and 1.05 mg N/L), respectively for use with the ORN/YEL sample tube, YEL/BLU NH_4Cl tube and sample concentrations of 0.50 - 1.00 mg N/L (nitrite + nitrate).

Dilute 2.0 mL of Stock Nitrate Standard to 100 mL with deionized water to yield concentrations of 100 µM N (1.40 mg N/L) for use with the ORN/GRN sample tube, YEL/BLU NH_4Cl tube and sample concentrations of 1.00 - 1.40 mg N/L (nitrite + nitrate).

Dilute 3.0, 5.0, 6.0 and 8.0 mL of Stock Nitrate Standard to 100 mL with deionized water to yield concentrations of 150, 250, 300 and 400 µM N (2.1, 3.5, 4.2 and 5.6 mg N/L) for use with the dilution loop and sample concentrations of 1.40 - 5.60 mg N/L (nitrite + nitrate). The nitrate sample is sampled originally with an ORN/WHT sample tube and YEL/YEL deionized water diluent tube which is then "resampled" to an ORN/YEL resample tube and YEL/BLU NH_4Cl tube.

Stock Nitrite Standard, 5,000 µM

Sodium nitrite ($NaNO_2$), dried at 45°C	0.345 g
Deionized water	up to 1000 mL
Chloroform ($CHCl_3$)	1 mL

In a 1000 mL volumetric flask, dissolve 0.345 g of sodium nitrite in ~600 mL of deionized water. Dilute to 1000 mL with deionized water. Add 1 mL of chloroform as a preservative (1 mL contains 5 µmoles N).

Working Nitrite Standard

Dilute 0.8 mL of Stock Nitrite Standard to 100 mL with deionized water to yield a concentration of 40 µM N (0.56 mg N/L).

SILICATE Revised 2/2004

This reaction is based on the reduction of silicomolybdate in acidic solution to "molybdenum blue" by ascorbic acid. Oxalic acid is added to minimize interference from phosphates.

Methodology

Technicon Industrial Method No. 811-86T. November 1986. Technicon Industrial Systems. Tarrytown, New York, 10591.

Instrumentation

Technicon TrAAcs-800 Nutrient Analyzer

Manifold Assembly

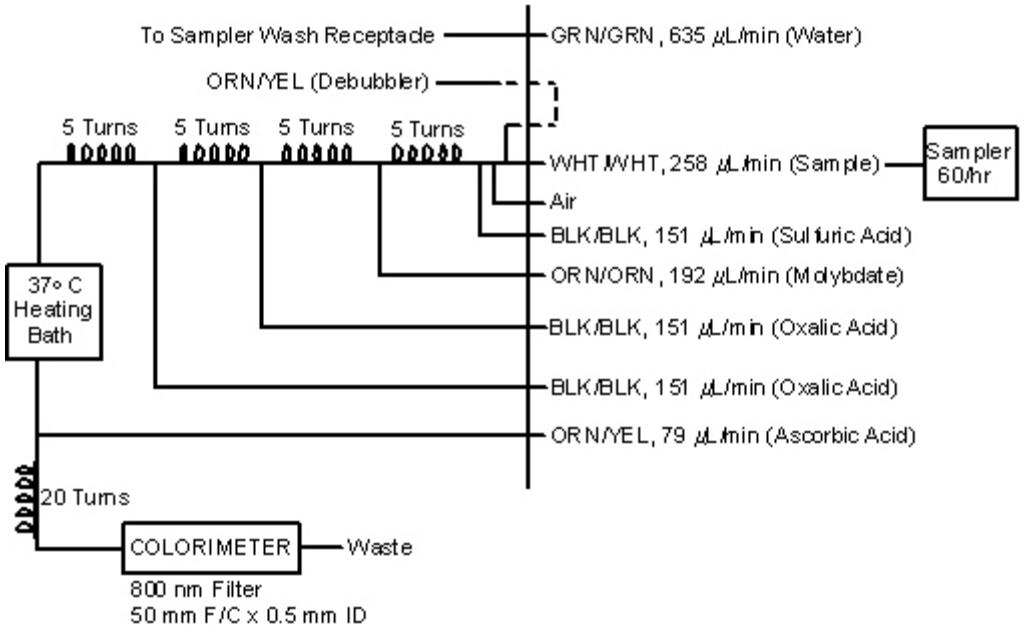


Figure 5. Manifold assembly for silicate method.

Specifications

Gain: 8
Baseline: 39 - 42
Sampling Rate: 60/hour
Filter: 800 nm
Flowcell: 50 mm
Relative Absorbance (75 µM Si): ~1.10
Interferences: Salinity may interfere.

Reagents

Oxalic Acid Solution

Oxalic acid ($H_2C_2O_4 \cdot 2H_2O$)	100 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 100 g of oxalic acid in ~900 mL deionized water and dilute to 1000 mL with deionized water.

Ascorbic Acid Solution

Oxalic acid ($H_2C_2O_4 \cdot 2\ H_2O$)	5 g
Ascorbic acid ($C_6H_8O_6$), U.S.P. quality	100 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 5 g of oxalic acid in ~800 mL of deionized water. Add 100 g of ascorbic acid and mix until dissolved. Dilute to 1000 mL with deionized water.

Ammonium Molybdate Solution

Ammonium molybdate [$(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$]	7.5 g
Deionized water	up to 250 mL

In a 250 mL volumetric flask, dissolve 7.5 g of ammonium molybdate in ~200 mL of deionized water. Dilute to 250 mL with deionized water.

Stock Phosphate Solution (Baseline Spike)

Potassium phosphate (KH_2PO_4), dried at 45°C	0.4394 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 0.4394 g of potassium phosphate in ~600 mL of deionized water. Dilute to 1000 mL with deionized water. Prepare fresh when making 0.7 N Sulfuric Acid Solution.

Sulfuric Acid Solution, 0.7 N

Sulfuric acid (H_2SO_4), concentrated (sp. gr. 1.84)	4.06 mL
Stock Phosphate Solution	21.4 mL
Sodium dodecyl sulfate, (SDS) [$CH_3(CH_2)_{11}OSO_3Na$; m.w. = 288.38; phosphate $\leq$0.0001 %]	~0.25 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, add 4.06 mL of concentrated sulfuric acid and 21.4 mL of Stock Phosphate Solution to ~600 mL of deionized water. Dilute to 1000 mL with deionized water. Add ~0.25 g of SDS.

Sodium Hydroxide Wash Solution

Sodium hydroxide (NaOH)	4 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 4 g of sodium hydroxide in ~800 mL of deionized water. Dilute to 1000 mL with deionized water.

Sampler (Baseline) Solution

Isopropanol [$(CH_3)_2CHOH$]	100 mL
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dilute 100 mL of isopropanol to 1000 mL with deionized water.

Standards

Stock Silicate Standard, 10,000 µM

Sodium silicofluoride (Na_2SiF_6), dried at 45°C	1.88 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 1.88 g of sodium silicofluoride in ~900 mL of deionized water. Dilute to 1000 mL with deionized water (1 mL contains 10 µmoles Si). Store in a plastic container.

Working Silicate Standards

Dilute 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.75 mL of Stock Silicate Standard to 100 mL with deionized water to yield concentrations of 5 µM Si (0.14 mg/L), 10 µM Si (0.281 mg/L), 20 µM Si (0.562 mg/L), 30 µM Si (0.843 mg/L), 40 µM Si (1.124 mg/L), 50 µM Si (1.41 mg/L) and 75 µM Si (2.10 mg/L), respectively.

NOTE:

1. The use of glassware with this method should be avoided because it may be a source of silica contamination. All chemicals used for reagents and the deionized water should be of good quality and low in silica.

2. Sampler (Baseline) Solution of isopropanol is used instead of deionized water as baseline solution to counter the effect of salt water on the system due to its high refractive index relative to deionized water.

Procedure

1. At start-up, check the level of all reagents to ensure an adequate supply.

2. With the molybdate line pumping air, place all other lines into their respective containers and start the proportioning pump.

3. After 2 - 3 minutes, place the molybdate line into its container. Allow ~10 minutes equilibration.

4. A typical tray protocol appears as follows:

P, 5C, H, 2L, *n*S, I,....

where:
P = primer (100% FS standard),
C = calibrant,
H = 100% FS standard,
L = 20% FS standard,
S = samples, and
I = intersample calibrant.

5. At shut-down, remove all reagent lines and place into the Sodium Hydroxide Wash Solution container. Allow to pump 5 minutes.

6. Remove all lines from the wash container and place into deionized water with SDS. Allow to pump 10 minutes.

SULFATE, CHLORIDE AND NITRATE Revised 2/2004

A small volume of filtered sample is introduced into an ion chromatograph (IC). The sample is pumped through a pre-column, separator column, suppressor column and conductivity detector. Anions are separated in the pre-column and separator column based on their affinity for resin exchange sites in the columns. The suppressor column converts the sample anions to their acid form. The separated anions are measured by the conductivity detector. The concentration of anions is determined by comparing peak areas of unknowns to a calibration curve generated from known standards.

Methodology

Pfaff, J.D., C.A. Brockhoff and J.W. O'Dell. August 1991. USEPA Test Method No. 300.0. The determination of inorganic anions in water by ion chromatography. Environmental Monitoring and Systems Laboratory, Cincinnati, OH 45268.

USEPA. 1987. Section 11.0. Determination of chloride, nitrate and sulfate by ion chromatography *in* Handbook of methods for acid deposition studies: Laboratory analyses for surface water chemistry. United States Environmental Protection Agency, Office of Research and Development. Washington, D.C.. Report # EPA 600/4-87/026 September 1987. 336pp.

Instrumentation

Dionex-120 Ion Chromatograph with PeakNet software (version 5.21) was brought on-line in January 2003. A Dionex AS-40 AutoSampler was added to the system in January 2004.

Reagents

Eluent, 3.5M Na_2CO_3/1.0M $NaHCO_3$ Solution

Sodium carbonate (Na_2CO_3)	0.742 g
Sodium bicarbonate ($NaHCO_3$)	0.164 g
Deionized water	up to 2000 mL

In a 2000 mL volumetric flask, dissolve 0.742 g of sodium carbonate and 0.164 g of sodium bicarbonate in approximately 1500 mL of deionized water. Dilute to 2000 mL with deionized water.

Regenerant

Sulfuric acid (H_2SO_4), concentrated (sp.gr. 1.84)	2.8 mL
Deionized water	up to 2000 mL

In a 2000 mL volumetric flask, add 2.8 mL of concentrated H_2SO_4 to approximately 1500 mL of deionized water. Dilute to 2000 mL with deionized water. The regenerant reservoir must be filled to the absolute top of the container. CBL's system holds six liters of acid.

This eluent/regenerant system is called displacement chemical regeneration. As the regenerant is used up, the used eluent takes its place in the reservoir. It is crucial that every time that the eluent is changed, that the regenerant is also changed.

Standards

Stock Sodium Chloride Standard

Sodium Chloride (NaCl), dried at 45° C	1.6485 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 1.6485 g of sodium chloride in ~ 800 mL deionized water. Dilute to 1000 mL with deionized water (1mL contains 1 mg Cl^-).

Stock Sodium Sulfate Standard

Sodium sulfate (Na_2SO_4), dried at 45° C	1.8141 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 1.8141 g of sodium sulfate in ~ 800 mL deionized water. Dilute to 1000 mL with deionized water (1mL contains 1 mg SO_4^{-2}).

Stock Sodium Nitrate Standard

Sodium nitrate ($NaNO_3$), dried at 45° C	6.0679 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 6.0679 g of sodium nitrate in ~ 800 mL deionized water. Dilute to 1000 mL with deionized water (1mL contains 1 mg NO_3^--N).

Working 5:5:0.5 Chloride/Sulfate/Nitrate Standard (mg/L)

Stock Sodium Chloride Standard	0.5 mL
Stock Sodium Sulfate Standard	0.5 mL
Stock Sodium Nitrate Standard	0.05 mL
Deionized water	up to 100 mL

In a 100 mL volumetric flask, dilute 0.5 mL of stock sodium chloride standard, 0.5 mL stock sodium sulfate standard and 0.05 mL stock sodium nitrate standard to 100 mL with deionized water. The final concentration of this standard is 5 mg Cl^-/L, 5 mg SO_4^{-2}/L and 0.5 mg NO_3^--N/L.

A comparable procedure may be used to make different working standards based on the concentrations of the samples to be analyzed.

Procedure

Each sample to be analyzed must be pre-treated to remove organic particles that may bind to the column beads, reducing column efficiency, reliability and also column life. Organic particles are removed by a BakerBond SPE C-18 column and filtration through a Gelman or Whatman 0.2 µm Acrodisk designed for use with an IC system.

1. To prepare the C-18 columns, pass one column volume of HPLC grade methanol through each column. Then pass three column volumes of deionized water through each column.

2. To prepare a sample, first remove all the deionized water from the column and then immediately place 1 mL of sample into the column.

3. Place an acrodisk at the end of the column containing the sample.

4. Pass the sample through the column using positive pressure (pushing air through a syringe). Discard the first five drops.

Capture the next 11 drops in a 0.5 Ml Dionex sample vial, place a Dionex sample cap on top of the vial and refrigerate until analysis.

ORGANIC ANALYTES

Rationale

Methods for measuring dissolved organic carbon, nitrogen and phosphorus are described in the following sections. Dissolved organic carbon concentrations are determined by high temperature combustion. For total dissolved nitrogen and phosphorus, potassium persulfate is added to a sample, which, when under heat and pressure, breaks down the organic constituents to inorganic forms. Inorganic fractions then are subtracted from the total dissolved sample to yield the dissolved organic concentration. See Figures 6 and 7.

Sampling and Storage

Collected water samples are filtered through Whatman GF/F filters (nominal pore size 0.7 µm) and placed in appropriate containers and preserved. Table 2 presents sampling and storage practices for organic analytes. Containers for dissolved organic carbon are acid washed in 10% HCl and rinsed thoroughly with deionized water. Containers for nitrogen and phosphorus are autoclaved with potassium persulfate solution before use, then rinsed thoroughly with deionized water only.

Table 2. Sampling and storage for organic analytes

ANALYTE	VOLUME (mL)	STORAGE	CONTAINER
Dissolved organic carbon Vial[1]	~30	-20°C	Teflon or Borosilicate Glass
Dissolved nitrogen/phosphorus Tube	10	-20°C	Borosilicate Glass Screw-cap

[1] Teflon vials can be used for all samples. Wheaton-33® low extractable borosilicate glass vials (recommended by EPA) are used for saltwater samples.

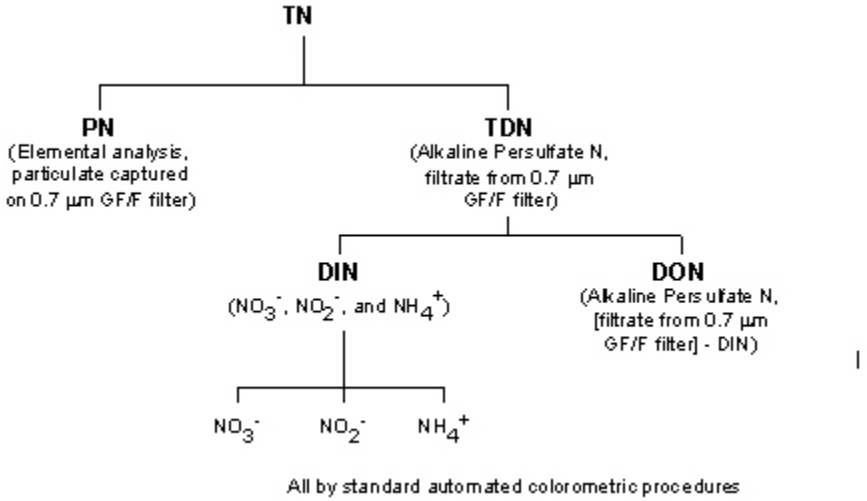


Figure 6. Flow diagram of nitrogen analysis (TN = total nitrogen, PN = particulate nitrogen, TDN = total dissolved nitrogen, DIN = dissolved inorganic nitrogen, DON = dissolved organic nitrogen).

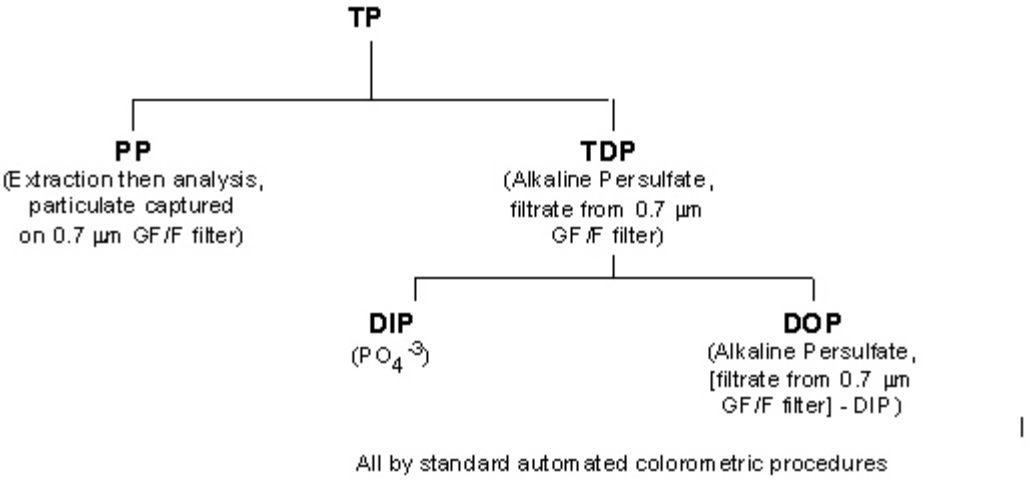


Figure 7. Flow diagram of phosphorus analysis (TP = total phosphorus, PP = particulate phosphorus, TDP = total dissolved phosphorus, DIP = dissolved inorganic phosphorus, DOP = dissolved organic phosphorus).

TOTAL DISSOLVED NITROGEN AND PHOSPHORUS

Revised 2/2004

This method is a persulfate oxidation technique for nitrogen and phosphorus where, under initially alkaline conditions, nitrate is the sole nitrogen product. Phosphate is the sole phosphorus product after acidic conditions are achieved following further autodecomposition of the persulfate in the heated oxidation tubes..

Digested samples are passed through a granulated copper-cadmium column to reduce nitrate to nitrite. The nitrite then is determined by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine dihydrochloride to form a colored azo dye. Color is proportional to nitrogen concentration.

Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex which is reduced to an intensely blue-colored complex by ascorbic acid. Color is proportional to phosphorus concentration.

Methodology

D'Elia, C.F., P.A. Steudler and N. Corwin. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. Limnol. Oceanogr. 22:760-764.

Valderrama, J.C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar. Chem. 10:109-122.

Instrumentation

Technicon AutoAnalyzer II

Manifold Assembly

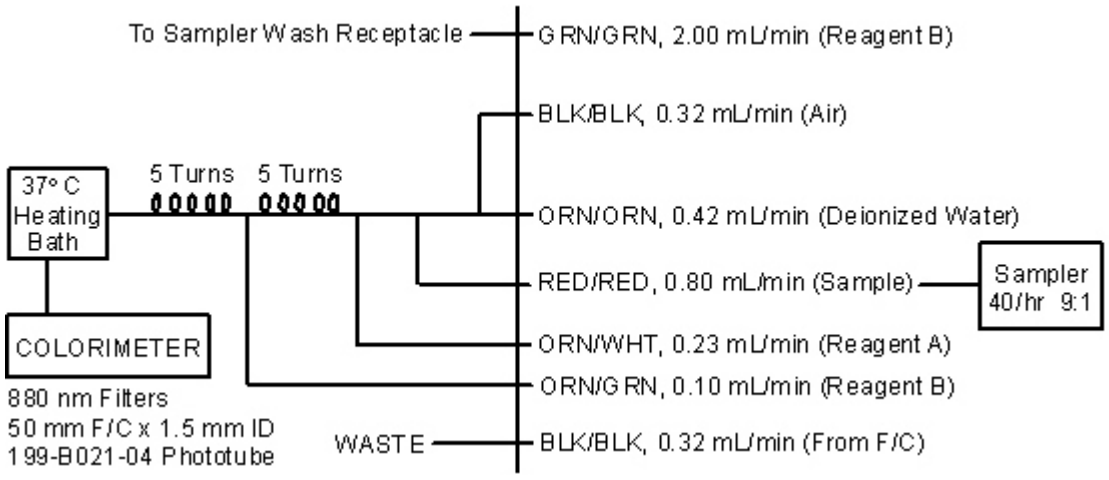


Figure 8. Manifold assembly for total dissolved phosphorus method.

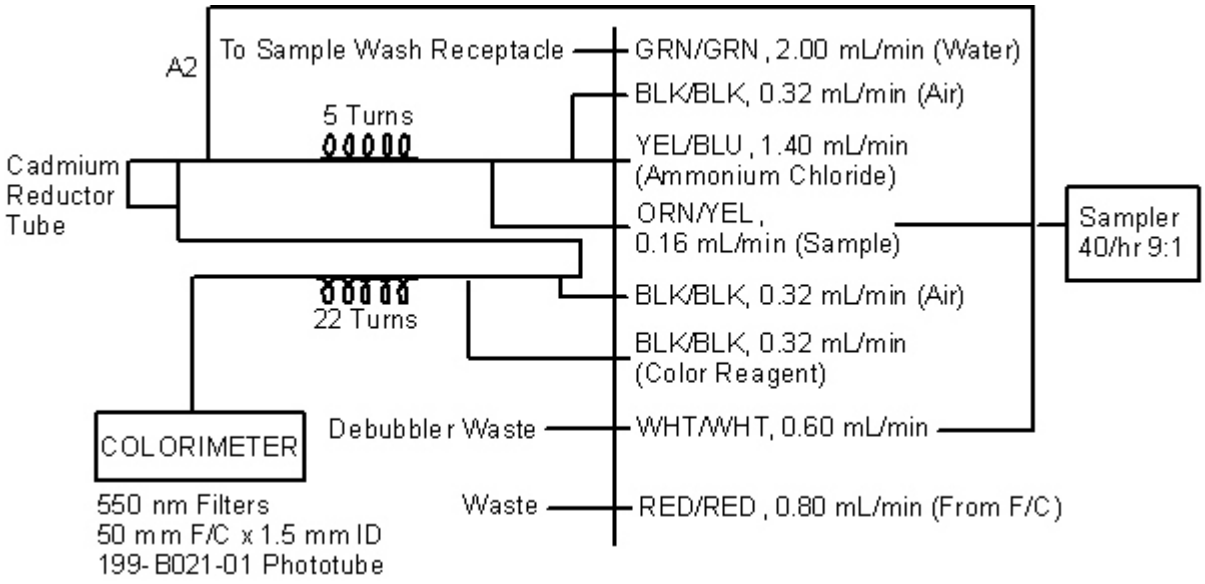


Figure 9. Manifold assembly for total dissolved nitrogen method.

Specifications

Damp: Normal
Sampling Rate: 40/hour, 9:1 sample/wash ratio
Filters: 550 nm for nitrate and 880 nm for phosphate
Phototubes: 199-B021-01 for nitrate and 199-B021-04 for phosphate
Flowcell: 50 mm
Relative Absorbance (40 µM NO_3^--N; ORN/YEL sample tube): ~.59
Relative Absorbance (18 µM P): ~.85

Interferences: Metal ions may interfere if present in sufficient concentrations. The presence of high concentrations of sulfate will reduce the sensitivity of the copper-cadmium column. Silicon at a concentration of 100 µM Si causes interferences equivalent to ~0.04 µM P.

Reagents

Oxidizing Reagent - Low, Moderate and High N and P Concentrations

Sodium hydroxide (NaOH)	3 g
Potassium persulfate ($K_2S_2O_8$), low N (<0.001 %)	20.1 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 3 g of sodium hydroxide and 20.1 g of potassium persulfate in ~800 mL of deionized water. Dilute to 1000 mL with deionized water. Make fresh daily.

Oxidizing Reagent - Very High N and P Concentrations

Sodium hydroxide (NaOH)	3 g
Potassium persulfate ($K_2S_2O_8$), low N (<0.001 %)	20.1 g
Deionized water	up to 3000 mL

In a 3000 mL volumetric flask, dissolve 3 g of sodium hydroxide and 20.1 g of potassium persulfate in ~800 mL of deionized water. Dilute to 3000 mL with deionized water. Make fresh daily.

Buffer Solution

Boric acid (H_3BO_3)	61.8 g
Sodium hydroxide (NaOH)	8 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 61.8 g of boric acid in ~300 mL deionized water. Add 8 g of sodium hydroxide and dilute to 1000 mL with deionized water. The solution is stable for several weeks.

Internal Standards

Stock Glutamic Acid Standard, 5,040 µM

Glutamic acid [$C_3H_2NH_2(COOH)_2$], dried at 45°C	0.3705 g
Deionized water	up to 500 mL
Chloroform ($CHCl_3$)	0.5 mL

In a 500 mL volumetric flask, dissolve 0.3705 g of glutamic acid in ~400 mL of deionized water and dilute to 500 mL with deionized water. Add 0.5 ml of chloroform as a preservative (1 mL contains 5.04 µmoles N).

Working Glutamic Acid Standard - Low, Moderate and High N and P Concentrations

Stock Glutamic Acid Standard	1 mL
Deionized water	up to 100 mL

In a 100 mL volumetric flask, dilute 1 mL of Stock Glutamic Acid Standard to 100 ml with deionized water to yield a concentration of 50.4 µM N (0.7056 mg N/L).

Working Glutamic Acid Standards - Very High N and P Concentrations

Stock Glutamic Acid Standard	6 mL
Deionized water	up to 100 mL

In a 100 mL volumetric flask, dilute 6 mL of Stock Glutamic Acid Standard to 100 mL with deionized water to yield a concentration of 302.4 µM N (4.23 mg N/L).

Stock Glycerophosphate Standard, 309 µM

β-Glycerophosphoric acid, disodium salt, 5-hydrate [$(HOCH_2)_2CHOPO_3Na_2 \cdot 5\ H_2O$]	0.0473 g
Deionized water	up to 500 mL
Chloroform ($CHCl_3$)	0.5 mL

In a 500 mL volumetric flask, dissolve 0.0473 g of β-Glycerophosphoric acid, disodium salt, 5-hydrate in ~400 mL of deionized water. Dilute to 500 mL with deionized water. Add 0.5 mL of chloroform as a preservative (1 mL contains 0.309 µmoles P).

Working Glycerophosphate Standard - Low, Moderate and High N and P Concentrations

Stock Glycerophosphate Standard	1 mL
Deionized water	up to 100 mL

In a 100 mL volumetric flask, dilute 1 mL of Stock Glycerophosphate Standard to 100 mL with deionized water to yield a concentration of 3.09 µM P (0.096 mg P/L).

Working Glycerophosphate Standard - Very High N and P Concentrations

Stock Glycerophosphate Standard	2 mL
Deionized water	up to 100 mL

In a 100 mL volumetric flask, dilute 2 mL of Stock Glycerophosphate Standard to 100 mL with deionized water to yield a concentration of 6.18 µM P (0.192 mg P/L).

Working Inorganic Standards

Stock Nitrate Standard, 5,000 µM

Potassium nitrate (KNO_3), dried at 45°C	0.5055 g
Deionized water	up to 1000 mL
Chloroform ($CHCl_3$)	1 mL

In a 1000 mL volumetric flask, dissolve 0.5055 g of potassium nitrate in 1000 mL of deionized water. Add 1 mL of chloroform as a preservative (1 mL contains 5 µmoles N).

Working Nitrate Standards - Low N and P Concentrations

Dilute 0.5, 1.0 and 1.5 mL of Stock Nitrate Standard to 100 mL with deionized water to yield concentrations of 25 µM N (0.35 mg N/L), 50 µM N (0.70 mg N/L) and 75 µM N (1.05 mg N/L), respectively.

Working Nitrate Standards - Moderate N and P Concentrations

Dilute 0.5, 1.0 and 2.0 mL of Stock Nitrate Standard to 100 mL with deionized water to yield concentrations of 25 µM N (0.35 mg N/L), 50 µM N (0.70 mg N/L) and 100 µM N (1.40 mg N/L), respectively.

Working Nitrate Standards - High N and P Concentrations

Dilute 1.0, 2.0 and 4.0 mL of Stock Nitrate Standard to 100 mL with deionized water to yield concentrations of 50 µM N (0.70 mg N/L), 100 µM N (1.40 mg N/L) and 200 µM N (2.80 mg N/L), respectively.

Working Nitrate Standards - Very High N and P Concentrations

Dilute 3.0, 6.0 and 8.0 mL of Stock Nitrate Standard to 100 mL with deionized water to yield concentrations of 150 µM N (2.1 mg N/L), 300 µM N (4.2 mg N/L) and 400 µM N (5.6 mg N/L), respectively.

Stock Orthophosphate Standard, 12,000 µM

Potassium phosphate, monobasic (KH_2PO_4), dried at 45°C	1.632 g
Deionized water	up to 1000 mL
Chloroform ($CHCl_3$)	1 mL

In a 1000 mL volumetric flask, dissolve 1.632 g of potassium phosphate in ~ 800 mL of deionized water. Dilute to 1000 mL with deionized water. Add 1 mL of chloroform as a preservative (1 mL contains 12 µmoles P).

Secondary Orthophosphate Standard

Stock Orthophosphate Standard	1 mL
Deionized water	up to 100 ml

In a 100 mL volumetric flask, dilute 1 mL of Stock Orthophosphate Standard to 100 mL with deionized water (1 mL contains 0.12 µmoles P).

Working Orthophosphate Standards - Low, Moderate and High N and P Concentrations

Dilute 0.5, 2.0 and 4.0 mL of Secondary Orthophosphate Standard to 100 mL with deionized water to yield concentrations of 0.6 µM P (0.0186 mg/L), 2.4 µM P (0.0744 mg/L) and 4.8 µM P (0.1488 mg/L), respectively.

Working Orthophosphate Standards - Very High N and P Concentrations

Dilute 2.0, 5.0 and 10.0 mL of Secondary Orthophosphate Standard to 100 mL with deionized water to yield concentrations of 2.4 µM P (0.0744 mg/L), 6.0 µM P (0.186 mg/L) and 12.0 µM P (0.372 mg/L), respectively.

Procedure

1. Select appropriate standards and oxidizing reagent for samples to be analyzed. For 0 - 200 µM N and 0 - 5.0 µM P, use 10 mL samples and 5 mL of Oxidizing Reagent for Low, Moderate and High N and P Concentrations, following steps 2 - 9. For 150 - 400 µM N and 2.4 - 12.0 µM P, use 5 mL samples and 15 mL of Oxidizing Reagent for Very High N and P Concentrations, following steps 2VH - 9VH.

2. Place 10 mL of filtered water (Whatman GF/F filter, 0.7 µm) in a 30 mL screw cap test tube and freeze.

2VH. Place 5 mL of filtered water (Whatman GF/F filter, 0.7 µm) in a 30 mL screw cap test tube and freeze.

3. Place 10 mL of each standard (3 replicates of each) in 30 mL screw cap test tubes and treat exactly as samples.

3VH. Place 5 mL of each standard (3 replicates of each) in 30 mL screw cap test tubes and treat exactly as samples.

4. When ready to analyze, thaw samples at room temperature.

5. Add 5 mL of Oxidizing Reagent. A precipitate will form with seawater samples. Cap test tubes fairly tightly. Invert twice.

5VH. Add 15 mL of Oxidizing Reagent. A precipitate will form with seawater samples. Cap test tubes fairly tightly. Invert twice.

6. Place test tubes in a pressure cooker at 100 - 110°C and 3 - 4 psi for 60 minutes. Bring back to atmospheric pressure over 1 hour.

7. Remove test tubes, tighten caps and cool to room temperature. Samples can be stored at this point.

8. Add 1 mL of Buffer Solution to each tube and shake. The pH of the sample should be 4 - 4.5 after the addition of the buffer solution. Transfer an aliquot of each sample to AutoAnalyzer cups using a Pasteur pipette.

9. Analyze for nitrite + nitrate and orthophosphate as described in the Dissolved Inorganic Analytes section. Use the pump tubes for low sample concentrations of 0.00 - 1.00 mg N/L; ORN/YEL NO_3^- sample tube and YEL/BLU NH_4Cl tube, for moderate sample concentrations of 0.35 - 1.4 mg N/L; ORN/GRN NO_3^- sample tube and YEL/BLU NH_4Cl tube, for high sample concentrations of 0.70 - 2.80 mg N/L; ORN/WHT NO_3^- sample tube and YEL/YEL deionzed water diluent on the

dilution loop "resampled" to a BLK/BLK resample tube and YEL/YEL NH_4Cl tube.

9VH. Analyze for nitrite + nitrate and orthophosphate as described in the Dissolved Inorganic Analytes section. Use a BLK/BLK NO_3^- sample tube and YEL/YEL deionzed water diluent on the dilution loop "resampled" to a BLK/BLK resample tube and YEL/YEL NH_4Cl tube.

NOTES:

1. The use of internal organic standards (glutamic acid and glycerophosphate) allows the check for percentage recovery and is incorporated into each digestion batch.

2. The procedure includes an internal dilution factor of samples and standards due to addition of reagents of 1:1.6 for low N and P concentration samples and 1:4.2 for very high N and P concentration samples.

3. Reagent Blanks: Oxidizing reagent only (3 replicates) is digested in 30 mL test tubes, and buffered. The analyzed peak heights of the nitrate and phosphate reagent blanks are normalized to the sample + reagent volume by adding 10 mL of deionized water immediately before analyzing for nitrite + nitrate and phosphate. The resultant reagent blank peak height then is subtracted from the sample peak heights before calculating the concentrations based on the regression from the peak heights of the standards. Reagent blank for N should be $\leq$8% of the peak height of the 75µM N standard. Reagent blank for P should be $\leq$6% of the 4.8 µM P standard.

4. Deionized Water Blanks: 10 mL of deionized water (3 replicates) are digested, and analyzed with the samples and standards. Their peak heights are included with the peak height of the digested inorganic working standards in the regression to obtain the slope used to calculate concentrations of the samples. The value of the reagent blank is generally about 0 - 5 chart lines less than the value of the y intercept obtained in the regression of inorganic working standards and deionized water blanks.

TOTAL AND DISSOLVED ORGANIC CARBON

Revised 02/2004

The Shimadzu TOC 5000/5000A utilizes high temperature combustion in determining organic carbon, and is used to analyze all ranges of salinity.

SHIMADZU TOC-5000 TOTAL ORGANIC CARBON ANALYZER METHOD

Methodology

Sugimura, Y. and Y. Suzuki. 1988. A high temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. Mar. Chem. 24:105-131.

Instrumentation

The Shimadzu TOC-5000/5000A uses a high temperature combustion method to analyze aqueous samples for TIC, TOC and non-purgeable organic carbon (NPOC). Samples are treated with hydrochloric acid and sparged with ultra pure carrier grade air to drive off inorganic carbon. High temperature combustion (680°C) on a catalyst bed of platinum-coated alumina balls breaks down organic carbon into carbon dioxide (CO_2). The CO_2 is carried by ultra pure air to a non-dispersive infrared detector (NDIR) where CO_2 is detected.

Specifications

Analytical Range: 100 ppb - 4000 ppm using 250 µL syringe and 4 - 100 µL injection volume, using regular sensitivity catalyst.
Calibration: Up to 4 concentrations may be used to calibrate the instrument

Reagents

Hydrochloric Acid, 2 N

Hydrochloric acid (HCl), concentrated	172 mL
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, add 172 mL of concentrated hydrochloric acid to ~600 mL of deionized water. Dilute to 1000 mL with deionized water.

Standard

Stock Potassium Hydrogen Phthalate (KHP) Standard, 1000 mg/L

Potassium hydrogen phthalate ($HOCOC_6H_4COOK$), dried at 45°C	2.125 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 2.125 g of potassium hydrogen phthalate in ~800 mL of deionized water. Dilute to 1000

mL with deionized water. Make fresh every 4 - 6 months. Store at 4°C.

Procedure

1. Samples are stored frozen at -20°C. When ready to analyze, thaw samples at room temperature and transfer to autosampler vials.

2. Make 3 working standards (generally <20 mg/L) from the Stock KHP Standard for the standard curve, and load into autosampler. Use deionized water as the zero concentration. Numerous standard concentrations may be analyzed as samples.

3. Acidify each sample with 100 µL of 2 N Hydrochloric Acid and add 250 µL of 2 N Hydrochloric Acid to each standard vial. It is possible to program the instrument to do this automatically.

4. The standards and samples are sparged with ultra pure carrier (UPC) grade Air for 6 minutes at 60 mL/minute, driving off any inorganic carbon in the sample.

5. Standards and samples are injected onto a catalyst bed of platinum-coated alumina balls and combusted at 680°C. The resulting CO_2 gas is carried to the NDIR detector by the UPC grade Air.

6. For the standard curve, use the area of the deionized water as the water blank in calculating the 4 standard concentrations.

7. Calculate a linear regression of the 4 standard concentrations, first subtracting the water blank from each standard.

8. The absolute value of the y-intercept is an indicator of the instrument blank and is subtracted from the area of the samples. The water blank includes the instrument blank, so only one value is subtracted from the standards.

Calculation of TOC

TOC concentration is calculated using the following equation:

$$\text{mg } TOC/L = \frac{(A_s - \|b\|)}{m}$$

where: A_s = area of the sample,
b = y-intercept, and
m = slope of the regression line.

Dissolved inorganic carbon, carbonate alkalinity, and hardness:

Dissolved inorganic carbon also can be determined with direct reaction with 25% phosphoric acid. Once the inorganic concentration is determined, carbonate alkalinity can be calculated using the following equation:

$$\text{mg } CaCO_3 / L = \frac{\text{mg DIC / L x } 100}{12}$$

NOTE: This method can also be used on saline waters of < 10 ppt salinity.
Titrimetric hardness determination is appropriate for freshwater samples (APHA, 1975).

Methodology

APHA. 1975. Method 309B. EDTA Titrimetric Method *in* Standard Methods for the Examination of Water and Wastewater, 14th Edition. American Public Health Association. Washington, D.C. 1193pp.

PARTICULATE ANALYTES

Rationale

The direct measurement of particulate carbon, particulate nitrogen and particulate phosphorus is the preferred method used by the Nutrient Analytical Services Laboratory. A large volume can be filtered onto the pad, yielding a representative sample. The alternative, subtraction of the dissolved concentration from the total sample concentration to determine the particulate carbon, nitrogen or phosphorus concentration, is imprecise, sometimes yielding negative concentrations. Direct measurement is rapid, sensitive and more precise.

Instrumentation

Particulate phosphorus and biogenic silica procedures require the use of a segmented continuous flow analyzer such as the AutoAnalyzer II, previously described in the section Dissolved Inorganic Analytes. Particulate carbon and particulate nitrogen procedures require the use of an elemental analyzer.

Sampling and Storage

A known volume of the collected water is filtered through Whatman GF/F filters (25 mm for particulate carbon and nitrogen, and 47mm for particulate phosphorus, nominal pore size 0.7 μm). The filter is folded, placed in an aluminum foil pouch and frozen until analysis. For biogenic silica, water is filtered through a 0.4 μm Nuclepore polycarbonate filter. The filter is placed in a 50 mL plastic centrifuge tube and stored in a refrigerator.

Sediment samples are collected, dried and ground with a mortar and pestle to thoroughly blend the sample.

PARTICULATE CARBON AND NITROGEN Revised 2/2004

Samples are combusted in pure oxygen (O_2) under static conditions. Products of combustion are passed over suitable reagents in the combustion tube where complete oxidation occurs. In the reduction tube, oxides of nitrogen (N) are converted to molecular N. The carbon dioxide (CO_2), water vapor and N are mixed and released into the thermal conductivity detector where the concentrations of the sample gases are measured.

Instrumentation

Exeter Analytical, Inc. (EAI) CE-440 Elemental Analyzer

Operating Principles

- Carbon (as CO_2), hydrogen (as H_2O) and nitrogen (N_2) content in organic and inorganic compounds can be determined.

- Combustion of the weighed or filtered sample occurs in pure O_2 under static conditions (Figure 13).

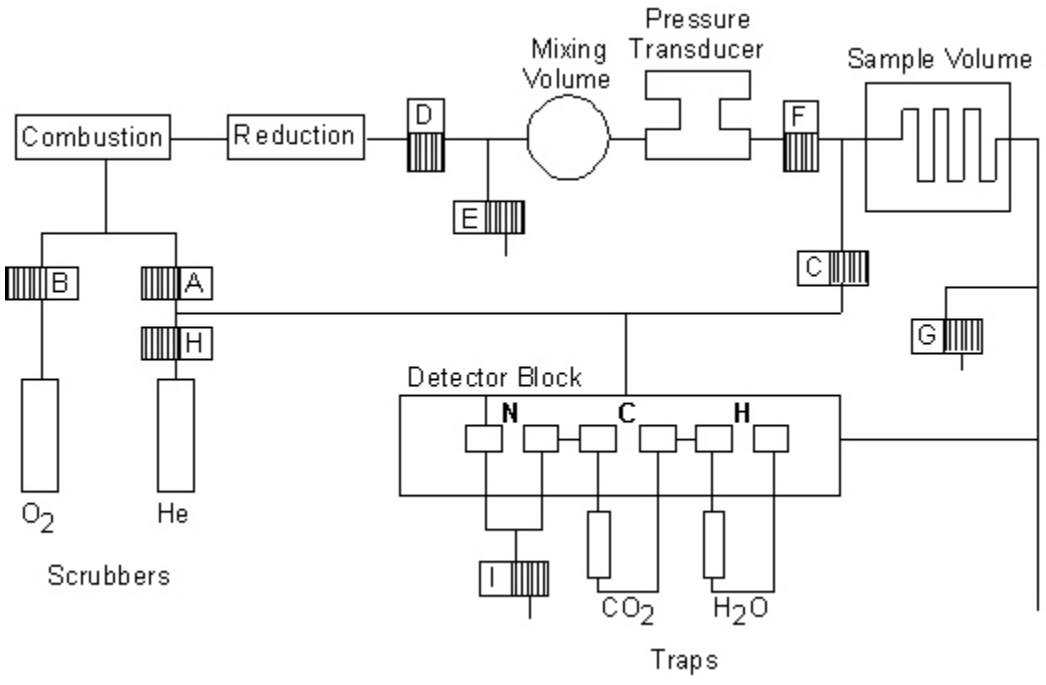


Figure 10. Schematic diagram of the Exeter Analytical, Inc. (EAI) CE-440 Elemental Analyzer.

- Helium (He, ultra-pure carrier grade) carries the combustion products through the analytical system to the atmosphere. Helium also purges the instrument.

- Solenoids A - G control the gas flow through the system. Valves H and I are used for automatic leak testing.

- Products of combustion pass over suitable reagents in the combustion tube where complete oxidation occurs.

- In the reduction tube, oxides of N are converted to molecular N. The CO_2, water vapor and N are then flushed into a mixing volume where they are thoroughly homogenized at a precise volume, temperature and pressure. The mixture is released through the sample volume into the thermal conductivity detector.

- Between the first of three pairs of thermal conductivity cells, an absorption trap removes water (H_2O) from the sample gas. The differential signal read before and after the trap reflects the amount of hydrogen (as H_2O) in the original sample. A similar measure is made of the signal output of a second pair of thermal conductivity cells between which a trap removes CO_2. The remaining gas consists only of N_2 and He. This gas passes through a thermal conductivity cell and the output signal is compared to a reference cell through which pure He flows. This gives the N_2 concentration.

Standard

Standard is acetanilide ($CH_3CONHC_6H_5$) stored at 45°C. Composition of acetanilide is 71.09% C, 6.71% H and 10.36% N.

Procedure

1. Filter a known volume of sample water through a Whatman GF/F filter pad (25 mm; 0.7 µm pore size; pre-combusted in oven set at 500°C for 90 minutes and cooled to room temperature in a dessicator. This setting on CBL's oven has been determined to be 550°C.) Filter samples in duplicate for back-up and 10% QA/QC.

2. Fold filter pads in half inward, and place in an aluminum foil pouch. Label and freeze for later analysis.

3. When ready for analysis, remove aluminum foil pouches from freezer and place in a drying oven at 45°C overnight.

4. Load nickel sleeves (pre-combusted at 875°C for 90 minutes and cooled to room temperature) into the filter ram. Load filter pads into the nickle sleeves. Place loaded nickel sleeves into the sample wheel.

5. Dry sediment samples at 45°C overnight and weigh into aluminum capsules (pre-combusted at 550°C for 90 minutes and cooled to room temperature). Load nickel sleeves into the sample wheel and place aluminum capsules containing the sediment samples into the nickel sleeves.

6. Load standards (~1500 µg acetanilide) and blanks (empty capsule for standards and weighed samples; filter pad for filtered samples) into the sample wheel. Approximately 50 samples can be run per day.

7. Place 3 standards at the beginning of each run to determine the K-Factor.

8. Insert standard checks between every 15 - 20 samples and at the end of each run.

The Run Cycle

1. At the start of each run, the entire system is flushed with helium (He) at a high flow rate while the sample is in the cool zone.

2. The injection box is automatically purged using the P valve.

3. The combustion train is filled with O_2 and the sample is injected.

4. Shortly after sample injection, D valve closes to seal off the combustion train from the rest of the analytical system that is still being flushed with He.

5. Combustion occurs under static conditions in an excess of O_2 at about 975°C. During this time, the mixing volume is being purged with E and F valves open.

6. Valve F closes to allow the pressure in the mixing volume to reach atmospheric pressure.

7. Close to the end of the combustion period, a high-temperature heating coil around the combustion tube vaporizes any condensates at the entrance of the combustion tube that may have been produced by diffusion of the sample during initial stages of combustion.

8. To assure complete combustion, the ladle is retracted ~1 inch, a small amount of O_2 is added and the ladle is fully injected.

9. During high heat, valve E closes, A and D re-open and the combustion products are completely flushed from the combustion train into the mixing volume.

10. When a pressure of 1500 mm Hg is reached, valve D closes, trapping the sample gas in the mixing volume. The time required to reach this pressure is called "fill time" and is usually 20 - 50 seconds.

11. The combustion train remains under positive pressure until the end of the complete cycle.

12. While the sampling gases are mixing, pure He flows from valve C through the sample volume and through the detectors.

13. The signal from each detector bridge is read and stored in memory to provide a baseline reading with no sample gas in the detector.

14. After mixing is complete and baseline reading is set, F and G open allowing the sample gas captured in the mixing volume to expand through the sample volume to the atmosphere. During this time, valve C is closed and there is low flow through the detector.

15. When sample gases are near atmospheric pressure, valves F and G close and C opens. The H_2O, CO_2 and N_2 concentrations of the sample are measured by displacing the sample gas through the detectors to the atmosphere.

16. The volume of sample gas in the system is large enough so that the He flow allows measurement of the contents of each detector in sequence, under steady-state conditions.

17. The sample gas passes through the detectors at a constant flow, pressure and temperature. This eliminates any variation in water vapor concentration due to changes in water adsorption on the walls of the pneumatic system.

18. While the sample gas is displaced through the detectors, the output signals are recorded.

19. The difference in microvolts (μV) between each "read" signal and the baseline ("zero") level for the same detector is directly proportional to the concentration of the sample gas measured.

20. At the end of a cycle, the exhaust valves open to allow the sample gases to escape to the atmosphere.

21. The computer then prints out the calculated results, places the instrument in standby with C valve open and waits for the next command.

22. With the HA automatic injector, the results are printed after each run. The run cycle continues until the pre-selected number of runs have been completed.

Definition of Terms

Blank: Blank value = blank read (see Read Signal below) minus blank zero (see Zero Value below).

Capsule: Pre-combusted aluminum or nickel container. Used for sealing samples with an accurate weight and maintains integrity prior to combustion.

Combustion Time: Time for sample to fully combust in O_2 environment.

Combustion Tube: Quartz tube used for packing reagents and for sample combustion.

Conditioner: Coats the walls of the system surfaces, particularly the mixing and sample volumes, with H_2O vapor, CO_2 and N_2 that simulate actual sample running conditions. The conditioner we use is acetanilide as recommended by the manufacturer. A sample is considered conditioner when placed before a blank or a standard in a run. Blanks should be run immediately after a conditioner.

Detector: The heart of the analyzer consisting of three bridges. Determines the percentages of C, H and N in the sample via thermal conductivity.

Detector Oven: Keeps the temperature of the detector, pressure transducer, mixing volume and sample volume constant.

Double Drop: On HA automation, two samples are dropped for one run used for >25 mm filters. Sample ID requires a "+" prefix.

Fill Time: Time required to build up the pressure in the mixing volume to 1500 mm Hg.

Furnace: Heats the reduction and combustion tubes to operating temperature.

Injection: Moving the ladle into the combustion furnace.

Injection Box: For the HA automation, the box assembly that houses the sample wheel.

K-Factor: Instrument sensitivity factor in μV/μg, calibrated using a chemical standard.

Ladle: Transports the boat or capsule containing the sample into the combustion furnace.

Mixing Volume: Spherical bottle in which sample gases become homogenous.

Mother Board: The main circuit board. Where all CE-440 power supplies are located.

Read Signal: Steady-state signal produced by the detector when sample gases are present in stable concentration.

Reduction Tube: Quartz tube with reduced copper that removes excess O_2 from the sample gas and reduces oxides of N_2 to free N.

Run: One sample analysis from start to finish, including print-out.

Run Cycle: The entire analytical sequence of runs from the first run to the last run in a day, including the transfer of the run cycle data to the disk.

Sample Volume: Tube where sample gas is exhausted from the mixing volume prior to entering the detector.

Scrubbers: Removes water and CO_2 from gas supplies.

Traps: Used for removing water and CO_2 from the sample gas.

Zero Value: Bridge signal with only pure He flowing through the detector.

Calibration

The following formula is used to calculate K-Factors, as well as C, N and H concentrations in unknown samples:

$$\% = \frac{(R - Z - B)}{K \times W} \times 100$$

where:
- K = calibration factor of the instrument,
- W = sample weight,
- R = read signal of sample gas,
- Z = zero reading or instrument baseline, and
- B = blank signal (instrument, ladle and capsules).

Always run a conditioner before a standard and before and after a blank.

K-Factors vary greatly among instruments but should be within the following ranges:

K_C = 18 - 25
K_H = 55 - 76
K_N = 7 - 10

PARTICULATE PHOSPHORUS AND PARTICULATE INORGANIC PHOSPHORUS

Revised 2/2004

Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex which is reduced to an intensely blue-colored complex by ascorbic acid. Color is proportional to phosphorus concentration.

Methodology and Related Reference

Aspila, I., H. Agemian and A.S.Y. Chau. 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. Analyst 101:187-197.

Keefe, C.W. 1994. The Contribution of Inorganic Compounds to the Particulate Carbon, Nitrogen, and Phosphorus in Suspended Matter and Surface Sediments of Chesapeake Bay. Estuaries 17:122-130.

Instrumentation

Technicon AutoAnalyzer II with IBM compatible Bran and Luebbe AACE ver. 5.22 Software data collection system.

Manifold Assembly

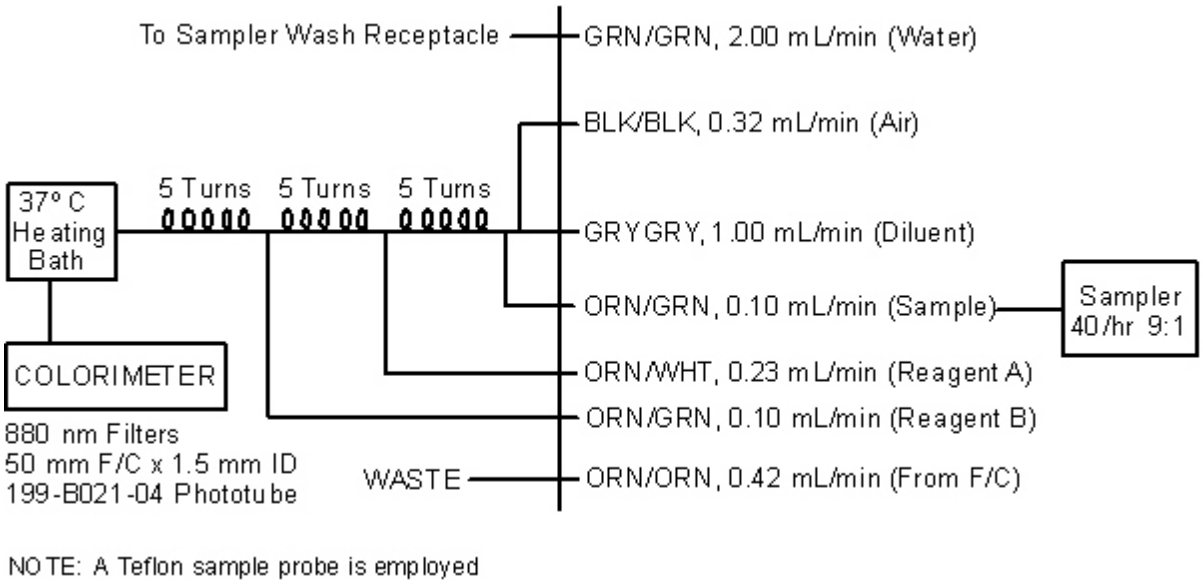


Figure 11. Manifold assembly for particulate phosphorus method.

Specifications

Standard Calibration Setting: 4.0
Damp: Normal
Sample Rate: 40/hour, 9:1 sample/wash ratio
Filter: 880 nm
Phototube: 199-B021-04
Flowcell: 50 mm
Relative Absorbance (48 µM P): ~.27
Interferences: Silicon (Si) at analysis temperature >40°C and/or <2.2 N sulfuric acid in the mixed reagent solution causes interference in the concentration range of >0.05 mg/mL Si in the extract. These conditions are avoided by maintaining an acid concentration of 2.45 N sulfuric acid in the reagents and analysis at 37°C.

Reagents

Hydrochloric Acid, 1 N

Hydrochloric acid (HCl), concentrated (sp. gr. 1.9)	86 mL
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, add 86 mL of concentrated hydrochloric acid to ~800 mL of deionized water. After cooled, dilute to 1000 mL with deionized water.

Deionized Water Diluent

Sodium dodecyl sulfate, (SDS) [$CH_3(CH_2)_{11}OSO_3Na$] m.w. = 288.38; phosphate ≤0.0001 %]	0.05 g
Deionized water	up to 500 mL

Add 0.05 g of sodium dodecyl sulfate to 500 mL deionized water. Mix well.

Sulfuric Acid Solution , 4.9 N

Sulfuric acid (H_2SO_4), concentrated (sp. gr. 1.84)	136 mL
Deionized water	up to 1000 mL

To a 1000 mL volumetric flask, add 136 mL of concentrated sulfuric acid to approximately 800 mL of deionized water while cooling (cold water bath). After the solution is cooled, dilute to 1000 mL with deionized water.

Ammonium Molybdate Solution

Ammonium molybdate [$(NH_4)_6Mo_7O_{24}\cdot 4\ H_2O$]	40 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 40 g of ammonium molybdate in 800 mL of deionized water. Dilute to 1000 mL with deionized water. Store in plastic bottle away from direct sunlight.

Ascorbic Acid Solution

Ascorbic acid ($C_6H_8O_6$)	18 g
Deionized water	up to 1000 mL
Sodium dodecyl sulfate, (SDS) [$CH_3(CH_2)_{11}OSO_3Na$]	1.66 g

In a 1000 mL volumetric flask, dissolve 18 g of ascorbic acid and 1.66 g of SDS in 800 mL of deionized water. Dilute to 1000 mL with deionized water and dispense ~40 mL into clean polybottles and freeze. Thaw overnight in refrigerator before use.

Antimony Potassium Tartrate Solution

Antimony potassium tartrate [$K(SbO)C_4H_4O_6 \cdot \frac{1}{2} H_2O$]	3 g
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 3 g of antimony potassium tartrate in 800 mL deionized water. Dilute to 1000 mL with deionized water.

Working Reagents

Reagent A

Sulfuric Acid Solution, 4.9 N	50 mL
Ammonium Molybdate Solution	15 mL
Antimony Potassium Tartrate Solution	5 mL
SDS	0.15 g

Reagent B

Ascorbic Acid Solution	30 mL
SDS	0.05 g

Standards

Stock Phosphorus Standard, 12,000 µM

Potassium phosphate, monobasic (KH_2PO_4), dried at 45°C	1.632 g
Deionized water	up to 1000 mL
Chloroform ($CHCl_3$)	1 mL

In a 1000 mL volumetric flask, dissolve 1.632 g of potassium phosphate in ~800 mL of deionized water. Dilute to 1000 mL with deionized water. Add 1 mL of chloroform to act as a preservative (1 mL contains 12 µmoles P).

Working Phosphorus Standards

Dilute 0.05, 0.1, 0.2, 0.3 and 0.4 mL of Stock Phosphorus Standard to 100 mL with 1 N Hydrochloric Acid to yield concentrations of 6 µM P (0.185 mg P/L), 12 µM P (0.37 mg P/L), 24 µM P (0.74 mg P/L), 36 µM P (1.11 mg P/L) and 48 µM P (1.48 mg P/L), respectively.

Particulate Phosphorus Procedure

1. Filter a known volume of water through a Whatman GF/F filter (47 mm; 0.7 µm pore size, pre-dried at 103 - 105°C for 24 hours, if also using pad for total suspended solids). Using forceps, fold filter pad in half, sample inside, and place in an aluminum foil pouch. Collect sediment samples in a clean container.

2. Freeze aluminum foil pouches and/or sediments.

3. If filters are used also for Total Suspended Solids, dry overnight at 103-105°C. Dry sediments at 50°C overnight or until dry. Grind sediment samples to blend thoroughly.

4. Fold filters in quarters using forceps and place in numbered crucibles with lids. Weigh sediment into crucibles. In our laboratory, generally about 25 mg of sediment is used. Cover crucibles. Record sample numbers and corresponding

crucible numbers. Prepare 5% of samples in duplicate.

5. Combust samples in crucibles in an oven set at 500 ° C for 90 minutes. This setting on CBL's oven has been determined to be 550 ° C.

6. When crucibles are cool, remove from muffle furnace. Place filter or sediment in a labelled 50 mL plastic screw cap centrifuge tube.

7. Add 10 mL of 1 N Hydrochloric Acid to each tube containing a filter. For sediments, add 20 mL to each tube.

8. Cap tubes and let stand for a minimum of 24 hours. Shake tubes several times during the 24-hour period.

9. Transfer supernatant to an AutoAnalyzer cup with a Pasteur pipette.

10. Analyze for phosphate using an AutoAnalyzer II system equipped with a Teflon sample probe. Spike 5% of samples 1:1 with 1.11 mg P/L standard solution and analyze.

11. Blank filter pads should be carried through the procedure above; approximately one blank per ~20 samples.

Calculation of Phosphorus Concentration

Phosphorus concentration is calculated using Bran and Luebbe AACE ver.5.22 software. The calculation is based on the following equation:

where: B = mean reading of blanks,
F = inverse of the regression slope of the standards,
V_E = volume of hydrochloric acid used for extraction (L)

$$\text{mg P/L} = \frac{[(\%\ \text{on AA Chart}) - B] \times F \times V_E}{V_F}$$

(i.e., 0.01 for filters and 0.02 for sediments), and
V_F = volume of sample filtered (L).

Technicon AutoAnalyzer II with 50 mm flowcell and STD CAL setting of 4.0 typically gives the following results:

slope	43.63
r^2	0.9999

Particulate Inorganic Phosphorus Procedure

1. Follow procedure for Particulate Phosphorus Steps 1 through 3. Do not combust the samples. Place filter or weighed sediment sample directly in labeled 50 mL screw cap centrifuge tube. Follow procedure for Steps 7 through 11.
2. Calculate Particulate Inorganic Phosphorus concentration.

PARTICULATE BIOGENIC SILICA Revised 2/2004

Silicomolybdate is reduced in acid solution to "molybdenum blue" by ascorbic acid. Oxalic acid is added to eliminate interference from phosphates. Detection of the silicomolybdate complex is by colorimetry.

Methodology

Technicon Industrial Method No. 186-72W/B†. Technicon Industrial Systems. Tarrytown, New York, 10591.

Paasche, E. 1973. Silicon and the ecology of marine plankton diatoms. I. *Thalassiosira pseudonana (Cyclotella nana)* grown in a chemostat with silicate as limiting nutrient. Mar. Biol. 19:117-126.

Instrumentation

Technicon AutoAnalyzer II with IBM compatible Bran and Luebbe AACE vers. 5.22 Software data collection system

Manifold Assembly

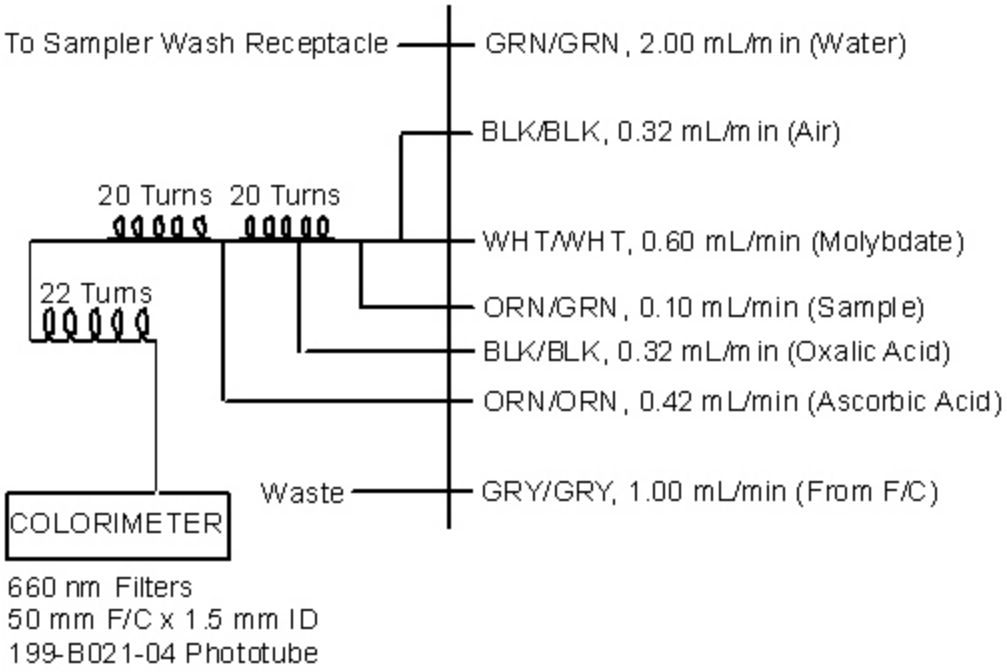


Figure 12. Manifold assembly for particulate biogenic silica method.

Specifications

Standard Calibration Setting: 5.0
Damp: Normal
Sampling Rate: 40/hour, 9:1 sample/wash ratio
Filter: 660 nm
Phototube: 199-B021-04
Flowcell: 50 mm
Relative Absorbance (42 µM Si): ~.27

Interferences: Tannin, large quantities of iron and sulfide, strong coloration and turbidity may interfere.

Digestion Equipment

2 digital timers
100°C water bath
ice bath
centrifuge tube racks
2 polypropylene re-pipettes
2 1000 mL polypropylene volumetric flasks

Digestion Reagents

Sodium Hydroxide , 0.2 N

Sodium hydroxide (NaOH)	8 g
Deionized water	up to 1000 mL

In a 1000 mL polypropylene volumetric flask, dissolve 8 g of sodium hydroxide pellets in ~800 mL of deionized water. Dilute to 1000 mL with deionized water.

Sulfuric Acid Solution, 1 N

Sulfuric acid (H_2SO_4), concentrated (sp. gr. 1.84)	28 mL
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, carefully add 28 mL of concentrated sulfuric acid to ~800 mL of deionized water. When cool, dilute to 1000 mL with deionized water.

Sample Collection

1. Filter a known volume (volume dependent on water source) of water through a 0.4 µm Nuclepore® polycarbonate filter.

2. Fold filter in half, sample inside, and place in a labelled 50 mL polypropylene centrifuge tube, cap and refrigerate.

Procedure

1. Fill water bath to depth that will cover liquid in tubes and heat to 100°C.

2. Prepare ice bath.

3. To the sample pad in the centrifuge tube, add 10 mL of 0.2 N Sodium Hydroxide from a polypropylene re-pipette. Make

sure that the filter pad is covered by the Sodium Hydroxide. Cap the tube leaving it loosened ¼ turn.

4. Place the centrifuge tube in the 100°C water bath for exactly 20 minutes.

5. After exactly 20 minutes, remove the tube from the hot water bath and place in ice bath for exactly 4 minutes.

6. Add 2.5 mL of 1 N Sulfuric Acid Solution to tube to neutralize. Cap and shake. The samples can be stored now, until analyzed.

7. Transfer extract to an AutoAnalyzer cup using a polyethylene Pasteur pipette. Avoid particulate pieces. Samples may need to be centrifuged at 3000 rpm for 10 minutes.

8. Analyze for silicon on an AutoAnalyzer II system.

9. Digest blanks and standards interspersed with samples. Digest 5% of samples in duplicate and spike 5% of sample duplicates with 0.4 mL of Secondary Silica Standard (2.5 µM Si/mL). Analyze blanks and standards before samples to establish standard curve.

Analysis Reagents

Oxalic Acid Solution

Oxalic acid ($H_2C_2O_4 \cdot 2H_2O$)	50 g
Deionized water	up to 1000 mL

In a 1000 mL polypropylene volumetric flask, dissolve 50 g of oxalic acid in ~900 mL deionized water and dilute to 1000 mL with deionized water.

Ascorbic Acid Solution

Ascorbic acid ($C_6H_8O_6$), U.S.P. quality	17.6 g
Acetone [$(CH_3)_2CO$]	50 mL
Sodium dodecyl sulfate, (SDS) [$CH_3(CH_2)_{11}OSO_3Na$], m.w. = 288.38; phosphate ≤0.0001 %]	0.3 g
Deionized water	up to 1000 mL

In a 1000 mL polypropylene volumetric flask, dissolve 17.6 g of ascorbic acid in 500 mL of deionized water and 50 mL of acetone. Mix and dilute to 1000 mL with deionized water. Add 0.3 g of sodium dodecyl sulfate. Dispense 200 mL aliquots into poly bottles and freeze.

Sulfuric Acid Solution, 0.07 N

Sulfuric acid (H_2SO_4), concentrated (sp. gr. 1.84)	1.96 mL
Deionized water	up to 1000 mL

In a 1000 mL polypropylene volumetric flask, add 1.96 mL of concentrated sulfuric acid to ~800 mL of deionized water. Dilute to 1000 mL with deionized water.

Ammonium Molybdate Solution

Ammonium molybdate [$(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$]	7 g
Sulfuric Acid Solution, 0.07 N	up to 1000 mL

Dissolve 7 g of ammonium molybdate in 1000 mL of 0.07 N Sulfuric Acid Solution. Store in an amber plastic container in the dark. Prepare fresh every few days.

Standards

Stock Silica Standard, 10,000 µM

Sodium silicofluoride (Na_2SiF_6), dried at 45°C	1.88 g
Deionized water	up to 1000 mL

In a 1000 mL polypropylene volumetric flask, dissolve 1.88 g of sodium silicofluoride in ~800 mL of deionized water. Dilute to 1000 mL with deionized water (1 mL contains 10 µmoles Si).

Secondary Silica Standard

Stock Silica Standard	25 mL
Deionized water	up to 100 mL

In a 100 mL polypropylene volumetric flask, dilute 25 mL of Stock Silica Standard to 100 mL with deionized water (1 mL contains 2.5 µmoles Si).

Working Silica Standards

To labelled 50 mL polypropylene screw cap centrifuge tubes, add 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mL of Secondary Silica Standard. Digest standards as samples. After digestion, standard tubes will contain 0, 7.0, 14.0, 21.1. 28.1, 35.1 and 42.2 µg Si, respectively.

NOTE: The use of glassware with this method should be avoided because it may be a source of Si contamination. All chemicals used for reagents and the deionized water should be of good quality and low in Si concentration.

Calculation of Silica Concentration

Silica concentration is calculated using Bran and Luebbe AACE ver. 5.22 software. The calculation is based on the following equation:

$$\text{mg Si/L} = \frac{[(\%\ \text{AA on Chart}) - B] \times F}{V}$$

where: B = mean reading of the blanks,
F = inverse of the regression slope of the standards, and
V = volume filtered (L)

Technicon AutoAnalyzer II with 50 mm flowcell and STD CAL setting of 5.0 typically gives the following results:

slope	1.60
r^2	0.9998

TOTAL SUSPENDED SOLIDS Revised 2/2004

Total suspended solids (TSS) is the retained material on a standard glass filter pad after filtration of a well-mixed sample of water. Results are expressed in mg/L.

Methodology

APHA. 1975. Method 208 D. Total Nonfilterable Residue Dried at 103 - 105 C (Total Suspended Matter) *in* Standard Methods for the Examination of Water and Wastewater, 14th Edition. American Public Health Association. Washington, D.C. 1193pp.

USEPA. 1979. Method No. 160.2 (with slight modification) *in* Methods for chemical analysis of water and wastes. United States Environmental Protection Agency, Office of Research and Development. Cincinnati, Ohio. Report No. EPA-600/4-79-020 March 1979. 460pp.

Specifications

Interferences: Excessive residue may form a water-trapping crust. Sample size should be limited to yield <200 mg of residue.

Sample Collection and Handling

1. Sequentially number Whatman 47 mm GF/F filters (0.7 µm pore size) along the outside edge, where the sample will not pass through, using a fine-tipped permanent marker.
2. Dry filters at 103 - 105°C overnight.
3. Weigh filters (in grams) to 4 decimal places to obtain pre-weight. Pre-weighed filters are stored in sealed containers until ready for use.
4. Filter a known volume of water through the filter pad (conducted in the field). Rinse filter with deionized water to remove salts. Fold pad in half, sample inside, and place in aluminum foil pouch.
5. Freeze filter pads for storage.

Procedure

1. Dry filters at 103 - 105°C overnight. Allow samples to cool to room temperature in dessicator.
2. Weigh filters and record weights (in grams).
3. Subsample a portion of the filters and replace them in the drying oven for a minimum of 1 hour.
4. Re-weigh subsampled filters. If there is >0.5 mg weight loss between the first and second weight of the subsampled filter pads, then all filter pads should be re-dried and re-weighed.
5. Repeat steps 3 and 4 as necessary.

Calculation of TSS

TSS concentration is calculated using the following equation:

$$\text{mg TSS/L} = \frac{(W_{post} - W_{pre}) \times 1000}{V_L}$$

where: W_{post} = dry weight of filter pad after filtering (g),
W_{pre} = dry weight of filter pad before filtering (g), and
V_L = volume of water filtered (L).

TOTAL VOLATILE SUSPENDED SOLIDS Revised 2/2004

Total volatile solids (TVS) is the volatilized material that is lost on ignition from Total Suspended Solids (TSS). It is calculated from the measurement of a total suspended solid (TSS) sample minus the measurement of the quantity remaining after combustion. Results are expressed in mg/L.

Methodology

APHA. 1975. Method 208 E (with modification). Total volatile and fixed residue at 550° C. in Standard Methods for the Examination of Water and Wastewater, 14 th Edition. American Public Health Association. Washington, D.C. 1193pp.

Sample Collection and Handling

1. Combust Whatman 47 mm GF/F filters (0.7 µm pore size) in an oven set at 500° C for 90 minutes. This setting on CBL's oven has been determined to be 550° C.
2. Weigh filters (g) to 4 decimal places to obtain pre-weight and store individual pads in a numbered petri dish.
3. Follow sample filtration procedure for total suspended solid sample. Make note of pad number on foil pouch.

Procedure

1. Dry filters overnight at 103°-105° C. Allow to cool to room temperature in dessicator.
2. Weigh filters and record weights (in g). Calculate TSS.
3. Transfer filter to a porcelain crucible and combust at 500° C for 90 minutes. Allow to cool to room temperature in dessicator.
4. Weigh filter and record weights (in g). Calculate TVS.

Calculation of TVS

$$\text{mg TVS / L} = \frac{(W_{combust(g)} - W_{post(g)}) \times 1000}{V_L}$$

where: $W_{combust}$ = dry weight of filter pad and sample after combustion (in grams).
W_{post} = dry weight of filter pad after filtering (in grams).
V_L = volume of water filtered (L)

CHLOROPHYLL *a* AND PHAEOPIGMENTS

Revised 2/2004

Two fluorometric methods are available for analysis of Chlorophyll *a*. Chlorophyll, in a measured volume of water, is concentrated by filtering through a glass fiber filter, and the pigments on the filter are extracted in 90% acetone. Fluorescence is proportional to chlorophyll concentration. In one method, fluorescence of the extract is measured before and after acidification using a fluorometer. In the second method, the fluorometer is equipped with specific wavelength filters which read only emissions from chlorophyll *a*, without interferences from chlorophyll *b* and phaeopigments. Therefore, the sample is not acidified.

Methodology

Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis. Bulletin 167 (2nd ed.). Fisheries Research Board of Canada, Ottawa, Canada.

Parsons, T.R., Y. Maita and C.M. Lalli. 1984. Determination of chlorophylls and total carotenoids: Spectrophotometric method. pp. 101 - 112 *in* Parsons, T.R., Y. Maita and C.M. Lalli. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford.

Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and phaeopigments. Limnol. Oceanogr., 39: 1985-1992.

Instrumentation

Acidification Method:
Fluorometer: Turner Designs Model TD700 calibrated against a spectrophotometer using pure chlorophyll *a* from spinach (Sigma Chemical Company, C 5753), or liquid standards from Turner Designs, #10-850.
Excitation filter: Turner Designs 10-050R, 340-500 nm
Emission filter: Turner Designs 10-051R, > 665 nm
Lamp: Daylight White Lamp, Turner Designs 10-045

Welschmeyer Technique:
Fluorometer: Turner Designs Model TD700 calibrated against a spectrophotometer using pure chlorophyll *a* from spinach (Sigma Chemical Company, C 5753), or liquid standards from Turner Designs, #10-850.
Excitation Filter: 436 nm
Emission Filter: 680 nm
Lamp: blue, F4T4.5B2 equivalent

Reagents

Acetone, 90%

Acetone [$(CH_3)_2CO$]	900 mL
Deionized water	100 mL

In a 1000 mL volumetric flask, add 900 mL of acetone and add 100 mL deionized water.

Hydrochloric Acid, 1N

Hydrochloric acid (HCl), concentrated	8.6 mL
Deionized water	up to 100 mL

In a 100 mL volumetric flask, add 8.6 mL of concentrated hydrochloric acid to ~70 mL of deionized water. Dilute to 100 mL with deionized water.

Procedure

1. Filter a known volume of water through a Whatman GF/F filter pad (nominal pore size 0.7 µm). Only a faint color is needed on the pad.

2. Fold pad in half, sample inside, wrap in aluminum foil, label and freeze for analysis within 4 weeks.

3. Before analysis, briefly thaw pads, then place in a 15 ml centrifuge tube. Add 10 mL of 90% Acetone.

4. Using a Teflon pestle, grind the filter against the side of the tube until the filter is well ground. Allow the sample to extract for 2 - 24 hours in the dark under refrigeration. Overnight is recommended.

5. Remove tubes from refrigerator and allow to warm to room temperature.

6. Shake tubes, centrifuge at ~2300 rpm for 5-10 minutes and pipette liquid into a 5 mL round cuvette for reading on the fluorometer. If a refrigerated centrifuge is used, a longer spin time may be used, but is not necessary.

7. If phaeopigments are to be measured, add 2 drops of 1N Hydrochloric Acid and read on the fluorometer again. If using the Welschmeyer technique, **do not** acidify the sample.

Calculation of Chlorophyll *a* and Phaeopigment Concentration

Chlorophyll *a*, phaeopigment, and active chlorophyll *a* concentrations are calculated using the following equations:

$$\mu g \text{ Total chlorophyll } a/L = \frac{F_s \times R_b \times 10}{V_L}$$

and

$$\mu g \text{ Phaeopigments}/L = \frac{F_s \times \left(\frac{r}{r-1}\right) \times ((R_a \times r) - R_b) \times 10}{V_L}$$

$$\mu g \text{ active chla} / L = \frac{\left(R_b - R_a\right) \times F_s \times \left(\frac{r}{r-1}\right) \times 10}{V_L}$$

where: R_b = fluorometric reading before adding acid,
R_a = fluorometric reading after adding acid,
F_s = calibrating std conc./reading of std, (C/R_b),
$r = R_b/R_a$ determined on a calibrating extract, and
V = volume filtered (mL).
10 = extract volume.

Calibration of the TD700: Determine the concentration of the chlorophyll *a* stock solution on the spectrophotometer, or use liquid standards from Turner Designs. Follow instructions in the manual to calibrate for raw fluorescence or concentration.

Calculation for the Welschmeyer Technique:

$$\mu g \ chl \ a/L = \frac{(Reading) \times 10 \times dilution}{mLs \ filtered}$$

The instrument can be calibrated to read in concentration. To correct for volume filtered, the above calculation is used.

Chlorophyll *a* Calibration of the Fluorometer:

1. Empty 1 mg of dried chlorophyll *a* into a 100 mL volumetric flask and dilute to 100 mL with 90% Acetone (10 mg/L).

2. Make serial dilutions of 1.0 mg/L, 0.1 mg/L, and 0.01 mg/L from the 10 mg/L solution (10 mL of each solution diluted to 100 mL with 90% Acetone).

3. Read the 10 mg/L and 1 mg/L solutions on a spectrophotometer with a 10 cm cell path length at 750 nm, 665 nm, 664 nm, 647 nm and 630 nm. Add 2 drops of 1N Hydrochloric Acid and read again. The reading at 750 nm is a turbidity blank. If its value is greater than .005, centrifuge the sample at 2300 rpm for 5 minutes and read it again. The reading at 665 nm is used in the determination of phaeopigments.

4. Read the 10, 1, 0.1 and 0.01 mg/L solutions on the fluorometer, using many dilutions.

5. The calculations for the Spec. numbers are as follows:

$$\text{mg Chl } a \text{ /L} = 11.85(R_{664}) - 1.54(R_{647}) - 0.08(R_{630})$$

where: R_n = Reading at *n* nm

Determination of the calibration factors:

Multiply the 1 mg/L value by 10, and average this value and the 10 mg/L value. By using this average and the fluorescence readings from each standard, including dilutions, calibration factors can be calculated.

$$\text{Factor} = \frac{C}{R_b}$$

C = concentration of standard.
R_b = fluorescence reading before adding acid.

Determination of r:

____Determine the ratio of R_b/R_a for all of the readings. Average.
____Use a rolling average of the last three calibrations as the working factor and r.

APPENDIX A

SAMPLE CUSTODY

Upon arrival at the laboratory, samples are counted, observed for potential problems (melting, broken containers, etc.) and placed in a freezer until analysis. Sample information and date of arrival are recorded on a log sheet, and entered in an ACCESS file for internal tracking.

INSTRUMENT MAINTENANCE

Analytical instruments are maintained on a regular basis and records are kept of hours of operation, scheduled maintenance, pump tube changes, etc.

A critical spare parts inventory is maintained for each instrument. Instrument down-time is minimized by troubleshooting instrument problems by telephone with manufacturers and service representatives. Spare parts can be received within 24 hours via next-day air service.

TEMPERATURE LOGS

The temperatures in freezers and refrigerators used for sample storage are monitored with NIST certified and calibrated thermometers. Temperatures in drying ovens are monitored with NIST certified and calibrated thermometers.

DATA HANDLING

Data produced by computer driven instruments are transferred electronically via LIM system to the main laboratory data base. Other data are manually entered in Lotus 123 © or Microsoft Excel © spreadsheets by data management personnel. A hard copy of manually entered data is produced and undergoes a manual point-by-point verification process. Any necessary corrections are made. Subsequently, all data are further checked by a series of programs to ensure the following:

- $NO_3^- + NO_2^- + NH_4^+ <$ Total Dissolved Nitrogen,
- $PO_4^{-3} <$ Total Dissolved Phosphorus,
- DOC < TOC, and
- $NO_2^- < NO_2^- + NO_3^-$.

Any data indicating errors are given specific error codes. Final printed data files are created and sent along with an electronic copy to the client.

APPENDIX B

STATEMENT ON INSTRUMENT COMPARABILITY

The Nutrient Analytical Services Laboratory develops a data quality maintenance program for each analyte whenever new instrumentation is acquired. It is the policy of the Nutrient Analytical Services Laboratory to report any data from new instrumentation only after thorough and satisfactory side-by-side comparisons with existing instrumentation are preformed.

No predetermined number of data pairs are used to make the assessment on data comparability between new and existing methodology. Even in the case of instrumentation with similar methods of detection (i.e., automated colorimetric), although no specific number of data pairs is used, there are at least 100 data pairs. Comparability at low and high concentrations, salinity and other possible matrix interferences, sensitivity and precision are all factors in determining the number of pairs that must be addressed before bringing an instrument on-line and in determining instrument comparability.

The analyst who performs these comparisons should be experienced, open-minded and impartial. This person can give an evaluation of ease of instrument operation and a very important general statement of comparability. This statement on comparability must then be substantiated via statistical analysis of the data. As previously mentioned, these data must encompass the entire concentration range, matrix interferences, percent recovery, results of standard reference material analyses, etc. The data interpretation must support comparability. The analyst and laboratory QA/QC officer must concur and finally, some sort of presentation (written or verbal) must be given to the contractor explaining what procedure was followed and the results that were obtained to bring this instrument on-line.

STATEMENT OF QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES

A constant consideration of Nutrient Analytical Services is assuring the quality of data generated by the procedures presented in this manual. Further, indication of data quality is accomplished by analyzing duplicates, spikes, standards-as-samples, standard reference materials; and participating in cross-calibration exercises.

Laboratory Duplicates

Approximately 5% of the total number of samples analyzed consist of laboratory duplicates. For dissolved analytes, after a sample is analyzed, the same sample container is placed farther along in the automatic sampler tray and re-analyzed. The mean of the two values is reported as the concentration for that sample. If a difference of >10% is observed between replicates, then all of the replicates for that particular analytical run are carefully reviewed. If only one of the duplicate pairs is in question, then only that sample is re-analyzed. If all show a similar trend, then instrumentation/reagent problems are suspected and the analytical run is halted until such time as the problem is resolved. This procedure is practiced for all dissolved analytes that are not consumed completely in the analytical procedure. For those that are completely consumed and for particulate analytes, duplicate analyses are actually duplicate samples collected in the field and analyzed in the same analytical run.

Values for each duplicate analyzed are recorded in a separate QA/QC data file along with the sample number, sample collection date and analysis date. The mean concentration and standard deviation of the

replicates are calculated in this data file.

In the case of particulate carbon and nitrogen, total suspended solids and chlorophyll *a*, 10% of the total number of samples are analyzed as duplicates. This generates sufficient quality assurance data to compensate for the omission of laboratory spikes for these non-aqueous samples.

Laboratory duplicates serve as an indicator of instrument stability, consistency in laboratory sample preparation and analysis, as well as an estimate of field proficiency.

Laboratory Spikes

Approximately 5% of the total number of samples analyzed consist of laboratory spikes. A spike is prepared by adding a known volume of standard to a known volume of pre-analyzed sample. We routinely add enough concentrated standard to provide a significant response on our instruments that is distinguishable from the original concentration of the sample. This concentrated standard is used to minimize any possible change in sample matrix by the addition of spike.

The spiked sample is analyzed and its expected concentration calculated as the sum of the original concentration and the spike concentration, normalized for the constituent volumes. A comparison is made between the actual value and the expected value. These concentrations (original, expected and actual) are recorded in a separate QA/QC data file along with sample number, sample collection date, analysis date and the amount of spike added. In the case of particulate phosphorus, the volume filtered is not used in the calculation to determine percentage recovery.

If a value of >115% or <85% is observed for percentage recovery of the spike, then all of the spikes for that particular analytical run are carefully reviewed. If only one of the spikes is in question, then only that sample is re-analyzed. If all show poor recovery, then instrumentation/reagent problems are suspected and the analytical run is halted until such time that the problem is resolved. This procedure is adhered to for all dissolved analytes and for the extracts of particulate phosphorus and biogenic silica.

Documentation of Slopes, etc.

A running record of the slopes of the standard curves (the so-called "F," "S" and "K" factors) is maintained for each analysis. Random up and down movement within a predetermined range as a function of time indicates the analysis is under control. Consistent upward or downward trend of these factors indicates the analysis is out of control and requires immediate attention.

Limits of Detection

Limits of detection, the lowest concentration of an analyte that the analytical procedure can reliably detect, have been established for all parameters routinely measured by Nutrient Analytical Services. The limit of detection is 3 times the standard deviation of a minimum of 7 replicates of a single low concentration sample.

Table 3 presents the current minimum limits of detection. These values are reviewed and revised periodically.

Table 3. Minimum limits of detection of CBL, October/November 1987, March 1994, February/December 1998, February 1999, January 2004 [a]

NUTRIENT	MEAN CONC. (mg/L)	STANDARD DEVIATION	DETECTION LIMIT (mg/L)	(μM)
Ammonium	0.0007	0.001	0.0030	0.21
Nitrite	0.0002	0.0001	0.0003	0.02
Nitrite + Nitrate	0.0011	0.00023	0.0007	0.05
Phosphate	0.0027	0.00025	0.0007	0.02
Dissolved Organic Carbon	3.5800	0.050	0.1500	12.50
Total Suspended Solids	13.4000	0.800	2.4000	
Total Volatile Solids	3.95	0.67	1.98	
Particulate Phosphorus	0.0187	0.0008	0.0024	0.08
Particulate Inorganic Phosphorus	0.0027	0.0002	0.0006	0.02
Total Dissolved Nitrogen	0.39	0.0096	0.0300	2.06
Total Dissolved Phosphorus	0.0057	0.0005	0.0015	0.05
Silicate	0.2500	0.003	0.0100	0.32
Particulate Nitrogen	0.3170	0.0041	0.0123	0.88
Particulate Carbon	2.2600	0.0253	0.0759	6.32
Particulate Biogenic Silica	0.1630	0.003	0.0090	0.32
Sediment C (10 mg)	2.1830%	0.044	0.1300%	
Sediment N (10 mg)	0.1950%	0.003	0.0084%	
Sediment P (34.8 mg)	0.0304%	0.003	0.0087%	
Chlorophyll *a*	24.26	0.22	0.65 (μg/L)	
FRESHWATER DETECTION LIMITS				
Chloride	5.84	0.08	0.23	6.57
Sulfate	4.90	0.03	0.09	2.80

[a] Results based on a minimum of seven replicates collected from one cubitainer and analyzed randomly on a typical day of analyses.

Standard as Sample

Standards are analyzed as samples throughout the analytical run. This is an excellent means of evaluating instrument performance during the course of an analytical run. Standards as samples are analyzed every 12 - 20 samples, depending on the instrument and analyte.

Standard Reference Materials

Particulate Carbon, Nitrogen and Phosphorus: BCSS-1 is a marine sediment reference material prepared by the National Research Council of Canada. It is certified by the Council for carbon content, gives a non-certified range of results for phosphorus, but no information for nitrogen. We have analyzed this

sediment for many years and maintain a substantial database for nitrogen and phosphorus, as well as carbon values. We analyze this sediment at least quarterly and compare these results to the certified value, non-certified range of values and our historical values.

Dissolved Analytes and Hardness: Standard reference materials for ammonium, nitrite + nitrate, nitrite, orthophosphate, dissolved nitrogen, dissolved phosphorus, dissolved organic carbon, sulfate and chloride and hardness are supplied by SPEX, a US EPA certified company. The samples arrive in ampules and we prepare final concentrations to approximate typical estuarine concentrations. The samples are then placed in pre-cleaned Teflon or poly bottles, frozen and analyzed on at least a quarterly basis.

The analysis of these frozen standard reference materials as a function of time also provides data on the effect of our preservation technique (freezing) on the integrity of the concentration of samples. The US EPA recommends a holding time of 28 days for many of the parameters we routinely analyze.

Dissolved organic carbon results provide a good example. SPEX standards (2 and 5 mg C/L) were prepared in deionized water on 31 May 1995. Several of each concentration were placed in pre-cleaned Teflon bottles and frozen for subsequent analysis. Results for the Shimadzu TOC-5000 Carbon Analyzer are presented below.

SHIMADZU TOC-5000 TOTAL ORGANIC CARBON ANALYZER
(Concentrations in mg C/L)

	ANALYSIS DATE			
Standard	6/14/95 (Frozen)	7/5/95 (Frozen)	8/16/95 (Frozen)	11/6/95 (Frozen)
5.00	5.30	5.23	5.32	5.42
2.00	2.11	2.13	2.15	2.13

Organic compounds are included with each dissolved nitrogen and dissolved phosphorus digestion to determine the completeness of the digestion procedure. Glutamic acid and glycerophosphate are used as the N and P sources, respectively. Figures 14 and 15 illustrate the percentage recoveries of these internal standards for a specific project in which we participated in 1994 - 1995.

Cross Calibration Exercises

Nutrient Analytical Services has participated in many cross calibration exercises. Participation in such programs is an excellent means of determining accuracy of results. Examples of such cross calibration exercises include the Chesapeake Bay Program Quarterly Split Samples, Chesapeake Bay Program Blind Audits, USGS Standard Reference Sample Project, US EPA Method Validation Studies and International Council for the Exploration of the Sea Intercomparison Exercise for Nutrients in Sea Water.

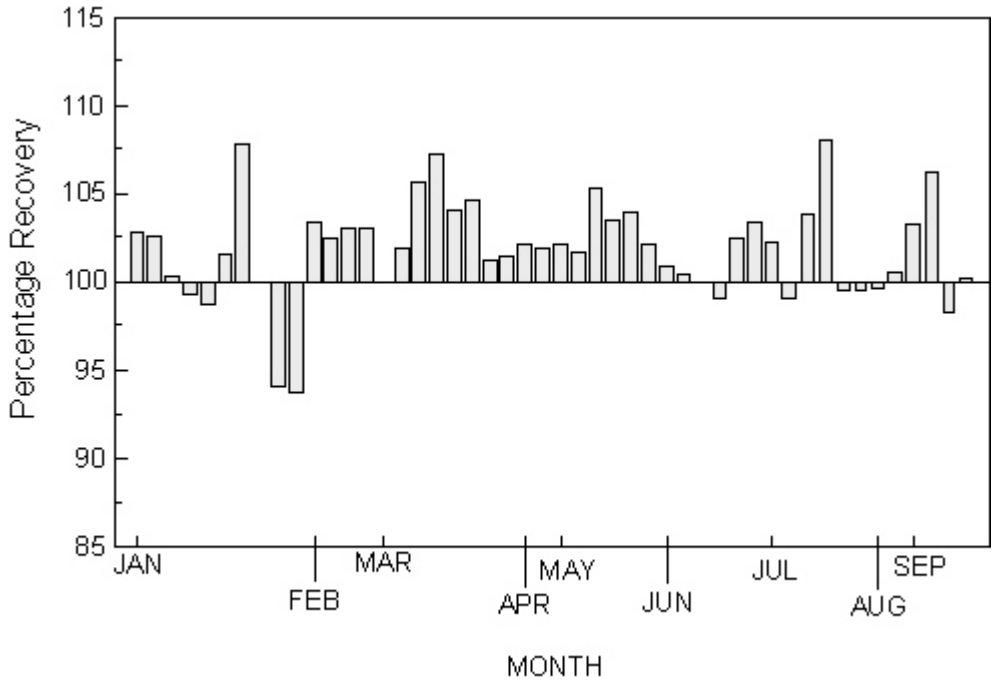


Figure 13. Percentage recovery of glutamic acid as an internal standard for total dissolved nitrogen digestion/analysis. (1995)

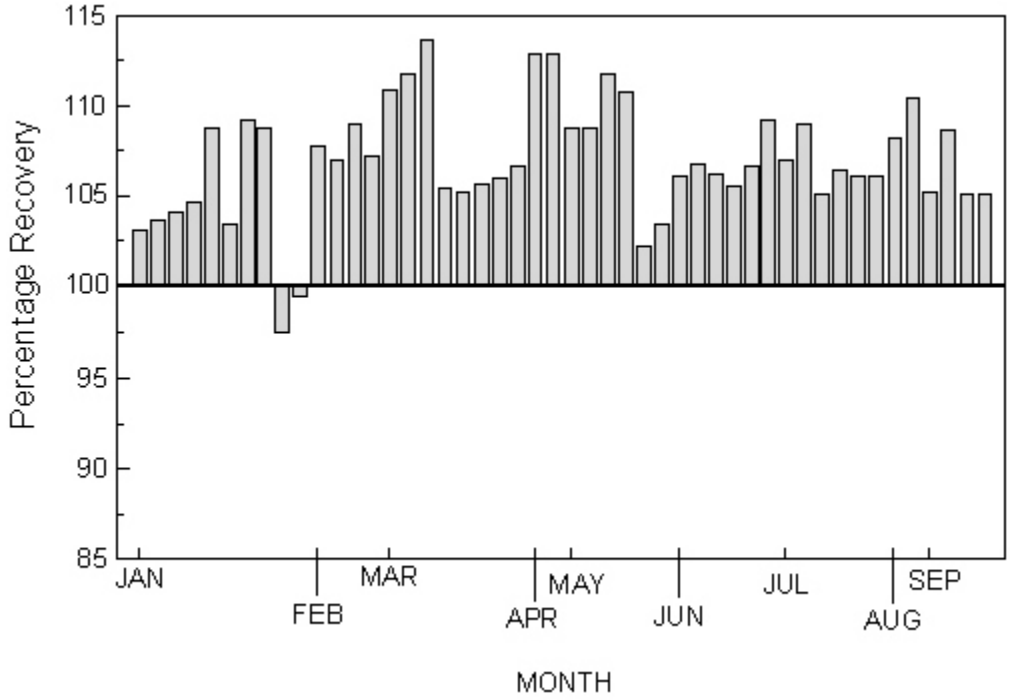


Figure 14. Percentage recovery of glycerophosphate as an internal standard for total dissolved phosphorus digestion/analysis. (1995)

ACKNOWLEDGEMENTS

We appreciate Dr. Christopher D'Elia's help in assembling the earlier versions of this manual. In 1978 he started acquiring equipment and hiring personnel to establish NASL to support research at CBL and, later, "outside" programs. This manual reflects his insistence on appropriate methods for the sample matrix to ensure data accuracy.

Erin Connor authored part of the 1997 manual, and assembled all the sections and diagrams in a consistent format. Adriene Capers helped assemble this manual and, also, the previous versions.

We thank Dr. Theodore Loder and Dr. Leonard Haas for their editorial suggestions and review of the 1997 version of this document.

Ammonium Method for Aquakem 250

Reagent Preparation:

A. Complexing Reagent:

Sodium Potassium Tartrate	3.9 g
Sodium Citrate	2.8 g
Sulfuric Acid (concentrated)	As required
Deionized water	Up to 100 mL

In a 150 mL beaker, dissolve 3.9 g sodium potassium tartrate and 2.8 g of sodium citrate in ~ 90 mL deionized water. Adjust the pH of the solution to 5.0 using concentrated sulfuric acid. Dilute to 100 mL with deionized water in a volumetric flask. Store in a light resistant container and refrigerate.

B. Alkaline Phenol Reagent:

Liquid Phenol (88%):	23.6 mL
Sodium Hydroxide (50% w/w)	18.0 g
Deionized water	Up to 250 mL

In a 250 mL volumetric flask, slowly add 23.6 mL of 88% phenol to ~150 mL of deionized water. Weigh out exactly 18g of 50% (w/w) sodium hydroxide, then while in an ice bath, slowly add the sodium hydroxide to the phenol/water solution. Dilute to 250 mL with deionized water. Store in a light resistant glass container and refrigerate. Make fresh weekly.

C. Sodium Hypochlorite, 1%

Sodium Hypochlorite (Clorox Ultra-6%)	44 mL
Deionized water	Up to 200 mL

In a 250 mL volumetric flask dilute 44 mL Clorox with 200 mL deionized water for a total volume of 244 mL. Make fresh every two days.

D. Sodium Nitroprusside (Sodium Nitroferricyanide), 0.05%

Sodium nitroprusside	0.5 g
deionized water	Up to 1000 mL

In a 1000 mL volumetric flask, dissolve 0.5 g of sodium nitroprusside in 900 mL deionized water. Dilute to 1000 mL with deionized water. Store in a light resistant container.

Procedure: The Aquakem 250 mixes 100 ul of sample with 55 ul Complexing Reagent and 33 ul Alkaline Phenol Reagent. An initial blank absorbance is measured prior to the addition of Sodium Hypochlorite and Sodium Nitroprusside. 26 ul Sodium Hypochlorite and 33 ul Sodium Nitroprusside are then added. After all reagents have been added, the sample is incubated for 7 minutes at 37 degrees C and the absorbance is measured at 630 nm. The Aquakem 250 software then automatically uses the initial blank measurement to correct the final absorbance and calculates the Ammonium concentration of the sample.

Standard Curve: The standard curve is prepared by automated series dilution of the highest standard. In the case of Ammonium the standards are:

Low Curve	**High Curve**
0.0168 mg N/L	0.168 mg N/L
0.0336 mg N/L	0.24 mg N/L
0.056 mg N/L	0.42 mg N/L
0.084 mg N/L	0.56 mg N/L
0.168 mg N/L	0.84 mg N/L
	1.68 mg N/L

Nitrite Method for Aquakem 250

Reagent Preparation:

A. Sulfanilamide: In a 500 mL volumetric flask containing 300 mL deionized water, add 2.5g sulfanilamide and 25 mL HCL and then bring up to volume. Transfer to a brown polybottle and refrigerate once dissolved.
B. N-1-Napthylethylenediamine Dihydrochloride (N-1-N): In a 500 mL volumetric flask, dissolve 0.25g N-1-N and bring to volume with deionized water. Transfer to a brown polybottle and refrigerate.
C. Stock Nitrite Standard

Sodium nitrite dried at 45 degrees C	0.345 g
Deionized water	Up to 1000 mL
Chloroform	1 mL

D. Secondary Nitrite Standard

Stock Nitrite Standard	0.8 mL
Deionized Water	Up to 100 mL

Procedure: The Aquakem 250 measures an initial blank absorbance for each sample prior to the addition of reagents. The Aquakem 250 then mixes 145 ul sample, 50 ul Sulfanilimide and 50 ul N-1-N. After reagents have been added, each sample is incubated for 7 minutes at 37 degrees C and the absorbance is measured at 540 nm. The Aquakem 250 software then automatically uses the initial blank measurement to correct the final absorbance and calculates the Nitrite concentration of the sample.

Standard Curve: The standard curve is prepared by automated series dilution of the highest standard. In the case of Nitrite the standards are:

Low Curve	**High Curve**
0.0042 mg NO2/L	0.0280 mg NO2/L
0.0084 mg NO2/L	0.0467 mg NO2/L
0.0140 mg NO2/L	0.0933 mg NO2/L
0.0210 mg NO2/L	0.1400 mg NO2/L
0.0420 mg NO2/L	0.2800 mg NO2/L

Orthophosphate Method for Aquakem 250

Reagent Preparation:

A. Triple Reagent:

1. Sulfuric Acid: Slowly add 27.2 mL conc. Sulfuric acid up to 100 mL using deionized water.
2. Ammonium Molybdate: Add 8.0 g Ammonium molybdate up to 100 mL deionized water.
3. Antimony Potassium Tartrate (KAT): Add 0.6 g antimony potassium tartrate up to 100 mL deionized water.

To 25 mL of the 9.8N Sulfuric Acid, SLOWLY add 7.5 mL NH4 Molybdate and 2.5 mL KAT. Mix thoroughly.

B. Ascorbic Acid: Add 3.6 g Ascorbic Acid up to 100 mL deionized water.

Procedure: The Aquakem 250 measures an initial blank absorbance for each sample prior to the addition of reagents. The Aquakem 250 then mixes 165 ul sample with 14 ul Triple Reagent and 7 ul Ascorbic Acid. After reagents have been added, each sample is incubated for 10 minutes at 37 degrees C and the absorbance is measured at 880 nm. The Aquakem 250 software then automatically uses the initial blank measurement to correct the final absorbance and calculates the Orthophosphate concentration of the sample.

Standard Curve: The standard curve is prepared by automated series dilution of the highest standard. In the case of Orthophosphate the standards are:

Low Curve	**High Curve** (For concentrations greater than 0.15 mg P/L)
0.0035 mg P/L	
0.0056 mg P/L	0.1488 mg P/L
0.0080 mg P/L	0.298 mg P/L
0.0112 mg P/L	0.372 mg P/L
0.0186 mg P/L	0.496 mg P/L
0.0279 mg P/L	0.744 mg P/L
0.0558 mg P/L	1.488 mg P/L

Particulate Phosphorus (PP) and Particulate Inorganic Phosphorus (PIP) Method for Aquakem 250

Reagent Preparation:

A. Triple Reagent:

1. Sulfuric Acid: In a 100 mL flask slowly add 27.2 mL conc. Sulfuric acid to 75 mL deionized water and then bring up to volume.
2. Ammonium Molybdate: In a 100 mL flask add 8.0 g Ammonium molybdate and bring up to volume with deionized water.
3. Antimony Potassium Tartrate (KAT): In a 100 mL flask add 0.6 g antimony potassium tartrate and bring up to volume with deionized water.

To 25 mL of the 9.8N Sulfuric Acid, SLOWLY add 7.5 mL NH4 Molybdate and 2.5 mL KAT. Mix thoroughly.

B. Ascorbic Acid: In a 100 mL flask add 3.6 g Ascorbic Acid and bring up to volume with deionized water.

Procedure: The Aquakem 250 measures an initial blank absorbance of 160 ul deionized water mixed with 16 ul sample for each sample prior to the addition of reagents. The Aquakem 250 then mixes 14 ul Triple Reagent and 7 ul Ascorbic Acid to each sample. After reagents have been added, each sample is incubated for 10 minutes at 37 degrees C and the absorbance is measured at 880 nm. The Aquakem 250 software then automatically uses the initial blank measurement to correct the final absorbance and calculates the PP/PIP concentration of the sample.

Standard Curve: The standard curve is prepared by automated series dilution of the highest standard. In the case of PP/PIP the standards are:

Low Curve	**High Curve**
0.0572 mg P/L	0.1488 mg P/L
0.0744 mg P/L	0.298 mg P/L
0.1063 mg P/L	0.372 mg P/L
0.1860 mg P/L	0.496 mg P/L
0.2480 mg P/L	0.744 mg P/L
0.3720 mg P/L	1.488 mg P/L
0.7440 mg P/L	

Silicate Method for Aquakem 250

Reagent Preparation:

A. Oxalic Acid: Dissolve 10.0 g Oxalic Acid up to 100 mL with deionized water.
B. Ascorbic Acid Solution: In a 100 mL volumetric flask, dissolve 0.5 g Oxalic Acid and 10.0 g Ascorbic Acid and bring to volume with deionized water.
C. Ammonium molybdate: Dissolve 3.0 g in 100 mL deionized water.
D. Stock Phosphate Solution

Potassium phosphate dried at 45 degrees C	0.4394 g
Deionized Water	Up to 1000 mL

E. Sulfuric Acid Solution (0.7 N)

Sulfuric Acid (concentrated)	4.06 mL
Stock Phosphate Solution	21.4 mL
Deionized water:	Up to 1000 mL

Procedure: The Aquakem 250 measures an initial blank absorbance for each sample prior to the addition of reagents. The Aquakem 250 then mixes 100 ul sample, 62 ul Oxalic Acid, 39 ul Ammonium molybdate, 31 ul Sulfuric Acid and 16 ul Ascorbic Acid. After reagents have been added, each sample is incubated for 10 minutes at 37 degrees C and the absorbance is measured at 660 nm. The Aquakem 250 software then automatically uses the initial blank measurement to correct the final absorbance and calculates the Silicate concentration of the sample.

Standard Curve: The standard curve is prepared by automated series dilution of the highest standard. In the case of Silicate the standards are:

Low Curve	**High Curve**
0.21 mg Si/L	1.05 mg Si/L
0.42 mg Si/L	2.10 mg Si/L
0.70 mg Si/L	3.50 mg Si/L
1.05 mg Si/L	5.25 mg Si/L
2.10mg Si/L	10.5 mg Si/L

DRAFT

Determination of Dissolved Organic Carbon (NPOC), Total Organic Carbon, and Dissolved Inorganic Carbon in waters of Fresh/Estuarine/Coastal Waters using High Temperature Combustion and Infrared Detection.

1. SCOPE and APPLICATION

1.1 High temperature combustion (680°C) is used to determine dissolved organic carbon (DOC), also known as non-purge able organic carbon (NPOC), total organic carbon (TOC), and total (TIC) or dissolved inorganic carbon (DIC), using a non-dispersive infrared detector (NDIR). The method is used to analyze all ranges of salinity.

1.2 A Method Detection Limit (MDL) of 0.18 mg/L DOC, and 0.03 mg/L DIC was determined using 3X the standard deviation of 7 replicates.

1.3 The quantitation limit for DOC and DIC was set at 0.05 mg/L C.

1.4 This procedure should be used by analysts experienced in the theory and application of organic carbon analysis. Three months experience with an experienced analyst, certified in the analysis using the organic carbon analyzer, is required.

1.5 This method can be used for all programs that require analysis of dissolved and total organic and inorganic carbon.

1.6 This procedure conforms to EPA Method 415.1.

2. SUMMARY

2.1 The Shimadzu TOC-5000/5000A uses a high temperature combustion method to analyze aqueous samples for TIC, TOC and non-purge-able organic carbon (NPOC).

2.2 NPOC samples are treated with hydrochloric acid and sparged with ultra pure carrier grade air to drive off inorganic carbon. TOC samples are injected directly onto the catalyst bed with no pretreatment. High temperature combustion (680°C) on a catalyst bed of platinum-coated alumina balls breaks down all carbon compounds into carbon dioxide (CO_2). The CO_2 is carried by ultra pure air to a non-dispersive infrared detector (NDIR) where CO_2 is detected.

2.3 Samples for inorganic carbon (TIC) are injected directly into a receptacle of 25% phosphoric acid where the carbonates are reduced to CO_2 and detected by the NDIR.

3. DEFINITIONS

3.1 Acceptance Criteria – Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)

3.2 Accuracy – The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)

3.3 Aliquot – A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)

3.4 Analytical Range - 100 ppb - 4000 ppm using 250 µl syringe and 4 - 100 µl injection volume, using regular sensitivity catalyst.

3.5 Batch – Environmental samples, which are prepared and /or analyzed together with the same process and personnel, using the same lot(s) of reagents. A **preparation batch** is composed of one to 20 environmental samples of the same matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An **analytical batch** is composed of prepared environmental samples (extracts, digestates, or concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)

3.6 Blank- A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)

3.7 Calibrate- To determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.8 Calibration – The set if operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.9 Calibration Curve – The graphical relationship between known values, such as concentrations, or a series of calibration standards and their analytical response. (NELAC)

3.10 Calibration Method – A defined technical procedure for performing a calibration. (NELAC)

3.11 Calibration Standard – A substance or reference material used to calibrate an instrument. (QAMS)

- 3.11.1 Initial Calibration Standard (STD) – A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.
- 3.11.2 Initial Calibration Verification (ICV) – An individual standard, analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve.

3.11.3 Continuing Calibration Verification (CCV) – An individual standard which is analyzed after every 10-15 field sample analysis.

3.12 Certified Reference Material – A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (ISO 17025)

3.13 Combustion tube – Quartz tube filled with platinum catalyst, heated to 680° C, into which the sample aliquot is injected.

3.14 Conditioning Blank – DI water run before the calibration curve to decrease the instrument blank and stabilize the column conditions.

3.15 Corrective Action – Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)

3.16 Deficiency – An unauthorized deviation from acceptable procedures or practices. (ASQC)

3.17 Demonstration of Capability – A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)

3.18 Detection Limit – The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.

3.19 Duplicate Analysis – The analyses of measurements of the variable of interest performed identically on two sub samples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage internal to the laboratory. (EPA-QAD)

3.20 External Standard (ES) – A pure analyte (potassium hydrogen phthalate (KHP)) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.

3.21 Field Duplicates (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout filed and laboratory procedures. Analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.22 Field Reagent Blank (FRB) – A aliquot of reagent water or other blank matrix that is places in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.23 Furnace – Heats the combustion tube to the operating temperature of 680° C.

3.24 Holding time – The maximum time that samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.

3.25 Injection – The sample aliquot that is drawn into the syringe and injected into the combustion tube.

3.26 Instrument Detection Limit (IDL) – The minimum quantity of analyte of the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time absorbance line, etc.

3.27 Laboratory Duplicates (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.28 Laboratory Reagent Blank (LRB) – A matrix blank (i.e., DI water) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, 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 instrument.

3.29 Laboratory Control Sample (LCS) – A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standard or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)

3.30 Limit of Detection (LOD) – The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank. (ACS)

3.31 Limit of Quantitation (LOQ) – The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD, depending on the degree of confidence desired.

3.32 Linear Dynamic Range (LDR) – The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).

3.33 Material Safety Data Sheets (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.

3.34 May – Denotes permitted action, but not required action. (NELAC)

3.35 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported with 98% confidence that the analyte concentration is greater than zero.
3.36 Must – Denotes a requirement that must be met. (Random House College Dictionary)
3.37 Non-Dispersive Infrared Detector (NDIR) – The detector found in the Shimadzu 5000/5000A TOC analyzer. Carbon dioxide is detected.
3.38 Precision – The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)
3.39 Preservation – Refrigeration, freezing, and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.
3.40 Quality Control Sample (QCS) – A sample of analytes of known and certified concentrations. 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.
3.41 Run – One sample analysis from start to finish, including printout.
3.42 Run Cycle – Typically a day of operation – the entire analytical sequence of runs from the first run to the last run and including the transfer of run cycle data to the disc.
3.43 Sample Volume – Amount of sample injected into the combustion tube.
3.44 Sensitivity – The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.
3.45 Shall – Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)
3.46 Should – Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)
3.47 Sparge Time – The time required to aerate an acidified sample with ultra pure air to remove inorganic carbon to determine the concentration of organic carbon.
3.48 Standard Reference Material (SRM) – Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique.

4. INTERFERENCES

4.1 Carbonates and bicarbonates may interfere with the determination of organic carbon by increasing the concentration of CO_2 detected. These are removed by adding enough 2N HCl to the sample to bring the pH to 2 or below, then sparging with ultra-pure air for a predetermined time.

5. SAFETY

5.1 Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats, safety glasses and enclosed shoes should be worn. In certain situations, it will be necessary to also use gloves and/or a face shield. If solutions come in contact with eyes, flush with water continuously for 15 minutes. If solutions come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed and also inform the CBL Business Manager of the incident. Contact the CBL Business Manager if additional treatment is required.

5.2 The toxicity or carcinogenicity of each reagent used in this procedure may not have 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 hazardous materials and procedures.

5.3 Do not wear jewelry when troubleshooting electrical components. Even low voltage points are dangerous and can injure if allowed to short circuit.

5.4 The following hazard classifications are listed for the chemicals used in this procedure. Detailed information is provided on Material Safety Data Sheets (MSDS).

Chemical	Health	Flammability	Reactivity	Contact	Storage
Potassium Hydrogen Phthalate	0	1	0	1	Green
Sodium Carbonate, Anhydrous	1	0	1	2	Green
Sodium Bicarbonate	1	1	1	1	Green
Phosphoric Acid	3	0	2	4	White
Hydrochloric Acid	3	0	2	4	White
Sodium Hydroxide	3	0	2	4	White Stripe
Platinum Catalyst on Alumina Beads	1	0	1	1	Green
Soda Lime	1	0	1	3	White

On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability, reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)
STORAGE
Red – Flammability Hazard: Store in a flammable liquid storage area.

Blue – Health Hazard: Store in a secure poison area.
Yellow – Reactivity Hazard: Keep separate from flammable and combustible materials.
White – Contact Hazard: Store in a corrosion-proof area.
Green – Use general chemical storage (On older labels, this category was orange).
Striped – Incompatible materials of the same color class have striped labels. These products should not be stored adjacent to substances with the same color label. Proper storage must be individually determined.

6. EQUIPMENT AND SUPPLIES

6.1 A Total Organic Carbon Analyzer capable of maintaining a combustion temperature of 680° C and analyzing for organic and inorganic carbon. The Shimadzu TOC5000 and the Shimadzu TOC5000A are used in this laboratory.
6.2 Freezer, capable of maintaining -20 ± 5° C.
6.3 Lab ware – All reusable lab ware (glass, Teflon, plastic, etc) should be sufficiently clean for the task objectives. This laboratory soaks all lab ware related to this method in a 10% HCl (v/v) acid bath overnight.

7. REAGENTS AND STANDARDS

7.1 Purity of Water – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to ASTM Specification D 1193, Type I. Freshly prepared water should be used for making the standards intended for calibration. The detection limits of this method will be limited by the purity of the water and reagents used to make the standards.
7.2 Purity of Reagents – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without compromising the accuracy of the determination.
7.3 Potassium Hydrogen Phthalate (KHP) C_6H_4 (COOK) (COOH) – primary standard for organic carbon.
7.4 Sodium Hydrogen Carbonate ($NaHCO_3$) and Sodium Carbonate (Na_2CO_3) – primary standard for inorganic carbon.

7.5 Hydrochloric Acid, 2 N –
Hydrochloric acid (HCl), concentrated, 172 ml
Deionized water, q.s. 1000 ml

In a 1000 ml volumetric flask, add 172 ml of concentrated hydrochloric acid to ~600 ml of deionized water. Dilute to 1000 ml with deionized water.

7.6 Organic Carbon Stock Standard: Potassium Hydrogen Phthalate (KHP) Standard, 1000 mg/l

Potassium hydrogen phthalate ($HOCOC_6H_4COOK$), Dried at 45° C	2.125 g
Deionized water	1000 ml

In a 1000 ml volumetric flask, dissolve 2.125 g of potassium hydrogen phthalate in ~800 ml of deionized water. Dilute to 1000 ml with deionized water. Make fresh every 4 - 6 months. Store at 4° C.

7.7 Inorganic Carbon Stock Standard: Sodium Hydrogen Carbonate/ Sodium Carbonate ($NaHCO_3/Na_2CO_3$) Standard, 1000 mg/l

Sodium Hydrogen Carbonate ($NaHCO_3$)	1.75 g
Sodium Carbonate, Anhydrous (Na_2CO_3)	2.205 g
Deionized H_2O	500 ml

In a 500 ml volumetric flask, dissolve 1.75 g NaHCO3 and 2.205 g Na2CO3 in ~300 ml deionized H2O. Dilute to 500 ml with deionized H2O. Make fresh every 4 months. Store at 4° C.

7.8 Blanks – Two blanks are used in calculations for this analysis.

7.8.1 ASTM D1193, Type I water is used for the Laboratory Reagent Blank. The LRB is comprised of the instrument blank plus the organic content found within the TYPE I water. The area of the LRB is subtracted from the area of the standards.

7.8.2 For ambient water samples, the LRB cannot be used, because the samples have no added Type I water. Because the instrument has an internal blank that is not constant, the instrument blank is determined to be the **absolute value** of the y intercept. This value is subtracted from the area of the samples.

7.8.3 If the ambient water sample is diluted with TYPE I water, then the LRB must be used in the calculation.

7.8.4 This does not apply to TIC samples. The instrument blank for inorganic carbon is insignificant, so no blank is subtracted from the samples.

7.9 Quality Control Sample (QCS) – For this procedure, the QCS can be any certified dissolved sample which is obtained from an external source. If a certified sample is not available, then use the standard material (KHP).

8 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Water collected for DOC and/or DIC should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 μm), or equivalent.
8.2 Water collected for DOC should be frozen at -20° C, or acidified with 2N HCl to a pH of ≤2. Water collected for DIC should not be acidified. The sample container should be either borosilicate glass or Teflon. Plastic containers may be used if well cleaned and aged. Freshwater samples should be frozen in Teflon or plastic to prevent breakage.
8.3 Frozen DOC and/or DIC samples may be stored longer than 28 days. It has been shown that frozen QCS samples up to a year old still fall well within the control limits.
8.4 DOC samples acidified with 2N HCL should be frozen, as above, or refrigerated at 4° C for no longer than 28 days.
8.5 DIC samples stored at 4° C should be analyzed within 28 days.

9 QUALITY CONTROL

9.1 The laboratory 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 continued analysis of laboratory instrument blanks and calibration standard material, analyzed as samples, as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.
9.2 Initial Demonstration of Capability

9.2.1 The initial demonstration of capability (DOC) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.
9.2.2 Quality Control Sample (QCS/SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 10% of the certified 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 analyses.
9.2.3 Method Detection Limits (MDLs) – MDLs should be established for DOC and DIC using a low level ambient water sample. To determine the MDL values, analyzed seven replicate aliquots of water. Perform all calculations defined in the procedure (Section 10) and report the concentration values in the appropriate units. Calculate the MDL as follows:

MDL = S x 3

Where, S = Standard Deviation of the replicate analyses.

9.2.4 MDLs should be determined yearly.

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. The LRB consists of Nanopure water treated the same as the samples. LRB data are used to assess contamination from the laboratory environment.

9.3.2 Quality Control Sample (QCS)/ Standard Reference Material (SRM) – when using this procedure, a quality control sample is required to be analyzed at the beginning of the run and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within $\pm 3\sigma$ of the certified 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 the analyses. The results of these samples shall be used to determine batch acceptance.

9.3.3 The QCS will be obtained from a source external to the laboratory and different from the source of calibration standards.

9.3.4 Control Charts – The SRM data is graphed, and the slope, y-intercept, and r squared data are compiled and tracked.

9.3.5 Continuing Calibration Verification (CCV) – Following every 12-15 samples, one or two CCVs are analyzed to assess instrument performance. The CCVs are made from the same material as calibration standards (KHP), and are to be within TV $\pm 3\sigma$. Failure to meet the criteria constitutes correcting the problem and reanalyzing the samples. If not enough sample exists, the data must be qualified if reported.

9.4 Assessing Analyte Recovery

9.4.1 Matrix spikes are performed on a 20% QA/QC basis.

9.4.2 0.5 ml of the highest KHP standard in the curve is added to 5.0 ml of sample for a total volume of 5.5 ml.

9.4.3 0.5 ml standard 0.5/5.5 = 0.09

9.4.4 0.09 X STD conc.

9.4.5 5.0 ml sample 5.0/5.5 = 0.91

9.4.6 (original sample conc. X 0.91) + (0.09 x std conc.) = (expected conc.) mg/L

9.5 Data Assessment and Acceptance Criteria for Quality Control Measures

9.5.1 The Acceptance Criteria for DOC is 0.9990. If the r^2 is less than acceptable, all blanks and standards analyzed during the run may be averaged into the curve.

9.6 Corrective Actions for Out of Control Data

9.6.1 If the acceptance criteria are still not met, the samples are to be rerun.

10 CALIBRATION AND STANDARDIZATION

10.1 Calibration – Daily calibration must be performed before sample analysis may begin. Four point calibration is used with the Shimadzu TOC 5000/5000A.

10.1.1 Type I water is used as the "zero point" in the calibration. Because even Type I water contains some organic carbon, the area count response is subtracted from the standards as the water blank, forcing the curve through zero. The standards are calculated by the following equation:

$$\text{mg TOC/L} = (A_{STD} - A_{H2OBLK})/ m$$

Where: A_{STD} = Area of the standard
A_{H2OBLK} = Area of water blank
m = slope of the regression line

TOC sample concentration is calculated using the following equation:

$$\text{mg TOC/L} = (A_S - | b |)/ m$$

Where: A_S = area of the sample,
b = y-intercept, and
m = slope of the regression line

11 PROCEDURE

11.1 How to run the Shimadzu 5000

11.1.1 Turn on the gas.

11.1.2 Turn on instrument. The power switch is located on the right side of the instrument.

11.1.3 Instrument checks when turning on: Check level of liquid in the humidifier, located in the lower right corner inside. The level should be between the two lines. If low—add DHOH. Unscrew cap on the side and squirt in up to upper line.

11.1.4 Turn on ASI. Press F5 to initialize the ASI. Make sure the turntable is in place to avoid error messages. If the ASI is turned on after opening the MAIN MENU, the instrument will automatically initialize the ASI. Samples may be loaded onto the carousel while the TOC-5000 is warming up. Just make sure the turntable is in place before turning on the ASI.

11.1.5 Use the F keys to navigate. Go to NEXT (F1) which opens the MAIN MENU. Press 3 and Enter for General Conditions.

11.1.6 Using arrow keys, scroll down to TOC furnace. Press 1 and Enter to turn on furnace. Return to MAIN MENU (F2). Make sure liquid in the TIC chamber —plastic reservoir inside in upper center— is bubbling. If not, there is a leak.

11.1.7 Press 6 and Enter to get to MONITOR SCREEN. This screen allows you to monitor instrument conditions. It takes 20-30 minutes to come to temperature.

11.1.8 Loading Samples: Decide on standard curve.

11.1.8.1 NPOC conditioning curve: Place a std vial of DHOH is position S1. (This vial may also be used as the zero std of the calibration curve.) Load at least 3 sample vials of DHOH in positions 1-3. 100 µl injection, range 1, 9 max 10 injections, 3 minute sparge.

11.1.8.2 NPOC: I use 2 curves: Low — 0-10 ppm ---- DHOH, 2.0, 5.0, 10.0 as calibration standards, and adding 0.5, 1.0, and 7.5 as well for stds as samples. 60 µL injection, range 1, 6 min sparge.
High----- 0-20 ppm ----- DHOH, 5.0, 10.0, 20.0 as calibrating stds and adding 1.0, 2.0, & 15.0 as stds as samples. 30 µL injection, range 1, 6 min sparge. The instrument allows up to a 4 point curve.

11.1.8.3 TIC: This analysis is not performed on a regular basis; therefore the samples are analyzed using a curve of 0-30 ppm Na_2CO_3/$NaHCO_3$ and an injection volume of 25 µl in range 1. If the samples fall within the low end of the curve, the curve range and injection volume are

adjusted accordingly. If the samples fall off scale, they are diluted.

11.1.9 Use the large sample vials for the curve stds. Examples of sample protocol can be found in the data sheet notebook.

11.1.10 I always load several DHOH samples (at least 3) as conditioning samples.

11.1.11 Fill std vials ~ 1/3 to 1/2 full.

11.1.12 Fill sample vials to within 3/4 - 1" of the top. Acidify the NPOC samples by adding 100 µl of 2N HCl. The instrument can be set up to automatically add acid to the standard cups. Make sure a standard cup filled with 2N HCl is in position S8. DO NOT acidify TIC samples.

11.1.13 Data sheets are found in the LOTUS TOC5000 folder, labeled TOC datasheet, high, low std. Print as many as needed. (Low std set to print 5; high std prints 2, and plain datasheet prints 1.) Examples are found before section 12.

11.1.14 When the turntable is loaded, place in the ASI. Line up pins to align properly. Put top on lining up pins carefully. Sample needles will Z if the top is not properly aligned.

11.1.15 Go to the MAIN MENU — Press 9 and Enter for the AUTOSAMPLER.

11.1.16 Determine analysis type: Press 1 for TC, 2 for IC, 3 for TOC, 4 for NPOC on each to be used, one line per curve. The instrument can store up to 18 curves.

11.1.17 Create 2 std curves for NPOC. Using the number keys, toggle the sample type to NPOC. Enter sample positions under IS and FS, and curve # under C1. Curve #1 is used as the conditioning curve. At least vials 1 -3 of DHOH and the std DHOH (S1). (9 max 10 injections, 100 µl, range = 1, 3 minute sparge, and curve not through zero.) Curves 2-18 are used as sample curves. See 11.1.19.

11.1.18 If analyzing TIC, create 1 std curve for TIC. Using the number keys, toggle the sample type to TIC.

11.1.19 Enter curve # under C1 and ENTER. This opens the screen to input curve data. Use arrow keys to navigate. Enter std conc. & position (S1, S2, etc.)

11.1.19.1 NPOC: Range = 1, inj vol 60 µL for low curve and 30 µL for high curve. 3 max 5 injections, 200 SD, 2.0% CV, 6 minute sparge, acid addition on, and curve forced through zero.

11.1.19.2 TIC: Range = 1, inj. vol 25 µl for 0-30 ppm curve. 3 max 5 injections, 200 SD, 2.0% CV.

11.1.20 Return to SAMPLE CONDITIONS. Make sure sample conditions match curve conditions. Ex: # of inj, sparge time, etc.

11.1.21 When all is set, press F1 (NEXT)

11.1.22 Decide whether to leave instrument in 1 (Finish), 2 (Running), or 3 (No Change)
11.1.23 Press F1 (NEXT)
11.1.24 Press Start
11.1.25 Check paper supply
11.1.26 Make sure the Rinse Reservoir is full.
11.1.27 Make sure the waste bottle has room.

11.2 Shutdown procedure for the TOC5000.

11.2.1 Make sure the Autosampler needles are in the home position.
11.2.2 Open MAIN MENU (F2).
11.2.3 Enter 7 for STANBY OPTIONS
11.2.4 Press STANBY (F1) to shutdown. This turns the furnace off and closes the main pressure valve.
11.2.5 Wait 30 minutes before turning off power.

11.3 How to run the Shimadzu 5000A

11.3.1 Turn on gas.
11.3.2 Turn on instrument.
11.3.3 Turn on laptop and open TOCControl. There is no password, so just hit ENTER. Turn on TOC5000A. The power switch is located on the left hand side behind the Autosampler.
11.3.4 Open communication to instrument by clicking on the connect button on top button bar. (Looks like two plugs connecting). The computer will go through a series of checks before connecting.
11.3.5 Instrument checks when turning on: Check level of liquid in the humidifier, located in the lower right corner inside. The level should be between the two lines. If low—add DHOH. Unscrew cap on the side and squirt in up to upper line. Make sure liquid in the TIC chamber —plastic reservoir in upper center— is bubbling. If not, there is a leak.
11.3.6 The furnace automatically turns on when connected. It takes 20-30 minutes to come to temperature.
11.3.7 To view instrument conditions, click on View /Background Monitor. When all conditions are OK, the system is ready to run.
11.3.8 Loading Samples: Decide on standard curve. I use 2 curves: Low — 0-10 ppm ---- DHOH, 2.0, 5.0, 10.0 as calibration standards, and adding 0.5, 1.0, and 7.5 as well for stds as samples. 60 µL injection. High----- 0-20 ppm ----- DHOH, 5.0, 10.0, 20.0 as calibrating STDs and

adding 1.0, 2.0, & 15.0 as STDs as samples. 30 µL injection. The instrument allows up to a 4 point curve.

11.3.9 Use the large sample vials for the curve stds. Examples of sample protocol can be found in the data sheet notebook.

11.3.10 I always load several DHOH samples (at least 3) as conditioning samples

11.3.11 Fill std vials ~ 1/3 to 1/2 full.

11.3.12 Fill sample vials to within 3/4 - 1" of the top.

11.3.13 Data sheets are found in the LOTUS TOC5000 folder on the desktop, labeled TOC datasheet, high, low std. Print as many as needed. (Low STD set to print 5; high STD prints 2, and plain datasheet prints 1.)

11.3.14 Acidify the samples by adding 100 µl of 2N HCl. The standard curve files are set up to automatically add acid to the standard cups. Make sure a standard cup filled with 2 N HCl is in position S8.

11.3.15 When the turntable is loaded, place in the ASI. Line up pins to align properly. Put top on lining up arrows carefully. Sample needles will Z if the top is not properly aligned.

11.3.16 Close the Background Monitor and a blank sample table will be opened.

11.3.17 Enter sample table by clicking on Edit/Insert Std. Click on CONDBLK.CAL. Then click on Edit/Autogenerate. In the method name slot, type CONDBLK.MET. Using the TAB, toggle through to fill in the blanks. This is an NPOC file. I usually put conditioning DHOH vials in positions 1-3. The standard curve is next. Several curves are already stored. Click on Edit/ insert STD again to choose the proper curve. LOSTD10 is a 0-10 ppm curve with DHOH, 2.0, 5.0, & 10 in positions S1, 2, 3, 4. HISTD20 is a 0-20 ppm curve with DHOH, 5.0, 10.0, 20.0 in positions S1, 2, 3, 4.

11.3.18 Click on Edit/ Insert Sample next. Most likely this is a new file, so click on NEW. You will then be prompted enter the conditions of the samples to be run. Enter a file name (up to 8 characters), and TAB to the next slot. Enter the sample information (ex. DNR Potomac 3/30/08). TAB again to enter sample name (ex. Potomac), TAB to next and hit backspace to clear. This leaves the SAMPLE ID column blank so ID's can be entered when the table is completed. TAB again, and using the down arrow, choose the NPOC option. Click on NPOC tab at top to enter standard information. Click on Browse to choose standard curve. (LOSTD10 or HISTD20). Injection volumes and other stored info will be displayed. Click OK on each of the screens that pop up. You will be prompted to save the method file. (Up to 8 characters. Ex: POT0330). At this point, one line is added to the sample table. The sample vial

position is blank. Type in the vial position (usually 4) from the bench sheet and hit enter. Move the cursor to the next line down, first column and click. To enter the rest of the sample table, click on Edit/ Autogenerate. Enter the file name.met and TAB to enter vial positions (starting with 5.) If you have more than one group of samples, you may use autogenerate several times to enter each group. EX: Potomac uses vial positions 5-46, Chincoteague 47-60, and MDE Loch Raven 61-78. Each time you enter autogenerate, be sure to change the name of the group. The method file remains the same.

11.3.19 When the sample table is entered, go back and enter the sample ID's. Save the table using the same name as the method file.

11.3.20 Click Start.

11.3.21 Choose whether to keep instrument on at end of run or to finish. Click OK. Click OK again to acknowledge that the acid container is in position S8.

11.3.22 Do not run instrument with computer hooked up to the network. It increases the chances of the system locking up.

11.3.23 Make sure the Rinse Reservoir is full.

11.3.24 Make sure the waste bottle has room.

11.3.25 When the run is finished, reconnect to the network and save the file to P:drive/Kaumeyer, so it can be accessed by the desktop.

11.4 Shutdown Procedure for the TOC5000A

11.4.1 Make sure the Autosampler needles are in the home position.

11.4.2 Make sure the software is in Standby. (Should say Standby in lower left corner of screen.)

11.4.3 Click on connection button (next to ? button, 2nd from right)

11.4.4 Click OK to disconnect instrument.

11.4.5 Close program - shutdown computer.

11.4.6 Turn off instrument (on left behind ASI).

11.4.7 <u>If instrument is not in standby:</u>

11.4.8 Click on Halt to stop program

11.4.9 .The ASI needles should rise and go to home position. (They don't always)

11.4.10 Click on Measure, then Standby.

11.4.11 Click on OK in screen to shutdown. Wait at least 30 minutes for the furnace to cool.

11.4.12 Click Close to remove that screen.

11.4.13 After 30 minutes, follow shutdown procedure above.

SHIMADZU DATA SHEET CRUISE :

TODAY'S DATE:

INSTRUMENT USED:

MANUAL/PC CONTROL ANALYST: NLK OTHER:

SPIKE CONC.: INJECTION VOLUME: FILE NAME:

VIAL/STD	AREA		VIAL/STD	AREA		VIAL/STD	AREA
cond std	S1		S4			S7	
	S2		S5			S8	ACID
	S3		S6				

VIAL/ID	AREA	VIAL	ID	AREA	VIAL	ID	AREA
1 COND		27			53		
2 COND		28			54		
3 COND		29			55		
4 DHOH		30			56		
5 DHOH		31			57		
6		32			58		
7		33			59		
8		34			60		
9		35			61		
10		36			62		
11		37			63		
12		38			64		
13		39			65		
14		40			66		
15		41			67		
16		42			68		
17		43	DHOH		69		
18		44			70		
19		45			71		
20		46			72		
21		47			73		
22		48			74		
23		49			75		
24		50			76		
25		51			77		
26		52			78		

SHIMADZU DATA SHEET CRUISE :
TODAY'S DATE:
INSTRUMENT USED:
MANUAL/PC CONTROL **ANALYST: NLK OTHER:**
INJECTION VOLUME: 60 FILE
SPIKE CONC.: 10 mg/L KHP uL NAME:

VIAL/STD	AREA	VIAL/STD	AREA		VIAL/STD	AREA
	S1					
cond std	**DHOH**	**S4 10.0 KHP**			**S7**	
	S2 2.0 KHP	**S5**			**S8**	**ACID**
	S3 5.0 KHP	**S6**				

VIAL/ID	AREA	VIAL	ID	AREA	VIAL	ID	AREA
1 COND		27			53		
2 COND		28			54		
3 COND		29			55		
4 DHOH		30			56		
5 DHOH		31			57		
6 0.5 KHP		32			58		
7 1.0 KHP		33			59		
8 2.0 KHP		34			60		
9 5.0 KHP		35			61		
10 7.5 KHP		36			62		
11 10.0 KHP		37			63		
12 DHOH		38			64		
13 DHOH		39			65		
14		40			66		
15		41			67		
16		42			68		
17		43	DHOH		69		
18		44			70		
19		45			71		
20		46			72		
21		47			73		
22		48			74		
23		49			75		
24		50			76		
25		51			77		
26		52			78		

SHIMADZU DATA
SHEET CRUISE :
TODAY'S DATE:
INSTRUMENT USED:
MANUAL/PC CONTROL ANALYST: NLK OTHER:

SPIKE CONC.: 20 mg/L KHP **INJECTION VOLUME: 30 uL** **FILE NAME:**

VIAL/STD	AREA	VIAL/STD	AREA		VIAL/STD	AREA
cond std	**S1 DHOH**	**S4 20.0 KHP**			**S7**	
	S2 5.0 KHP	**S5**			**S8**	**ACID**
	S3 10.0 KHP	**S6**				

VIAL/ID	AREA	VIAL	ID	AREA	VIAL	ID	AREA
1 COND		27			53		
2 COND		28			54		
3 COND		29			55		
4 DHOH		30			56		
5 DHOH		31			57		
6 1.0 KHP		32			58		
7 2.0 KHP		33			59		
8 5.0 KHP		34			60		
9 10.0 KHP		35			61		
10 15.0 KHP		36			62		
11 20.0 KHP		37			63		
12 DHOH		38			64		
13 DHOH		39			65		
14		40			66		
15		41			67		
16		42			68		
17		43	DHOH		69		
18		44			70		
19		45			71		
20		46			72		
21		47			73		
22		48			74		
23		49			75		
24		50			76		
25		51			77		
26		52			78		

12. Maintenance Schedule for the TOC5000/5000A

12.1 **Daily**:

12.1.1 Check liquid level in humidifier. Add DHOH to top line if level is too low. Keep level between top and bottom scored lines on vessel.

12.1.2 Check paper supply if using either instrument in Stand Alone Mode.

12.1.3 Make sure that the liquid in the IC pot is bubbling once the furnace is on. Lack of bubbling means a leak is present.

12.1.4 Main gas pressure setting @ 4.5 kg/cm^2.

12.1.5 Carrier gas setting @ 150 cc.

12.1.6 Sparge gas setting @ ~30-60 cc when in use.

12.1.7 Check the level of the rinse container.

12.1.8 Carrier gas pressure. Use Ultra Zero grade Air from Airgas or comparable grade. UZ Air is a synthetic blend containing 20-22% oxygen, < 1 ppm CO + CO_2 combined, < 2% H_2O, < 0.1% THC.

12.2 **Monthly**, approximately or after 15-18 analytical batches:

12.2.1 Consumables parts list:

12.2.1.1 PN 017-42801-01 TC catalyst, regular sensitivity

12.2.1.2 PN 036-11209-84 Black o-rings, injection port; 5/pk

12.2.1.3 PN 036-11408-84 Teflon o-rings (white), 5/pk

12.2.1.4 PN 630-01565-00 injection port needle

12.2.1.5 PN 638-41323-00 TC combustion tube

12.2.1.6 PN 220-91101-00 syringe plunger w/tip

12.2.1.7 PN 630-00105-01 platinum screens, 2/pk

12.2.1.8 PN 630-02674-01 mist trap filter ball

12.2.1.9 PN 035-62994-03 Teflon ferrules, TOC5000 only

12.2.1.10 PN 630-00707-00 cooling coil, TOC5000 only

12.2.1.11 PN 200-91532-02 printer paper

12.2.1.12 PN 638-41314-00 cooling coil, TOC5000A only

12.2.1.13 PN 638-41284-00 ASI sampling needles

12.2.1.14 PN 630-01566-00 Teflon coated o-ring

12.2.1.15 PN 630-00635-01 KHP, primary std

12.2.1.16 PN 630-00962-01 Na_2CO_3, primary std

12.2.1.17 PN 630-00963-01 NaHCO3, primary std

12.2.1.18 JT Baker PN 1820-01 Cupric Oxide Wire, Baker Analyzed, A.C.S. Reagent grade or equivalent.

12.2.2 Make sure oven is off and cooled to room temperature.

12.2.3 Remove the old column by unscrewing the two side screws on the mounting plate.

12.2.4 Remove the injection port slide, and the injection block.

12.2.5 Release the TC gas line from the side of the block.

12.2.6 Remove the syringe from under the 4 port valve.

12.2.7 Rinse the syringe and replace the old plunger and tip with a new plunger and tip.

12.2.8 Remove the old mist trap filter ball and replace with a new filter, taking care not to touch with bare fingers.

12.2.9 Remove the ultra pure water trap, rinse well, and return. There is no need to fill with water.

12.2.10 The catalyst and the cupric oxide may be used straight from the bottle, but it is recommended that each be pre-combusted between 680° and 850° C for 90 minutes. This reduces the time needed to condition the column. Left over pre-combusted catalyst and cupric oxide may be stored in a desiccator.

12.2.11 Place 2 platinum screens in the bottom of the column. Cover with a very thin layer of quartz wool. Note: pressure problems may arise if the quartz wool is too thick.

12.2.12 Pour 120 mm of catalyst into the tube. Top with 2-4 mm of cupric oxide. Note: The CuO is used to prevent back splashing of the vaporized sample, which causes doublet peaks.

12.2.13 Smear a thin layer of high vacuum silicone grease 1-2 mm below the top of the column. Set aside.

12.2.14 Remove the old orings from the top of the injection block. Rinse the block. Put a thin layer of silicone grease on the new black oring and put into place. Lay a new (white) Teflon oring on top. Do not grease. Note: the Teflon coated oring on the underside of the injection block needs to be replaced twice yearly.

12.2.15 Remove the injection needle from the injection slide. Rinse the slide, Replace with a new needle (remove the wire from inside the new needle). Adjust the needle so that only a millimeter or so is showing through the slide, and then tighten the knurled nut. The tip of the needle should not be visible when holding the slide on a horizontal plane. It will score the Teflon oring if it is out too far.

12.2.16 Slide the air tubing back onto the needle.

12.2.17 Insert the column into the bottom of the injection block. Place into the furnace opening, making sure that the drain

tube is properly aligned. Insert into the cooling coil and hand tighten. Note: the TOC5000 requires new Teflon ferrules at this point.

12.2.18 Adjust the column height with mounting plate screws. On the 5000, allow enough space to slide a folded piece of paper underneath the coil. On the 5000A, adjust the height by the bottom platform.

12.2.19 Return the TC gas line to its proper position.

12.2.20 Secure the injection port slide.

12.2.21 Return the syringe to its proper position.

12.2.22 Turn gas and instrument on. Liquid in the IC block should bubble. If not, check and tighten everything that was loosened.

12.2.23 Turn the furnace on. Refer to Section 11.1 for TOC5000, using Curve #1, and Section 11.2 for TOC5000A instrument instructions, using the file COLCOND.CAL and COLCOND.MET.

12.2.24 Fill auto-sampler tubes 1-78 with ASTM Type I water, and acidify with 100 µl 2N HCl. Acidify the water in position S1 standard cup with 300 µl 2N HCl.

12.2.25 Pull up the maintenance screen and do a Zero Point Detection.

12.3 Semi-annual maintenance:

12.3.1 Replace the Teflon coated oring.

12.3.2 Replace the NaOH solution in the humidifier with a 0.3N NaOH solution: 1.2 g NaOH/100 ml H_2O.

12.4 Annual maintenance:

12.4.1 Replace the halogen scrubber and acrodisc filter.

12.4.2 Replace the soda lime scrubber.

12.4.3 Replace the 4 port valve.

12.4.4 If not used frequently, replace the IC port orings and needle. If used regularly, follow the monthly schedule.

12.4.5 Replace the cooling coil.

12.4.6 Replace the ASI needles.

12.5 Pollution Prevention and Waste Management:

12.5.1 Liquids generated by this method are safe to put down the sink.

12.5.2 Spent catalyst may be disposed of in the trash.

12.5.3 Spent CO_2 absorber (Soda Lime) must be disposed in a proper manner. It should be taken to the Storage Facility on campus to be dealt with as hazardous waste.

13.0 References:

13.1 EPA Method 415.1. Determination of Total Organic Carbon in Water using Combustion or Oxidation.

13.2 Sugimura, Y. and Y. Suzuki. 1988. A high temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. Mar. Chem. 24:105-131.

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DRAFT

Determination of Dissolved Inorganic Nitrate plus Nitrite (NO3+NO2) in Fresh/Estuarine/Coastal Waters Using Enzyme Catalyzed Reduction

1. SCOPE and APPLICATION

1.1 Enzyme catalyzed reduction is used to quantitatively reduce dissolved nitrate to nitrite which is then measured by colorimetric quantitative analysis of a highly colored azo dye. The method is used to analyze all ranges of salinity.

1.2 A Method Detection Limit (MDL) of 0.005 mg NO_3+NO_2-N/L was determined as three times the standard deviation of seven low level replicates.

1.3 The Quantitation Limit for NO_3+NO_2 was set at 0.0175 mg NO_3+NO_2-N/L, or ten times the standard deviation of the MDL calculation.

1.4 This procedure should be used by analysts experienced in the theory and application of aqueous inorganic analysis. Three months experience with an experienced analyst, certified in the analysis of nitrate plus nitrite in aqueous samples by enzyme catalyzed reduction is required.

1.5 This method can be used for all programs that require analysis of dissolved inorganic nitrate plus nitrite.

2. SUMMARY

2.1 Filtered samples are mixed with Nitrate Reductase (an enzyme isolated from the plant *Arabidopsis thaliana*) and NADH (*β*-Nicotinamide adenine dinucleotide reduced form disodium salt). The nitrite, both that which was reduced from nitrate and nitrite that was originally present, is then determined by diazotizing with sulfanilamide and coupling with N-1-napthylethylenediamine dihydrochloride to form a colored azo dye. Filtered samples with concentrations found to be below the method detection limit are analyzed via cadmium reduction with a Technicon Bran & Luebbe AutoAnalyzer II.

3. DEFINITIONS

3.1 Acceptance Criteria – Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)

3.2 Accuracy – The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)

3.3 Aliquot – A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)

3.4 Analytical Range – 0.035 to 5.6 mg NO_3+NO_2-N/L. The overall analytical range is comprised of three distinct yet overlapping concentration ranges. A separate calibration is performed for each range. These ranges include 0.035 to 0.28 mg NO_3+NO_2-N/L, 0.07 to 0.70 mg NO_3+NO_2-N/L and 0.56 to 5.6 mg NO_3+NO_2-N/L. Three sub-ranges are utilized so that samples can be analyzed on the most appropriate scale possible.

3.5 Batch – Environmental samples, which are prepared and /or analyzed together with the same process and personnel, using the same lot(s) of reagents. A **preparation batch** is composed of one to 200 environmental samples of the same matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 8 hours. An **analytical batch** is composed of prepared environmental samples (extracts, digestates, concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)

3.6 Blank- A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)

3.7 Calibrate- To determine, by measurement or comparison with a standard, the correct value of each scale reading on a device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.8 Calibration – The set of operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.9 Calibration Curve – The graphical relationship between known values, such as concentrations, or a series of calibration standards and their analytical response. (NELAC)

3.10 Calibration Method – A defined technical procedure for performing a calibration. (NELAC)

3.11 Calibration Standard – A substance or reference material used to calibrate an instrument. (QAMS)

3.11.1 Initial Calibration Standard (STD) – A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.

3.11.2 Initial Calibration Verification (ICV) – An individual standard, analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve.

3.11.3 Continuing Calibration Verification (CCV) – An individual standard which is analyzed after every 15-20 field sample analysis.

3.12 Certified Reference Material – A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (ISO 17025)

3.13 Corrective Action – Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)

3.14 Deficiency – An unauthorized deviation from acceptable procedures or practices. (ASQC)

3.15 Demonstration of Capability – A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)

3.16 Detection Limit – The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.

3.17 Duplicate Analysis – The analyses of measurements of the variable of interest performed identically on two sub samples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage internal to the laboratory. (EPA-QAD)

3.18 External Standard (ES) – A pure analyte (potassium nitrate (KNO_3)) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.

3.19 Field Duplicates (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.20 Holding time – The maximum time that samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.

3.21 Laboratory Duplicates (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated

with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.22 Laboratory Reagent Blank (LRB) – A blank matrix (i.e., DI water) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, 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 instrument.

3.23 Laboratory Control Sample (LCS) – A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standard or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)

3.24 Limit of Detection (LOD) – The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank. (ACS)

3.25 Limit of Quantitation (LOQ) – The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD, depending on the degree of confidence desired.

3.26 Linear Dynamic Range (LDR) – The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).

3.27 Material Safety Data Sheets (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.

3.28 May – Denotes permitted action, but not required action. (NELAC)

3.29 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 (Standard Methods).

3.30 Must – Denotes a requirement that must be met. (Random House College Dictionary)

3.31 Photometer – measures the absorbance of the solution in the cell in a multicell cuvette. Light passes from the lamp through the condensing lenses to the interference filter. The plane surface of the first condensing lens is coated with a material which reflects heat and infrared light. The filters are mounted on a filter wheel. There are 15 positions for filters. Each filter corresponds to a wavelength of interest. The 540 nm filter is specified by the test definition for nitrate plus nitrite. After passing through the filter the light is converted into

a stream of light pulses by a chopper. Then the light is directed via a quartz fiber through a focusing lens and a slit to the beam divider. The beam divider divides the light into two parts. A specified portion is reflected to the reference detector, which monitors the light level fluctuations. The remaining major portion of the light beam goes through the liquid in the cell to the signal detector, which measures the amount of light absorbed.

3.32 Precision – The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)

3.33 Preservation – Refrigeration, freezing, and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.

3.34 Quality Control Sample (QCS) – A sample of analyte of known and certified concentration. 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.

3.35 Run Cycle – Typically a day of operation – the entire analytical sequence from sampling the first standard to the last sample of the day.

3.36 Sample Segment – Bar-coded metal tray that holds up to fourteen four milliliter auto analyzer vials containing samples or standards. The user identifies each vial in the operating software.

3.37 Sample Segment Holder – An automated temperature controlled carousel that contains up to six sample segments. This carousel spins in clockwise or counterclockwise manner to move the sample segments into position for analysis. This carousel format allows for continuous processing.

3.38 Sensitivity – The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.

3.39 Shall – Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)

3.40 Should – Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)

3.41 Standard Reference Material (SRM) – Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique.

3.42 Test Definition – A photometric test consisting of a user defined testing sequence, reagent additions, calibration standards, incubations and absorption results.

3.43 Test Flow – Functions to define the parameter for reagent and sample dispensing, dilution, incubation and measurement.

4 INTERFERENCES

4.1 Suspended matter in the sample will scatter light as it passes through the cuvette to the detector. High blank responses will result. The identified sample will be reanalyzed.

4.2 Blemishes in the cuvette, as result of the manufacturing process, will result in high blank responses. The identified sample will be reanalyzed.

5 SAFETY

5.1 Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats, safety glasses and enclosed shoes should be worn. In certain situations, it will be necessary to also use gloves and/or a face shield. If solutions come in contact with eyes, flush with water continuously for 15 minutes. If solutions come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed and also inform the CBL Business Manager of the incident. Contact the CBL Business Manager if additional treatment is required.

5.2 The toxicity or carcinogenicity of each reagent used in this procedure may not have 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 hazardous materials and procedures.

5.3 Do not wear jewelry when troubleshooting electrical components. Even low voltage points are dangerous and can injure if allowed to short circuit.

5.4 The following hazard classifications are listed for the chemicals used in this procedure. Detailed information is provided on Material Safety Data Sheets (MSDS).

Chemical	Health	Flammability	Reactivity	Contact	Storage
Nitrate Reductase (AtNaR2) from *Arabidopsis thaliana*	0	0	0	0	Green
NADH (β-Nicotinamide adenine dinucleotide reduced form disodium salt)	0	0	0	0	Green
Potassium hydroxide	3	0	2	4	White

					Stripe
Sulfanilamide	0	1	1	1	Green
N-1-napthylethylenediamine dihydrochloride	2	1	1	2	Green
Hydrochloric Acid	3	0	2	4	White
Potassium nitrate	2	0	3	2	Yellow
Sodium nitrite	2	0	3	2	Yellow
Potassium phosphate	0	0	0	1	Green
EDTA (Ethylenediamine tetraacetic acid	1	0	0	1	Green

On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability, reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)
STORAGE
Red – Flammability Hazard: Store in a flammable liquid storage area.
Blue – Health Hazard: Store in a secure poison area.
Yellow – Reactivity Hazard: Keep separate from flammable and combustible materials.
White – Contact Hazard: Store in a corrosion-proof area.
Green – Use general chemical storage (On older labels, this category was orange).
Striped – Incompatible materials of the same color class have striped labels. These products should not be stored adjacent to substances with the same color label. Proper storage must be individually determined.

6 EQUIPMENT AND SUPPLIES

6.1 Aqaukem 250 multi-wavelength automated discrete photometric analyzer. Aquakem 250 control software operates on a computer running Microsoft Windows NT or XP operating system.
6.2 Freezer, capable of maintaining -20 ± 5° C.
6.3 Lab ware – All reusable lab ware (glass, Teflon, plastic, etc) should be sufficiently clean for the task objectives. This laboratory cleans all lab ware related to this method with a 10% HCl (v/v) acid rinse.

7 REAGENTS AND STANDARDS

7.1 Purity of Water – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D 1193, Type I. Freshly prepared water should be used for making the standards intended for calibration. The detection limits of this method will be limited by the purity of the water and reagents used to make the standards.
7.2 Purity of Reagents – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of

sufficiently high purity to permit its use without compromising the accuracy of the determination.

7.3 Ethylenediamine tetraacetic acid (EDTA, 25 mM) 9.3 g

In a 1 L volumetric flask add approximately 800 mL deionoized water. Dissolve 9.3 g ultrapure EDTA in deionized water and bring to volume. Store the flask at room temperature out of direct sunlight. The reagent is stable for one year.

7.4 Phosphate Buffer-

Potassium di-hydrogen phosphate (KH_2PO_4)	1.88 g
Potassium hydroxide (KOH)	0.7 g
EDTA (25 mM)	5.0 mL

In a 500mL volumetric flask dissolve 1.88 g KH_2PO_4 , 0.7g KOH and 5.0 mL EDTA (25mM) in approximately 400 mL deionized water. Bring flask to volume. Store flask at room temperature. The reagent is stable for six months.

7.5 Nitrate Reductase (AtNaR2)-

Nitrate reductase from *Arabidopsis Thaliana*	3.0 unit vial
Phosphate Buffer	20 mL

Transfer 1mL phosphate buffer to the 3.0 unit vial of AtNaR2 to affect dissolution. Shake several times over a thirty minute period. Transfer this to the 20mL reagent bottle quantitatively with four 1 ml aliquots of the phosphate buffer. Add 15mL of phosphate buffer to the reagent bottle. Shake bottle to complete the reagent preparation. This is enough reagent for approximately 300 analyses. This reagent is stable for eight hours in the refrigerated reagent compartment of the instrument.

7.6 NADH-

(β-Nicotinamide adenine dinucleotide reduced form disodium salt)	2.4 g vial
Phosphate Buffer	11 mL

Carefully transfer NADH crystals from vial to 20 mL reagent bottle. Place 1 mL phosphate buffer in vial and shake thoroughly. Transfer to reagent bottle. Add 10 mL phosphate buffer to the reagent bottle. Shake to complete reagent preparation. This is enough reagent for approximately 300 analyses. This reagent is stable for eight hours in the refrigerated reagent compartment of the instrument.

7.7 Sulfaniamide-

Sulfanilamide	10 g
Hydrochloric Acid (concentrated)	300 mL

Add 500 mL deionized water to a 1 L volumetric flask. Carefully add 300 mL concentrated hydrochloric acid to the flask. Then add 10 g sulfanilamide to the flask. Bring the flask to volume with deionized water. Once dissolution is complete transfer reagent to a brown poly-bottle and store in the refrigerator. This reagent is stable for six months.

7.8 N-1-napthylethylenediamine dihydrochloride –

N-1-napthylethylenediamine dihydrochloride	1.0 g

Place 1.0 g N-1-napthylethylenediamine dihydrochloride in a 1 L volumetric flask. Bring flask to volume with deionized water. Once dissolution is complete transfer reagent to a brown poly-bottle and store in refrigerator. This reagent is stable for six months.

7.9 Nitrate Stock Standard, 5000 µM –

Potassium nitrate (KNO_3), primary standard grade, dried at 45ºC 0.5055 g

In a 1 L volumetric flask, dissolve 0.5055 g of potassium nitrate in approximately 800 mL deionized water. Bring flask to volume with deionized water (1 mL contains 5 µmoles N). Make fresh every 4 months.

7.10 Stock Nitrite Standard –

Sodium nitrite ($NaNO_2$), primary standard grade, dried at 45ºC 0.345 g

In a 1 L volumetric flask, dissolve 0.345 g of sodium nitrite in approximately 800 mL of deionized water. Dilute to volume with deionized water (1 mL contains 5 µmoles N). Add 1 mL of chloroform as a preservative. Make fresh every 4 months.

7.11 Secondary Nitrite Standard –

Stock Nitrate Standard 0.80 mL

In a 100 mL volumetric flask, dilute 0.80 mL of Stock Nitrite Standard to volume with deionized water to yield a concentration of 40 µM NO_2 –N/L (0.56 mg N/L).

8 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Water collected for NO_3+NO_2 should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 µm), or equivalent.

8.2 Water collected for NO_3+NO_2 should be frozen at -20° C. The sample container should be clean and sample rinsed.

8.3 Frozen NO_3+NO_2 samples may be stored longer than 28 days. It has been shown that frozen QCS samples up to a year old still fall well within the control limits.

8.4 NO_3+NO_2 samples may be refrigerated at 4° C for no longer than one day.

9 QUALITY CONTROL

9.1 The laboratory 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 continued analysis of laboratory instrument blanks and calibration standard material, analyzed as samples, as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2 Initial Demonstration of Capability

9.2.1 The initial demonstration of capability (NO_3+NO_2) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.

9.2.2 Quality Control Sample (QCS/SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 10% of the certified 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 analyses.

9.2.3 Method Detection Limits (MDLs) – MDLs should be established for NO_3+NO_2 using a low level ambient water sample. To determine the MDL values, analyze seven replicate aliquots of water. Perform all calculations defined in the procedure (Section xx) and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = s \times 3$$

Where, s= Standard Deviation of the replicate analyses.

9.2.4 MDLs shall be determined yearly and whenever there is a significant change in instrument response, a significant change in instrument configuration, or a new matrix is encountered.

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. The LRB consists of Nanopure water treated the same as the samples. Analyte found in LRB indicates possible reagent or laboratory environment contamination. LRB data are used to assess and correct contamination from the laboratory environment.

9.3.2 Quality Control Sample (QCS)/ Standard Reference Material (SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning of the run and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 3s of the certified 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 the analyses. The results of these QCS/SRM samples shall be used to determine batch acceptance.

9.3.3 The QCS are obtained from a source external to the laboratory and different from the source of calibration standards.

9.3.4 Control Charts – The Accuracy Control Chart for QCS/SRM samples is constructed from the average and standard deviation of the 20 most recent QCS/SRM measurements. The accuracy chart includes upper and lower warning levels (WL=±2*s*) and upper and lower control levels (CL=±3*s*). These values are derived from stated values of the QCS/SRM. The standard deviation (*s*) is specified relative to statistical confidence levels of 95% for WLs and 99% for CLs. Set up an accuracy chart by using percent recovery since the concentration of the QCS/SRM varies. Enter QCS/SRM results on the chart each time the sample is analyzed

9.3.5 Continuing Calibration Verification (CCV) – Following every 18-23 samples, one CCV of 10 µM NO_3 (0.14 mg N/L) NiRMID, 20 µM NO_3 (0.28 mg N/L) NiRHI , 200 µM NO_3 (2.8 mg N/L) NiRXH is analyzed to assess instrument performance. The CCVs are made from the same material as calibration standards (KNO_3), and are to be within TV ± 3*s*. Failure to meet the criteria requires correcting the problem, including reanalysis of any affected samples. If not enough sample exists, the data must be qualified if reported.

9.4 Assessing Analyte Recovery - % Recovery

9.4.1 Analyte recovery is assessed through percent recoveries of laboratory spikes.

9.4.2 % Recovery = (Found Value/True Value) X 100

9.5 Assessing Analyte Precision – Relative Percent Difference

9.5.1 Analyte replication is assessed through duplicate analyses of samples – Relative Percent Difference.

9.5.2 RPD = (Laboratory Duplicate Result 1 – Laboratory Duplicate Result 2)/[(Laboratory Duplicate Result 1 + Laboratory Duplicate Result 2)/2] X 100

9.6 Corrective Actions for Out of Control Data

9.6.1 Control limit – If one measurement exceeds Accuracy Control Chart CL, repeat the analysis immediately. If the repeat measurement is within the CL, continue analyses; if it exceeds the CL, discontinue analyses and correct the problem.

9.6.2 Warning limit – If two out of three successive points exceed Accuracy Control Chart WL, analyze another sample. If the next point is within WL, continue analyses; if the next point exceeds the WL, evaluate potential bias and correct the problem.

9.6.3 Trending – If seven successive Accuracy Control Chart measurements are on the same side of the central line, discontinue analyses and correct the problem.

9.6.4 When external QCS samples are out of control, correct the problem. Reanalyze the samples analyzed between the last in-control measurement and the out-of-control one.

9.6.5 When external CCV samples are out of control, correct the problem. Reanalyze the samples analyzed between the last in-control measurement and the out-of-control one.

9.7 General Operation - To assure optimal operation and analytical results, the Reagent Blank and CCV are tracked daily in the raw data file, copied to Reagent Blank and CCV Control Charts.

10 CALIBRATION AND STANDARDIZATION

10.1 Calibration – Daily calibration must be performed before sample analysis may begin. Eight point calibrations are used with each of the three sub-calibrations that cover the analytical range. Five working nitrate standards are used to produce the calibrators for each set of three calibration curves. The instrument performs serial dilutions of working standards to produce the eight calibrators defined for each curve. The following outlines the preparation of the working standards and the following table describes the subsequent serial dilutions the instrument performs to make each standard for each of the three calibration curves.

NO23 Working Standards:

NiRMID

Working Standard 0.7 mg N/L (1.0 mL stock to 100 mL)
Working Standard 0.28 mg N/L (0.4 mL stock to 100 mL)

NiRHI

Working Standard 2.8 mg N/L (4 mL stock to 100 mL)
Working Standard 0.7 mg N/L (1.0 mL stock to 100 mL)

NiRXHI

Working Standard 5.6 mg N/L (8.0 mL stock to 100 mL)
Working Standard 22.4 mg N/L (32 mL stock to 100 mL)

NO23 Calibrators:

	Working Standard mg/L N	Dilution Factor	Concentration mg/L N
NiRMID	0.28	10	0.028
	0.28	6	0.047
	0.28	5	0.056
	0.28	3	0.093
	0.28	2	0.140
	0.7	4	0.175
	0.7	3	0.233
	0.28	1	0.280
NiRHI	0.7	10	0.070

	0.7	6	0.117
	0.7	5	0.140
	0.7	3	0.233
	0.7	2	0.350
	2.8	6	0.467
	2.8	5	0.560
	0.7	1	0.700
NiRXH	5.6	10	0.560
	5.6	6	0.933
	5.6	5	1.120
	5.6	3	1.867
	5.6	2	2.800
	22.4	6	3.733
	22.4	5	4.480
	5.6	1	5.600

10.2 The instrument software prepares a standard curve for each set of calibrators. A graph plotting measured absorbance against standard concentration is presented for review and approval. If acceptance criteria are not met the entire curve can be reanalyzed or individual standards can be reanalyzed. The coefficient of determination (Person's r value) for the calibration curve as well as the calculated concentration of each calibrator is reviewed. The calculated value of each calibrator must be within ten percent of the expected value.

11 Procedure – Daily Operation

11.1 Turn on computer. Computer will automatically initiate Konelab software. Once software is running, turn on instrument and allow connection between instrument and computer to complete.

11.2 Discard any water remaining in the water reservoir from the previous analytical run. Fill the water reservoir with fresh deionozed water.

11.3 Remove from freezer samples that will be analyzed that day. Allow samples to begin thawing. Begin daily bench sheet documentation. Remove SRM from freezer as well and allow to thaw. Also remove nitrate reductase and NADH vials from freezer.

11.4 Once water reservoir is full, "perform washes" – complete five wash cycles and then initiate "start-up" at main menu.

11.5 Gather working standards and reagents from refrigerator during startup. Assess standards and reagents. Remake anything that has exceeded the time over which it is considered stable. Nitrate reductase and NADH reagents are to be made fresh for every analytical run.

11.6 Once startup is complete, check that the instrument water blank has performed within acceptance limits. If any of the instrument functions are outside their predefined and software controlled limits, the user will be notified on the main menu page. User takes corrective action to return instrument functions to controlled limits.

11.7 Load reagents into reagent carousel and place into refrigerated reagent compartment.

11.8 Load working standards into a sample segment, identify the standards in their positions from the drop down menus at the individual segment positions, and load into instrument.

11.9 Select the methods to be calibrated. Three methods will be calibrated – NiRMID, NiRHI and NiRXH are the method names to be selected in the software.

11.10 Begin calibration – See test flow below for stepwise instrument functions for the analysis of standards and samples.

Test Flow – Method of Analysis, Stepwise

- 55 μL NiR AtNaR to cuvette
- 5 μL sample to cuvette with mixing
- 15 μL NiR NADH to cuvette with mixing
- Incubation, 600 seconds
- 25 μL sulfanilamide (SAN) reagent to cuvette with mixing
- Incubation, 120 seconds
- 25 μL N-1-Napthylethylenediamine dihydrochloride (NED) reagent to cuvette with mixing
- Incubation, 120 seconds
- End point absorbance measurement, 540 nm
- Side-wavelength measurement, 700 nm
- Software processes absorbance value and uses calibration curve to calculate analyte concentration (mg/L N as NO2)
- User is notified if any measured values used to calculate final concentration are outside preset limits. If so, user has options to accept results, rerun the sample or rerun the sample diluted to a user or software specified factor.

11.11 Organize samples, reagent blanks, check standards and all quality control samples while instrument performs calibrations.

11.12 As calibration curves are produced by the instrument, review them for acceptability. The instrument software prepares a standard curve for each set of calibrators. A graph plotting measured absorbance against standard concentration is presented for review and approval. If acceptance criteria are not met, either the entire curve shall be reanalyzed or individual standards shall be reanalyzed, depending on the violation.

11.13 Once calibration curves are accepted, samples are loaded into the sample segments and loaded into the instrument for analysis. The first samples analyzed should be an ICV (initial calibration verification) samples. There should be one sample for each calibration curve, of a concentration close to

the middle of each range. The following are the usual ICV samples for each curve: 0.14 mg N/L NiRMID, 0.28 mg N/L NiRHI and 2.8 mg N/L NiRXH.

11.14 Samples are loaded into the segments and analyzed. CCV (Continuing Calibration Verification) samples (one for each of the three calibration ranges) follow every 18-23 samples. Standard Reference Material (SRM) samples as well as Laboratory Reagent Blanks (LRB) are scattered throughout the analytical batch. Throughout the analytical batch, samples are chosen as laboratory duplicates and laboratory spikes to assess analyte precision and analyte recovery, respectively. The total number of duplicates and spikes performed will be equal or greater to ten percent of the total number of samples in the analytical batch.

11.15 As sample analysis is complete, results must be reviewed and accepted manually. If results fall outside acceptance limits, the sample should be reanalyzed. If sample result exceeds the highest standard of the calibration range it was run within, the samples can be automatically diluted by the instrument and reanalyzed. If the result is such that it will fall within a higher calibration range, it should be reanalyzed in that range. If the result is such that it will fall within a lower calibration range, it should be reanalyzed within that range. If the result falls below the lowest standard of the lowest calibration range, the result should be discarded and the sample should be analyzed via cadmium reduction method.

11.16 Upon completion of all analysis, results should be saved to a daily report file. The file is named by the run date. The daily report file for analytical batch of January 1, 2005 would be named 010105. The file is converted to Microsoft Excel for data work up. Remaining samples are discarded.

11.17 All reagents are removed from the reagent chamber and returned to the refrigerator. Reagents that have exceeded their stability period are discarded.

11.18 A cleaning solution is inserted into the instrument and shut down procedures are initiated. Daily files are cleared from the instrument software, the software is exited and the instrument is shut down. The computer is shut down.

11.19 The waste is flushed down the drain with copious amounts of tap water. The waste cuvette box is moved to the fume hood. The incubator cover plate is removed. The incubator is wiped clean. The cover is cleaned and returned to its original position.

Draft

Determination of Carbon and Nitrogen in Particulates and Sediments of Fresh/Estuarine/Coastal Waters, Plant and Animal Tissue, and Soils Using Elemental Analysis

1. SCOPE and APPLICATION

1.1. Elemental analysis is used to determine particulate carbon (PC), and particulate nitrogen (PN) in fresh, estuarine and coastal waters and sediments as well as for plant and animal tissue and soils. The method measures the PC and PN irrespective of source (organic or inorganic.)

1.2. A Method Detection Limit (MDL) of 0.0759 mg C/l and 0.0123 mg N/l, for filtered samples, and 0.130 %C and 0.008% N for sediment samples, were determined using three times the standard deviation of seven replicates.

1.3. The quantitation limit for PC and PN has not been determined.

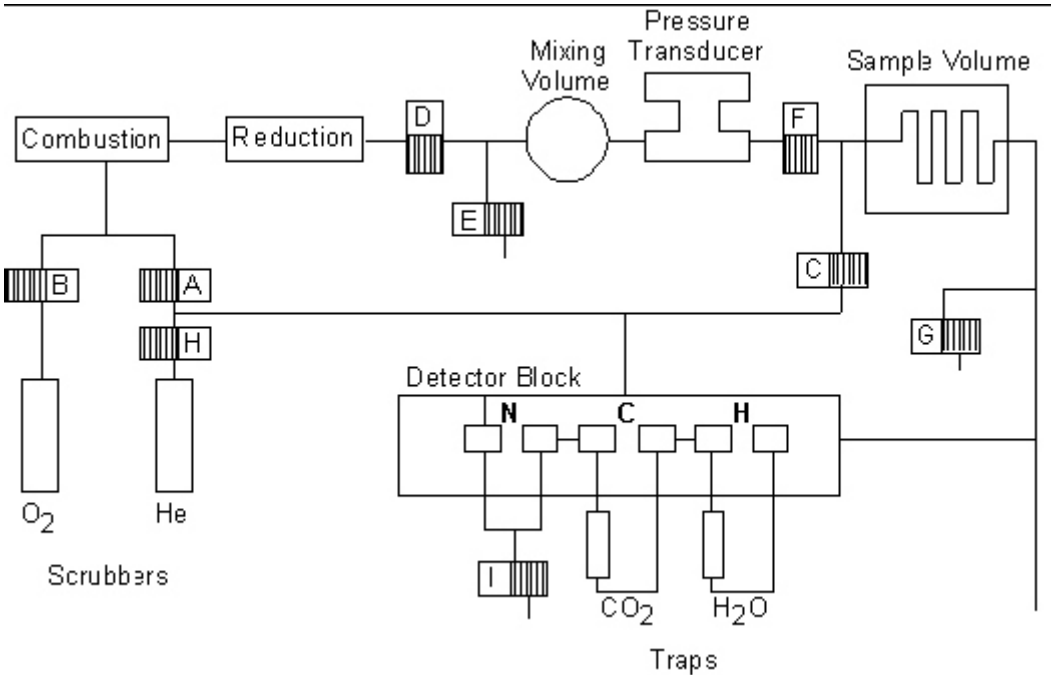
1.4. This procedure should be used by analysts experienced in the theory and application of elemental analysis. A minimum of 3 months experience with an elemental analyzer is recommended.

1.5. This method is for use by all programs that require analysis of particulate carbon and nitrogen in water and sediment, soils and tissues. The need to determine the organic fraction of the total particulate carbon and nitrogen in samples depends on the data-quality objectives of the study. Section 11.2.5 outlines the procedure used to ascertain the organic fraction.

2. SUMMARY

2.1. In the Exeter Analytical, Inc. Model CE-440 Elemental Analyzer, the carbon and nitrogen content in organic and inorganic compounds can be determined. Combustion of the sample occurs in pure oxygen under static conditions. The combustion train and analytical system are shown below in the CE-440 flow diagram. Helium is used to carry the combustion products through the analytical system to atmosphere, as well as for purging the instrument. Helium was selected for this purpose because it is chemically inert relative to tube packing chemicals, and it has a very high coefficient of thermal conductivity. The products of combustion are passed over suitable reagents in the combustion tube to assure complete oxidation and removal of undesirable by-products such as sulfur, phosphorus and halogen gases. In the reduction tube, oxides of nitrogen are converted to molecular nitrogen and residual oxygen is removed. In the mixing volume the sample gasses are thoroughly homogenized at precise volume, temperature, and pressure. This mixture is released through the sample volume into the thermal conductivity detector. Between the first of three pairs of thermal conductivity cells an absorption trap removes water from the sample gas. The differential signal read before and after the trap reflects the water concentration and, therefore, the amount of hydrogen in the original sample. A similar measurement is made of the signal output of a second

pair of thermal conductivity cells, between which a trap removes carbon dioxide, thus determining the carbon content. The remaining gas now consists only of helium and nitrogen. This gas passes through a thermal conductivity cell and the output signal is compared to a reference cell through which pure helium flows. This gives the nitrogen concentration.



Schematic diagram of the Exeter Analytical, Inc. (EAI) CE-440 Elemental Analyzer

3. DEFINITIONS

3.1. **Acceptance Criteria -** Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)

3.2. **Accuracy -** The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)

3.3. **Acetanilide** - Used as a standard or conditioner in the analyzer for calibration purposes. It has known percentages of C, H, and N.

3.4. **Aliquot -** A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)

3.5. **Batch -** Environmental samples, which are prepared and/or analyzed together with the same process and the same personnel using the same lot(s) of reagents. A preparation batch is composed of one to 20 environmental samples of the same

matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An analytical batch is composed of prepared environmental samples (extracts, digestates, or concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrixes and can exceed 20 samples. (NELAC/EPA)

3.6. **Blank -** A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)

3.7. **Blank** - Blank value = blank read minus blank zero. An indicator of the stability of the system. (Exeter)

3.8. **Bridge** - Electrical configuration of the thermal conductivity filaments.(Exeter)

3.9. **Calibrate -** To determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.10. **Calibration -** The set of operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.11. **Calibration Method -** A defined technical procedure for performing a calibration. (NELAC)

3.12. **Calibration Standard -** A substance or reference material used to calibrate an instrument. (QAMS)

3.12.1. **Initial Calibration Standards (STD) -** A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.

3.12.2. **Initial Calibration Verification (ICV) -** An individual standard, analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve.

3.12.3. **Continuing Calibration Verification (CCV) -** An individual standard which is analyzed after every tenth field sample analysis.

3.13. **Capsule** - Aluminum container. Used for containing samples and standards with an accurate weight and maintains integrity prior to combustion.

3.14. **Calibration Standard (CAL)** - An accurately weighed amount of a certified chemical used to calibrate the instrument response with respect to analyte mass. For this procedure the calibration standard is acetanilide, 99.9%+ purity.

3.15. **Certified Reference Material -** A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (ISO 17025)

3.16. **Combustion Time** - Time for sample to fully combust in an oxygen environment.

3.17. **Combustion Tube** - Quartz tube packed with reagents and used for sample combustion.

3.18. **Conditioner** - A standard chemical which is not necessarily accurately weighed that is used to coat the surfaces of the instrument with the analytes (water vapor, carbon dioxide, and nitrogen).

3.19. **Corrective Action -** Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)

3.20. **Deficiency -** An unauthorized deviation from acceptable procedures or practices. (ASQC)

3.21. **Demonstration of Capability** -A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)

3.22. **Detection Limit -** The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.

3.23. **Detector** - The heart of the analyzer consisting of three bridges. Determines the percentages of carbon, hydrogen, and nitrogen in the sample via thermal conductivity.

3.24. **Detector Oven** - Keeps the temperature of the detector, pressure transducer, mixing volume, and sample volume constant.

3.25. **Double Drop** - Two samples are dropped for one run - used for filter and inorganic applications. Sample requires a + prefix.

3.26. **Duplicate Analyses -** The analyses or measurements of the variable of interest performed identically on two sub samples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage internal to the laboratory (EPA-QAD)

3.27. **External Standard (ES)** - A pure analyte (atropine) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.

3.28. **Field Duplicates (FD1 and FD2)** - Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.29. **Fill Time** - Time required to build-up the pressure in the mixing volume to 1500 mm Hg.

3.30. **Filtered Sample –** An accurately measured amount of water from fresh, estuarine or coastal samples, filtered through a 25 mm Whatman GF/F filter or equivalent, which has been precombusted at 500^{o} C for 90 minutes.

3.31. **Furnace** - Heats the reduction and combustion tubes to operating temperature.

3.32. **Heated Line** - Connects the reduction tube outlet to the inlet of the mixing volume. Heated to prevent condensation of gases on tube walls.

3.33. **Holding Time -** The maximum time which samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.

3.34. **Inject Solenoid** - Solenoid used on the automated injection system to actuate the rotation of the sample wheel.

3.35. **Injection** - Moving the ladle, containing a capsule with the sample into the combustion furnace.

3.36. **Injector Box** - The box assembly that houses the sample wheel.

3.37. **Instrument Detection Limit (IDL)** - The minimum quantity of analyte or the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time, absorbance line, etc.

3.38. **K-Factor** - Instrument sensitivity factor in microvolts per microgram, calibrated using an external standard.

3.39. **Laboratory Duplicates (LD1 and LD2)** - Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.40. **Laboratory Reagent Blank (LRB)** - A matrix blank (i.e., a precombusted filter or sediment capsule) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, 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.

3.41. **Ladle** - Transports the capsule with the sample into a combustion furnace.

3.42. **Laboratory Control Sample (LCS) -** A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standards or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)

3.43. **Limit of Detection (LOD) -** The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank. (ACS)

3.44. **Limit of Quantitation (LOQ) -** The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD, depending on the degree of confidence desired.

3.45. **Linear Dynamic Range (LDR)** - The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).

3.46. **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.

3.47. **May -** Denotes permitted action, but not required action. (NELAC)

3.48. **Method Detection Limit (MDL)** - The minimum concentration of an analyte that can be identified, measured, and reported with 98% confidence that the analyte concentration is greater than zero.

3.49. **Mixing Volume** - Spherical bottle in which sample gases become homogenous.

3.50. **Mother Board** - The main printed circuit board. All CE 440 power supplies are located here.

3.51. **Must** - Denotes a requirement that must be met. (Random House College Dictionary)

3.52. **Precision** - The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)

3.53. **Preservation –** Refrigeration, freezing and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.

3.54. **Pressure Transducer** - Used to check for leaks in the system and to monitor pressure in the mixing volume.

3.55. **P Valve** - The valve on the injector box of the horizontal auto-injector (HA) used to automatically purge the box.

3.56. **Profile** - Generated by the bridge signal. Used to help determine if a leak or malfunction occurs in the system.

3.57. **Quality Control Sample (QCS)** - A sample of analytes of known and certified concentrations. 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.

3.58. **Reduction Tube** - Quartz tube with reduced copper that removes excess oxygen from the sample gas and reduces oxides of nitrogen to free nitrogen.

3.59. **Response Factor (RF)** - The ratio of the response of the instrument to a known amount of analyte.

3.60. **Run** - One sample analysis from start to finish, including printout.

3.61. **Run Cycle** - Typically a day or half day of operation - the entire analytical sequence of runs from the first run to the last run on the Sample Wheel.

3.62. **Sample Volume** - Tube where sample gas is exhausted from the mixing volume prior to entering the detector.

3.63. **Sample Wheel –** Sample holding device which contains up to 64 blanks, standards and samples. One wheel equals roughly 6 hours of run time, which is called the Run Cycle.

3.64. **Scrubber** - Removes water and CO_2 from the gas supplies.

3.65. **Sediment (or Soil) Sample** - A fluvial, sand, or humic sample matrix exposed to a marine, estuarine or fresh water environment.

3.66. **Sensitivity** - The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.

3.67. **Shall** - Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)

3.68. **Should** - Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)

3.69. **Sleeve** - Nickel - to maintain integrity of the sample capsule and to protect the quartz ware from devitrification (to destroy the glassy qualities by prolonged heating).

3.70. **Standard Reference Material (SRM)** - Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique.

3.71. **Trap** - Used for removing water and CO_2 from the sample gas.

3.72. **Tissue sample** – Plant or animal tissue dried and ground ready for weighing.

3.73 **Zero Value** - Bridge signal with only pure helium flowing through the detector.

4. INTERFERENCES

4.1. There are no known interferences for fresh, estuarine or coastal water or sediment samples. The presence of C and N compounds on laboratory surfaces, on fingers, in detergents and in dust necessitates the utilization of careful techniques (i.e., the use of forceps and gloves) to avoid contamination in every portion of this procedure (EPA.)

5. SAFETY

5.1. Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats and safety glasses and enclosed shoes must always be worn. In certain situations it may also be necessary to use gloves and goggles. If solutions or chemicals come in contact with eyes, flush with water continuously for 15 minutes. If solutions or chemicals come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed and also inform the CBL Business Manager of the incident. Contact the CBL Business Manager if additional treatment is required.

5.2. The toxicity or carcinogenicity of each reagent used in this procedure may not have 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 and procedures.

5.3. High current and voltages are exposed near the furnaces, furnace control card, and mother board even while the 440 is OFF. If non-electrical trouble shooting is desired, remove the 440 line cord from the wall receptacle.

5.4. The combustion tube is brittle since it is fused quartz. Do not put any unnecessary stress on it.

5.5. The exterior of the furnace becomes extremely hot; do not touch it or the heat shield unless wearing appropriate gloves.

5.6. Do not wear any jewelry if electrically troubleshooting. Even the low voltage points are dangerous and can injure is allowed to short circuit.
5.7. The following hazard classifications are listed for the chemicals regularly used in this procedure.

Chemical	Health	Flammability	Reactivity	Contact	Storage
Acetanilide	1	1	0	2	Green
Magnesium Perchlorate	1	0	3	2	Yellow
Ascarite	3	0	2	4	White Stripe
Silver vanadate on Chromosorb	3	0	0	3	White
Silver oxide/Silver tungstate on Chromosorb	3	0	0	3	White
Silver tungstate/Magnesium oxide on Chromosorb	3	0	0	3	White
Copper wire	0	0	0	1	Green
On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)					
STORAGE Red - Flammability Hazard. Store in a flammable liquid storage area. Blue - Health Hazard. Store in a secure poison area. Yellow - Reactivity Hazard. Keep separate from flammable and combustible materials. White - Contact Hazard. Store in a corrosion-proof area. Green - Use general chemical storage (On older labels, this category was orange). Striped - Incompatible materials of the same color class have striped labels. These Products should not be stored adjacent to substances with the same color label. Proper storage must be individually determined.					

6. EQUIPMENT AND SUPPLIES

6.1. An elemental analyzer capable of maintaining a combustion temperature of 975°C and analyzing particulate and sediment samples for elemental carbon and nitrogen. The Exeter Model 440 is used in this laboratory.
6.2. A gravity convection drying oven, capable of maintaining 47^{o}C ± 2°C for extended periods of time.
6.3. Muffle furnace, capable of maintaining 875°C +/- 15^{o}C.
6.4. Ultra-micro balance that is capable of accurately weighing to 0.1 ug.
6.5. Vacuum pump or source capable of maintaining up to 10 in. Hg of vacuum.
6.6. Freezer, capable of maintaining -20^{o}C± 5°C.
6.7. 25-mm vacuum filter apparatus made up of a glass filter tower, fritted glass disk base and 2-L vacuum flask.
6.8. Flat blade forceps.
6.9. Labware - All reusable labware (glass, quartz, polyethylene, PTFE, FEP, etc.) must be sufficiently clean for the task objectives. Clean glassware by rinsing with deionized water; soaking for 4 hours or more in 10% (v/v) HCl and then rinsing with deionized water. Store clean. All traces of organic material must be removed to prevent carbon and nitrogen contamination.

7. REAGENTS AND STANDARDS

7.1. **Purity of Water** – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to ASTM Specification D 1193, Type I.

7.2. **Purity of Reagents** – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.3. **Acetanilide, 99.9% + purity**, C_8H_9NO (CASRN 103-84-4) - Primary standard

7.4. **Blanks** – Three blanks are used for the analysis. Two blanks are instrument related. The instrument zero response (ZN) is the background response of the instrument without sample holding devices such as capsules and sleeves. The instrument blank response (BN) is the response of the instrument when the sample capsule, sleeve and ladle are inserted for analysis without standard or sample. The BN is also the laboratory reagent blank (LRB) for standards and sediment or other weighed samples. The LRB for water samples includes the sleeve, ladle and a precombusted filter without standard or sample. These blanks are subtracted from the uncorrected instrument response used to calculate concentration. The third blank is the laboratory fortified blank (LFB.) For sediment or other weighed sample analysis, a weighed amount of acetanilide or other standard is placed in an aluminum capsule and analyzed. For aqueous samples, a weighed amount of acetanilide or other standard is placed on a glass fiber filter the same size as used for sample filtration, and analyzed.

7.5. **Quality Control Sample (QCS)** – For this procedure, the QCS can be any assayed and certified sediment or particulate sample which is obtained from an external source. BCSS-1 from the National Research Council of Canada is used by this laboratory.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1. **Water Sample Collection** – Samples collected for PNC analysis from fresh, estuarine and coastal waters are normally collected from a boat or pier using one of two methods; hydrocast or submersible pump systems. Follow the recommended sampling protocols associated with the method used. Whenever possible, immediately filter the samples as described in Section 11.1.1. Store the filtered sample in an aluminum foil pouch and freeze at -20°C or store in a low temperature (47°C) drying oven after drying at 47°C ± 2°C, until use. If storage of the unfiltered water sample is necessary, place the sample into a clean bottle and store at 4°C until filtration is performed. Dry samples in a low temperature (47°C+/-2°C) drying oven prior to analysis.

8.2. The volume of water sample collected will vary with the type of sample being analyzed. Table 1, see 8.3.2., provides a guide for a number of matrices of interest. If the matrix cannot be classified by this guide, collect 1 L of water from each site.

8.3. Sediment, Tissue, or Soil Sample Collection – Sediment samples are collected with benthic samplers. The type of sampler used will depend on the type of sample

needed by the data-quality objectives. Tissue and soil samples are collected by a variety on methods. Store the wet sample in a clean jar and freeze at -20°C until ready for analysis. Dry samples in a low temperature (47°C+/-2°C)) drying oven, and grind to a homogenous powder with a mortar and pestle, prior to analysis.

8.3.1. The amount of solid material collected will depend on the sample matrix. A minimum of 1 g is recommended.

8.3.2. Filtration Volume Selection Guide

Sample Matrix	25mm Filter
Open Ocean	500 – 1000 ml
Coastal	400 – 500 ml
Estuarine (Low particulate)	250 – 400 ml
Estuarine (High Particulate)	25 – 200 ml

9. QUALITY CONTROL

9.1. The laboratory 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 continued analysis of laboratory instrument blanks field duplicates, and calibration standards analyzed as samples as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2. **Initial Demonstration of Capability**

9.2.1. **The initial demonstration of capability (DOC**) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.

9.2.2. **Quality Control Sample (QCS/SRM)** – When using this procedure, a quality control sample is required to be analyzed at the beginning or middle and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 10% of the certified values, performance of the determinative

step of the method is unacceptable. The source of the problem must be identified and corrected before proceeding with the initial determination of MDLs.

9.2.3. **Method Detection Limits (MDLs)** – MDLs should be established for PC and PN using a low level estuarine water sample, typically three to five times higher than the estimated MDL. The same procedure should be followed for sediments or other weighed samples. To determine the MDL values, analyze seven replicate aliquots of water or sediment and process through the entire analytical procedure. Perform all calculations defined in the procedure (Section 12) and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = 3 \times S$$

Where, S = Standard deviation of the replicate analyses.

9.2.4. MDLs should be determined annually, whenever there is a significant change in instrumental response, change of operator, or a new matrix is encountered.

9.3. **Assessing Laboratory Performance**

9.3.1. **Laboratory Reagent Blank (LRB)** – The laboratory must analyze at least one LRB (Section 3.40) with each batch of samples. For sediment samples the LRB consists of the ladle, sample sleeve and sample capsule, as there are no reagents involved in this procedure. For aqueous samples the LRB is a precombusted filter of the same type and size used for samples. LRB data are used to assess contamination from the laboratory environment. For sediment samples, the blank value for carbon should not exceed 150 uv and the blank value for nitrogen should not exceed 50 uv. For aqueous samples, the blank value for carbon should not exceed 375 uv and the blank value for nitrogen should not exceed 50 uv.

9.3.1.1. If the nitrogen blank during a BLANK analysis is in excess of 2000% the nitrogen blank in memory the "COPPER APPEARS SPENT" is printed. If the nitrogen blank increased over 100 uv over BN in memory and the first STANDARD KC/KN is more than any following STANDARD KC/KN by 0.2 uv/ug, then a "COPPER APPEARS SPENT" warning will be printed either during a BLANK analysis or a STANDARD analysis.

9.3.2. **Quality Control Sample (QCS)/ Standard Reference Material (SRM)** - When using this procedure, a quality control sample is required to be analyzed at the beginning and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 3σ of the certified 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 the analyses. Corrective action documentation is required for all data outside ± 3σ.

The sample weight of the SRM should mirror that of the unknown samples (~10 mg).

9.3.3. The laboratory must use QCS analyses data to assess laboratory performance against the required accuracy control limits of ± 3σ. The QCS will be obtained from a source external to the laboratory and different from the source of calibration standards. The standard deviation data should be used to establish an on-going precision statement for the level of concentrations included in the QCS. This data must be kept on file and be available for review. Values for QCSs should be plotted with the other control data.

9.4. **Assessing Analyte Recovery**

9.4.1. Percent recoveries cannot be readily obtained from particulate samples. Consequently, accuracy can only be assessed by analyzing check standards as samples and quality control samples (QCS).

9.5. **Data Assessment and Acceptance Criteria for Quality Control Measures**

INDICATOR	ACCEPTANCE LIMITS	ACTION
K-factor	KC = 18 to 25 +/- 3σ 18 to 25 μv/μg is manufacturers recommended limits. KN = 7 to 10 μv/μg 7 to 10 μv/μ is manufactures recommended limits.	The k-factors must be within the specified limits or the standard must be reanalyzed. (see 10.3)
System Blank	BC < 150 μv BN < 50 μv	If the blank value is greater the acceptable value, replace the capsules and rerun the blanks.
External QC (QCS) start or middle and end of run cycle	± 3σ	Qualify data if not within acceptance limits. Rejection criteria for batch.
Standard Reference Material (SRM) (when required by data user)	± 3σ	If SRM is outside acceptance limits, qualify the data for all samples back to last acceptable SRM or QCS.
Duplicate analysis (when available)	± 50%	Duplicate sample data must be within ± 50% or be qualified. All duplicates for this procedure are field duplicates and are more a measure of field collection and filtration techniques.

9.6. **Corrective Actions for Out-Of-Control Data**

9.6.1. All samples must be qualified when external QC samples are out of control.

9.6.2. All samples between QCSs that are out of control must be qualified.

9.6.3. All problems with analytical runs must be documented on the bench sheet.

9.7. **General Operation**

9.7.1. To assure optimal operation and analytical results, it is advisable to track the stability of the instrument. Of primary importance is the precision and repeatability of standard and blank values during the

course of a day of operation. Thus, a standard (as an unknown) should be inserted approximately every twenty runs. Try to use different standards for QA in order to assure the validity of the calibration values over the entire operating range of the instrument.

10. CALIBRATION, STANDARDIZATION and CALCULATIONS

10.1.1. Calibration - Daily calibration procedures must be performed and evaluated before sample analysis may begin. Single point calibration is used with the Exeter Model 440 Analyzer.

10.1.2. Establish single calibration factors (K) for each element (carbon, hydrogen, and nitrogen) by analyzing three weighed portions of calibration standard (acetanilide). The mass of the calibration standard should provide a response within 20% of the response expected for the samples being analyzed. Calculate the (K) for each element using the following formula:

$$K - factor\,(\mu v / \mu g) = \frac{RN - ZN - BN}{M(T)}$$

Where: RN = Instrument response to standard (μv)
ZN = Instrument zero response (μv)
BN = Instrument blank response (μv)
M = Mass of standard matter in μg
T = Theoretical % C, N, or H in the standard. For acetanilide %C = 71.09, %N = 10.36 and %H = 6.71.

10.2. The detector generates a signal directly proportional to the compound of interest in the sample. The following formula is used to calculate carbon, nitrogen and hydrogen concentrations in unknown samples.

$$\% = \frac{1}{K} X \frac{1}{W} X \left(R - Z - B\right) X\ 100$$

Where

K = calibration factor for the 440 instrument
W = sample weight
R = read signal of sample gas
Z = zero reading or base line of instrument
B = blank signal generated by instrument itself, including ladle and capsules

All the calculations are done by the PC computer but the operator decides which values to enter into the computer memory.

10.3. The K-factor is established by running samples of a known standard. The default value is for acetanilide, which we will use for our standard:

Acetanilide	C = 71.09%	H = 6.71%	N = 10.36%

If another standard is used, the values will need to be entered into the computer using The Edit Standards function in the Customizing Menu.

10.3.1. Once the blank values have been established and entered into memory, proceed to run known standards to arrive at the calibration factors for carbon and nitrogen for the instrument.

10.3.2. Run a minimum of three standards, average the results, and enter into computer memory, or use the automatic enter mode. During the run, standards may be entered as samples to verify the K-factors and blanks.

10.3.3. Any time a STD1 is entered as sample ID the computer calculates and enters a new set of operating Ks based on a weighted formula using the last three sets of Ks in memory. This occurs only if all three Ks fall within the following windows:

New KC = KC in memory ± 1.0

KN = KN in memory ± 0.5

10.3.3.1. It is important that the Ks in memory be close to expected values or new Ks generated will not be within the window and therefore will not be accepted for automatic insertion.

10.3.3.2. The weighted formula for calculating the Ks:

$$K = k^1 + \left(0.5 \times k^2\right) + \frac{\left(0.25 \times k^3\right)}{1.75}$$

where:

k^1 = k found in this run

k^2 = Next k in memory

k^3 = Last k in memory

10.4. **Conditioner** - Before running any samples or blanks, it is necessary to run one or more conditioners. The purpose of the conditioner runs is to coat the walls of the system surfaces, especially the mixing and sample volume, with water vapor, carbon dioxide and nitrogen which simulates actual sample running conditions. To simulate this condition as closely as possible, it is advisable to use conditioners of approximately the same weight as the samples to the run.

10.5. **Blanks** - The blank values used in the calculation is the total signal generated by the system including the ladle and sample capsule. This blank should always be run immediately after a weighed conditioner to represent a true blank of the instrument. Never use the blank value after an empty run since the system dries up and the blank value would be lower than normal.

10.5.1. The blanks will only be accepted if they fall within the following:

New BC < 500

New BN < 250

10.6. **K-Factors** - Once the blank values have been established and entered into memory, proceed to run known standards in order to establish the calibration factors for carbon, hydrogen and nitrogen. Always run a conditioner before a standard. The computer will calculate K-factors as long as STD# has been entered as the sample ID. Run a minimum of three (3) standards, average the results, and enter into the computer memory, or use the automatic enter mode. The instrument is now ready for running samples. Standards should be analyzed as unknowns during each run to verify the K-factors and blank values.

11. PROCEDURE

11.1. Aqueous Sample Preparation

11.1.1. Water Sample Filtration

Precombust 25-mm GF/F glass fiber filters at 500°C for 1.5 hours. Store filters covered, if not immediately used. Place a precombusted filter on a fritted filter base of the filtration apparatus and attach the filtration tower. Thoroughly shake the sample container to suspend the particulate matter. Measure and record the required sample volume using a graduated cylinder. Pour the measured sample into the filtration tower. Filter the sample using a vacuum no greater than 10 in. of Hg. Vacuum levels greater than 10 in. of Hg can cause filter rupture. Do not rinse the filter following filtration. It has been demonstrated that sample loss occurs when the filter is rinsed with an isotonic solution or the filtrate. Air dry the filter after the sample has passed through by continuing the vacuum for 30 sec. Using flat-tipped forceps, fold the filters in half while still on the fritted glass base of the filter apparatus. Store filters as described in Section 8.1.

11.1.2. If the sample has been stored frozen in foil pouches, place in a drying oven at $47^{o}C \pm 2°C$ for 24 hours before analysis. Slightly open the pouch to allow drying. When ready to analyze, fold, and insert the filter into a precombusted nickel sleeve using forceps. Tap the filter pad down into the nickel sleeve using a stainless steel rod. The sample is ready for analysis.

11.2. Sample Analysis

11.2.1. As the filters are packed into the nickel sleeves they are placed into the 64 position sample wheel. The calibration series must be placed at the beginning of the batch. The sample schedule consists of a conditioner, a blank, a conditioner and three standards. ACS grade acetanilide must be used to calibrate the instrument.

11.2.2. Set up the sample tray in the following manner (used for aqueous samples):

Position #	Contents	Notes	Schedule Entry	Weight
1	Capsule + sleeve	Blank	Blank	0
2	Conditioner	Acetanilide (1500-2500 µg)	Conditioner	Weight of Acetanilide
3	Capsule + sleeve	Blank	Blank	0
4	Conditioner	Acetanilide (1500-2500 µg)	Conditioner	Weight of Acetanilide
5	Standard	Acetanilide (1500-2500 µg)	STD1[a]	Weight of Acetanilide
6	Standard	Acetanilide (1500-2500 µg)	STD1	Weight of Acetanilide
7	Standard	Acetanilide (1500-2500 µg)	STD1	Weight of Acetanilide
8	Sleeve + filter pad	Filter Blank	LRB	0
9	Sleeve + filter pad	Filter Blank	LRB	0
10-31	Samples			Volume filtered/10
32	Sleeve + filter pad	Atropine (1500-	LFB	Weight of

	+ standard	2500ug)		Atropine
33-61	Samples			Volume filtered/10
62	Capsule + Sleeve	Blank	Blank	0
63	Sleeve + filter pad + standard	Atropine (1500-2500ug)	LFB	Weight of Atropine
64	Capsule + Sleeve	Blank	Blank	0

[a] Always use STD1 in the Standard position. The system recognizes this as acetanilide and makes the appropriate calculations for the K factor.

11.2.3. By entering volume filtered/10 for the weight of the aqueous filtered samples, results are printed out which represent micrograms of carbon or nitrogen per liter. This corresponds directly to the known amount of liquid that has passed though the filter. The maximum sample capacity per run is approximately 4,000 to 5,000 micrograms of carbon on the filter pad. Filters containing more than that amount can be cut in half and analyzed separately and the results added.

11.2.4. Filter Preparation for Analysis

11.2.4.1. Work on a clean, non-contaminating surface.

11.2.4.2. Using two pairs of clean forceps, fold the filter in half so that the exposed surface is inside. Continue folding the filter in half until you have a compact package.

11.2.4.3. Place a pre-combusted 7 x 5 mm nickel sleeve into the filter loading die, which functions as a holding device. Use the clean 4 mm loading plunger to force the compressed filter through the clean loading funnel and into the nickel sleeve.

11.2.4.4. Make sure no excess filter protrudes above the lip of the sleeve.

11.2.4.5. Place loaded sleeve in the 64-sample wheel.

11.2.5. Determination of Particulate Organic and Inorganic Carbon

11.2.5.1. Thermal Partitioning is the method used. The difference found between replicate samples, one part of which has been analyzed for total PC and PN and the other which was muffled at 550^{o}C for three hours to drive off organic compounds, and analyzed is the particulate organic component of that sample. This method of thermally partitioning organic and inorganic PC may underestimate slightly the carbonate minerals' contribution in the inorganic fraction since some carbonate minerals decompose below 500^{o}C, although $CaCO_3$ does not. This method is used for filtered samples where at least two filters per sample must be supplied. For sediment samples at least 1 g of sample is required and at least 0.5g of sample is weighed into a crucible of known weight. The weight is recorded. The crucible is then muffled as above, and weighed again. The percent remaining of the ash is calculated and multiplied times the %C in the ash which is then determined by the 440.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Raw results for each run are printed by the dot matrix printer attached to the instrument. These data are then manually entered into a LOTUS 123 spreadsheet. Results are reported in mg/l for aqueous samples, and in % for sediment or other weighed samples, standards and SRMs or QCSs.

12.2. Recalculation of data (if necessary)

12.2.1. The software gives the analyst the opportunity to recalculate values generated by the run. This option can be useful for adjusting the values of the data due to explained or unexpected changes in the blank or calibration (K) factor during an analytical run cycle. Blanks can change due to sample handling, different capsules or sleeves, small leaks in the system and contamination. K factors should remain stable but can drift due to flow changes caused by variable pressure drops in the traps or helium scrubber, or by changing delivery pressure at the helium regulator.

12.2.2. Before the analyst can change calibration values and recalculate the results, there must be a valid reason. When data is recalculated, always document the incident.

12.3. Example of LOTUS spreadsheet of results:

	A	B	B	C	D	E
1	2/29,3/3/08					
2	Jane Doe					
3	DNR MAINSTEM SPLITS					
4	2/08					
5	SAMPLE	MG N/L	MG C/L			
6	56	0.1440	0.9520			
7	57	0.1510	0.9980			
8	58	0.1440	0.9460			
9	59	0.1430	0.9260			
10	BCSS1, 2/29	0.20	2.09	%		
11	BCSS1, 3/3	0.20	2.14	%		
12	LAB DUPS	PN		PC		
13	SAMPLE	DUP 1	DUP 2	DUP 1	DUP 2	
14	56	0.1430	0.1460	0.9360	0.9670	
15	58	0.1430	0.1450	0.9470	0.9450	
17	BLANKS N= 16		K VALUE N= 7.493			
18	C= 140		C= 20.771			
19	BLANKS N= 15		K VALUE N= 7.393			
20	C= 126		C= 20.352			
21	ATROPINE 2/29 N= 4.85 %					
22	ATROPINE 2/29 C= 70.23 %					
23	ATROPINE 3/3 N= 4.90 %					

24 ATROPINE 3/3 C= 70.35 %

12.3.1. Cell 1A - Analysis date
12.3.2. Cell 2A – Analyst's name
12.3.3. Cell 3A – Sample source or client
12.3.4. Cell 4A – Sample date
12.3.5. Cell 5A – Column heading for Sample
12.3.6. Cell 5B – Column heading for N concentration
12.3.7. Cell 5C - Column heading for C concentration.
12.3.8. Cells 5A to 11D – Sample Results table.
12.3.9. Cells 10 D and 11 D - % to indicate that BCSS-1 is reported in %N or C
12.1.10. Cells 12A to 15E – QC table for field duplicates. The mean of these values is reported in the sample results table.
12.1.11. Cells 17A to 20F – Instrument values for the Blanks, and Ks.
12.1.12. Cells 21A to 24B- Values for LRB (atropine) for each day of analyses and middle and end of analytical run.

12.2. Sample data should be reported in units of mg/L as carbon or nitrogen for aqueous samples, and as percent carbon or nitrogen for sediment samples.

12.3. Report analyte concentrations to three significant figures for both aqueous and sediment samples.

12.4. For aqueous samples, calculate the sample concentration using the following formula:

$$Concentration\,(mg/L) = \frac{Corrected\ sample\ response\,(\mu g/L)}{1000 mls/L}$$

12.5. For sediment samples, % N or %C are already calculated by the instrument software.

13. METHOD PERFORMANCE

13.1. The procedure validation MDL, based on seven filtrations of a sample, was found to be 0.0633 mg/L for carbon and 0.0105 mg/L for nitrogen.

13.1. Twenty analyses of the BCSS-1 Marine Sediment QC, from 7/2007 to 3/2008, produced an average value of 2.13 +/- 0.4% C. The true value for the QC is 2.19 +/- 0.09% C. The true value for %N is not given, but the value obtained by our procedure was 0.194 +/- 0.008%N.

13.2. Forty analyses of the LRB (acetanilide), from 7/2007 to 3/2008, produced the following values for carbon and nitrogen: The true value for carbon in acetanilide is 71.09%. The average value over the time period was 70.35% ± 0.70%. The true value for nitrogen in acetanilide is 10.36%. The average value over the time period was 10.31% ± 0.10%.

13.3. Atropine became the standard used for LRB analyses as of 3/15/08. The true value for carbon is 70.56%. The average value form 3/15-4/7/08 was 70.14 ± 0.42%. The true value for nitrogen in atropine is 4.84%. The average value for the period was 4.92 ± 0.03%.

14. POLLUTION PREVENTION

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

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

15. WASTE MANAGEMENT

15.1. The reagents used in this procedure are minimal and are not hazardous with the exception of the Ascarite and magnesium perchlorate. Due to the small quantity of Ascarite and magnesium perchlorate used, the spent reagent can be flushed down the drain with running water.

15.2. For further information on waste management consult The Waste Management Manual for Laboratory Personnel, available from the American Chemical Society.

16. REFERENCES

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17. DETAILED PROCEDURE

17.1. Exeter 440 Operation

17.1.1. The following sequence should be followed when initially starting up the system or when restarting after a shutdown.

17.1.1.1. Make sure the power switches on the computer and on the CEC- 490 (Interface) are off.

17.1.1.2. Remove the 440 cover from instrument.

17.1.1.3. Check that the helium regulator is set at 18 psig and oxygen at 20 psig and open the in-line gas valves.

17.1.1.4. If restarting, check that the combustion and reduction tubes, scrubber and traps are not exhausted.

17.1.1.5. Turn the selector switch to SYSTEM. Turn on the CEC-490 and the computer. The monitor will now display the Menu. If this is a cold re-start, set combustion and reduction furnace temperature controls to values previously established. Wait until the reduction furnace has reached operating temperature. DO-NOT PUSH DETECTOR RESET BUTTON AT THIS TIME!

17.1.1.6. With the combustion to reduction tube end connector removed, go to "Tube Replacement" in the Service Menu, then follow the directions under "Combustion Tube Replacement" to purge the helium and oxygen regulators twice. This will also serve the purpose of conditioning the reduction and combustion tubes. Then go to Main Menu and install the end connectors.

17.1.1.7. After allowing the 440 oven to reach operating temperature (about one hour) go to the Service Menu and select Calibrate CEC-490. Calibrate all and follow instructions.

17.1.1.8. Run 2 to 3 blank runs to establish a fill time of about 20 to 40 seconds. If the fill time has been exceeded, increase the helium pressure by ½ psig, and repeat running until fill time is achieved. If the system still aborts after the helium pressure has been increased to 22 psig, go through the leak test mode.

17.1.1.9. After the first complete run, push DET RESET. High concentrations of air or oxygen in the analytical system will damage the filaments in the detectors if power is applied. To protect the detectors, a detector safety circuit is provided which shuts off power when the helium carrier gas becomes contaminated with air or oxygen at levels generating an imbalance of about 450 uv or higher. The safety circuit will

activate should leaks develop or when the helium supply is depleted. The safety circuit monitors the gross imbalance between the two sides of the nitrogen bridge. If air or oxygen is present on both sides of the bridge, the safety circuit may not activate and damage to the detectors may occur.

Make certain that helium gas is flowing and that the instrument is purged before pressing the DETECTOR RESET button.

The safety circuit is also activated when accidental or deliberate power interruption occurs. If power has been interrupted for more than 5 minutes, do not push DETECTOR RESET until the system has been run as if to run a blank. Do not hold the DETECTOR RESET button in or more than one second. If the light stays on when the button is released, further running is necessary before pushing the button again. Go through one blank run before turning on the detector.

17.1.1.10. After the last run go to the Service Menu and monitor the bridge readings. Adjust the "zero" reading to approximately 2500 μv by turning the respective potentiometers on the Bridge Balance Card located in the left rear corner of the "Motherboard". Typically the bridges should be set well above negative or zero to approximately + 2500 μv. This is after the instrument has stabilized. Stability is based on furnace and oven temperatures being steady for a period of not less than 1 hour.

17.1.1.11. Check the furnace and oven temperatures. If these have reached operating levels, let the instrument go through another three sets of runs in order to purge the system and condition the reagents. This can be done through the CHN Run Mode (Run Menu).

17.1.1.12. Turn off the B-valve using the Parameters mode in the Customize pull-down menu. Continue running helium blanks until the base line (zero reading) is steady and/or until the blank for nitrogen and carbon is less than 200 μv, and for hydrogen less than 1500 μv.

17.1.1.13. Turn ON the B-valve and run oxygen blanks until consecutive runs agree within 10 μv for nitrogen and carbon, and 50 μv for hydrogen.

17.1.1.14. Go to the Service pull down Menu and calibrate all of the CEC-490 again.

17.1.1.15. The instrument is now ready for system calibration with known standards.

17.1.2. Standby Mode - To reduce helium consumption and minimize wear on the terminal screen, the overnight or short term standby mode is used.

17.1.2.1. Select the overnight standby mode (in the Run pull-down menu).

17.1.1.1. Return to normal operation.

17.1.1.1.1. Select Stop Overnight Standby in the Run pull-down menu

17.1.2. Powering Down - It is preferable for the system to remain powered up at all times since this will extend the life time of the glassware, reagents, and electronics. However, helium and power will be consumed during this standby and it might be necessary to power down the 440 instrument. To assure minimum disruption for a future start up after a power down, proceed as follows:

17.1.2.1. Turn the furnace temperature controllers to zero.

17.1.2.2. Allow several hours for the furnace temperatures to drop below 100°C.

17.1.2.3. Turn off the power to the instrument as well as gas valves between the instrument and the regulators.

17.1.2.4. Turn off the gas on the cylinder.

17.2. 440 Software Summary

17.2.1. Run Pull-Down Menu

17.2.1.1. Carbon, Hydrogen, Nitrogen Run

17.2.1.2. Oxygen

17.2.1.3. Sulfur

17.2.1.4. Overnight Standby (save carrier gas)

17.2.1.5. Change Blanks and Ks

17.2.1.6. Balance Interface Weight Entry

17.2.2. Service Pull-Down Menu

17.2.2.1. Datalog Signals

17.2.2.2. Leak Test

17.2.2.3. Profiles

17.2.2.4. Tube Replacement (Includes packing and installing)

17.2.2.5. Valve Rebuild

17.2.2.6. Maintenance Schedule

17.2.2.7. Maintenance Log

17.2.2.8. Bridges

17.2.2.9. Test Injector Drive

17.2.2.10. Calibrate CEC-490

17.2.2.11. Diagnostics

17.2.2.12. Balance Interface Test

17.2.3. Calculate Pull-Down Menu (Manipulating existing data)

17.2.3.1. Recalculate data and statistics

17.2.3.2. BTU/lb.

17.2.3.3. Dry, Dry Ash Free

17.2.3.4.H/C, N/C, C/H, C/N Ratio
17.2.3.5.C/C, H/H, N/N, O/O, S/S Ratio
17.2.3.6.Empirical Formula

17.2.4. Customize Pull-Down Menu (Customizing software)
17.2.4.1.Set parameters
17.2.4.2.Users
17.2.4.3.Edit Standards (names, weights, percents)
17.2.4.4.Create Report Format
17.2.4.5.Change Infinite Run Counter
17.2.4.6.Set Automation Type

17.2.5. Help

17.3. Run Pull-Down Menu

17.3.1. Select "Carbon, Hydrogen, Nitrogen Run"

17.3.2. Select "Yes" for a new run

17.3.3. Enter message for this run series
17.3.3.1.Check "Enter the Ks and Blanks automatically".
17.3.3.2.Enter date followed by AM or PM as appropriate
17.3.3.3.Press "Enter Data"

17.3.4. Sample Entry Screen
17.3.4.1.Enter Weight (µg)
17.3.4.1.1. When entering the weight of the sample press [ENTER] to use the present weight or enter a new weight. If a weight of zero [0] is entered then the ID is assumed to be a blank. If a weight of 100 has been entered the results will be reported in micrograms (µg). When analyzing aqueous samples, enter the volume filtered/10 as the weight. The results will be reported in ug/l. When analyzing sediment samples or weighed QC samples, enter the weight in ug. The result will be reported in %.
17.3.4.2.Enter Sample ID
17.3.4.2.1. Enter the sample ID as either STD1, blank, or any other text. If STD is entered as the first three letters, then Ks will be calculated on the result report. If blank is entered, then blanks will be calculated. If a weight of 100 has been entered, the results will be reported in micrograms (µg). If a "weight" of volume filtered/10 has been entered, the results will be in ug/l. If a weight of ug has been entered, the result will be reported in %.
17.3.4.3.Worksheet

Position #	Sample ID	Weight or volume/10	Comment, Sample Date or Client
1	Capsule+sleeve		

2	Conditioner		
3	Capsule+sleeve		
4	Conditioner		
5	STD1		
6	STD1		
7	STD1		
8	Capsule+Filter		
9	Capsule+Filter		
10	FD1		
11	FD2		
12			
13			
14			
15			
16			
17			
18			
19			
20			
21	FD1		
22	FD2		
23			
24			
25			
26			
27			
28			
29			
30			
31			
32	LFB Atropine		
33			
34			
35			
36			
37			
38			
39			
40			
41			
42	FD1		
43	FD2		
44			
45			
46			
47			
48			
49			
50			
51			
52			
53	FD1		
54	FD2		

55			
56			
57			
58			
59			
60			
61			
62	Capsule+sleeve		
63	LFB Atropine		
64	Capsule+sleeve		

17.3.4.4. Press "Start Run"

17.3.4.5. Loading the Sample Wheel into the Injector Box

This mode opens the ADF and C valves allowing helium to enter the injection box and minimize air in this area while installing the sample wheel for the 64 sample automatic injector. The pressure will build up and eventually equilibrate to the helium tank pressure if the instrument is left in this mode for a long period of time. This is not recommended, therefore, do not delay carrying out the following steps:

17.3.4.5.1. Open the manual purge valve on the injector box (right side, behind the P valve) to relieve the internal pressure. **NOTE: The injector housing should not be opened while pressurized. Vent the housing with the manual purge valve prior to opening the lid.**

17.3.4.5.2. Loosen the 4 cover screws and lift the lid. Remove the empty wheel from the sample chamber.

17.3.4.5.3. Vacuum out, or blow out with canned air, any material that might be in the box from the previous run (Loose material from the previous batch can contaminate samples, blanks and standards).

17.3.4.5.4. Insert the loaded sample wheel with the locking pin in place. Tilt the wheel slightly, line up the scribe mark on the wheel with the ratchet in the housing, lower the wheel and make sure that it is properly seated. Place the locking pin in the center hold. Check that the o-ring of the cover is clean and well seated in the groove before closing the cover.

17.3.4.5.5. Close the cover, and tighten equally on all four screws. This should be performed in an alternating sequence to achieve a uniform seal.

Never over-tighten or use any tools on the screws.

17.3.4.5.6. Open and remove any spent capsules in the capsule receiver. Re-grease the gasket and re-install cover.

17.3.4.5.7. Close the purge valve, let pressure build up for about 30 seconds. Re-open the purge valve for about 5 seconds and then close again.

17.3.4.5.8. Select "OK" to continue operation.

17.3.4.6. The Sample Run

17.3.4.6.1. The sample is automatically injected into the combustion tube at the appropriate time. Upon completion of the fill time the ladle is retracted and allowed to cool. At the end of the run the results are printed and the soft key commands are followed if any has been selected. The screen returns to sample entry.

17.3.4.7. Run Display and Commands

Once the run begins, the screen displays the following information:

17.3.4.7.1. Run number, Sample Weight and ID., the operating K and B values, the preset combustion and purge times, valve status, and the elapsed time in minutes:seconds.

17.3.4.7.2. Temperatures and Pressure are also displayed near the bottom of the screen. These numbers may not be updated all of the time as time critical sections of a run occur. Run counters for the various tubes are displayed above the valve status diagram. The run counters will change from blue to red when they approach 10% within the thresholds set by the user.

17.3.4.7.3. During the run the analyst has various options available through the buttons at the top of the screen (accessed via simply selecting one). If a key is actuated, the button changes from grey to white. The buttons are for the following functions:

a. **Ks & Bs** - To access the Ks and Bs table at the end of the current run. This allows the operator to change the operating values.

b. **PARAMETERS** - Goes to parameters table at the end of the current run.

c. **LEAK TEST** - The leak test program is activated at the end of the run cycle.

d. **STANDBY -** At the end of the run cycle the instrument will go into overnight standby.
e. **DATALOG** -At the end of the run cycle a datalog is printed every half hour. A, D, and F valves are turned on, as in the overnight standby mode.
f. **SSI -** An HA function to activate the SSI (single sample inject) program after the completion of the current run. The HA program will automatically resume after the SSI run (unless SSI is pressed again).
g. **MENU NEXT -** Goes to the Analytical Menu at the end of the current run. The data will be stored on the data disc at that point.
h. **STOP -** Aborts the current operation and goes to the Analytical Menu. This is typically only used during emergency operations. If you exit an HA run cycle prematurely and you wish to start over or resume the HA run with the sample IDs and weights already in memory, then DO NOT exit the Analytical Menu. If you exit or reboot the Analytical Menu then the IDs and weights will be erased.
i. **NONE –** Nothing at end of run or run cycle.

17.4. Tube Replacement

17.4.1. This mode is used when one or more of the reagent tubes in the 440 need to be changed, as indicated by the maintenance schedule, poor analytical results or in the case of a cold restart.

17.4.2. Go to the Service Pull-down Menu. Select "Tube Replacement." "Select CHN Analysis." Another menu will be displayed that will contain options for tube packing information or for replacement of any tubes used for that analysis. If a new gas cylinder or regulator is to be replaced, select the appropriate tank changing from the menu.

17.4.2.1. Tube packing. By selecting the tube of interest the appropriate tube packing information is graphically displayed. In the individual tube replacement options, follow the step by step instruction shown on the screen. If the procedure is followed correctly and to its conclusion, the Maintenance Schedule Information for that tube will be

reset. You can return to the Service Menu at almost any point by pressing "End."

17.4.2.2. For the CHN Analysis there are instructions for:

17.4.2.2.1. Tube Packing Information
17.4.2.2.2. Helium Scrubber Replacement
17.4.2.2.3. Oxygen Scrubber Replacement
17.4.2.2.4. Carbon Dioxide Trap Replacement
17.4.2.2.5. Water Trap Replacement
17.4.2.2.6. Combustion Tube Replacement
17.4.2.2.7. Reduction Tube Replacement
17.4.2.2.8. Combustion & Reduction Tubes Replacement at the same time

17.4.3. Combustion Tube

17.4.3.1. Hold the tube vertically with the short end from the indentation up. Roll up a piece of platinum gauze so that it will fit snugly into the combustion tube. Slide the gauze plug into the tube and up against the indentation.

17.4.3.2. Add a small plug of quartz wool. (Quartz wool may be muffled for one hour at 850 °C to remove any residual carbon).

17.4.3.3. Add 1½" of silver tungstate/magnesium oxide on chromosorb. Gently tap the tube to prevent the reagent from channeling.

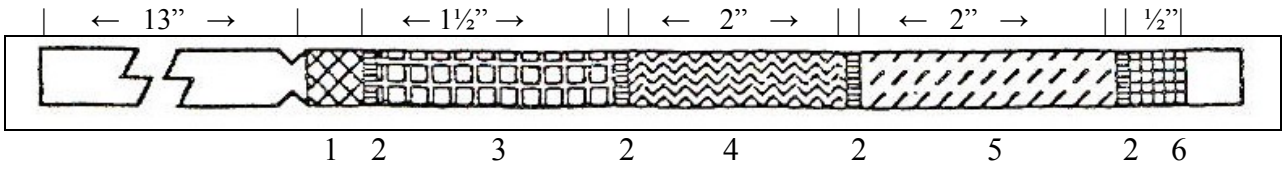
17.4.3.4. Add a small plug of quartz wool.

17.4.3.5. Add 2" of silver oxide/silver tungstate on Chromosorb tap the tube and add another small plug of quartz wool.

17.4.3.6. Slide a rolled-up piece of silver gauze into the tube and pack against the quartz wool. Make sure that there is no less than ½" of space between the end of the tube and the silver gauze since the silver gauze will conduct heat and damage the o-ring on the end connector.

17.4.3.7. The amount of each reagent used can be varied to suit the type of materials to be analyzed. For example, if predominantly fluoridated compounds are run proportionately more silver tungstate/magnesium oxide should be packed into the tube.

17.4.3.8. There is rarely such a thing as a "too tightly" packed combustion tube. Loosely packed combustion tubes can cause non-linearity.



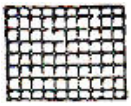
 #1 - Platinum gauze

 #2 - Quartz wool

 #3 - Silver tungstate / Magnesium oxide on Chromosorb

 #4 - Silver oxide / Silver tungstate on chromosorb

 #5 - Silver vanadate on Chromosorb

 #6 - Silver gauze

17.4.3.9.Function of Combustion Tube Packing Material

17.4.3.9.1. Silver Vanadate on Chromosorb

Reacts with and removes chlorine, bromine, iodine and sulfur contained in the combustion gases. When absorbing sulfur, it changes color from yellow to dark brown when saturated. In absorbing halogens, exhaustion of the silver vanadate is indicated by color changes on the surface of the silver gauze at the end of the combustion tube. Each element forms a distinctively colored salt deposit – silver chloride is gray, silver bromide is brown, and silver iodide is purple. The gauze can be rejuvenated by heating in the upper, reducing portion of a Bunsen burner or muffling at 550^{o}C for 90 minutes.

17.4.3.9.2. Silver Tungstate / Magnesium Oxide on chromosorb: Removes fluorine, phosphorus, and arsenic.

17.4.3.9.3. Silver Oxide / Silver Tungstate on chromosorb: Removes sulfur and halogens (except fluorine).

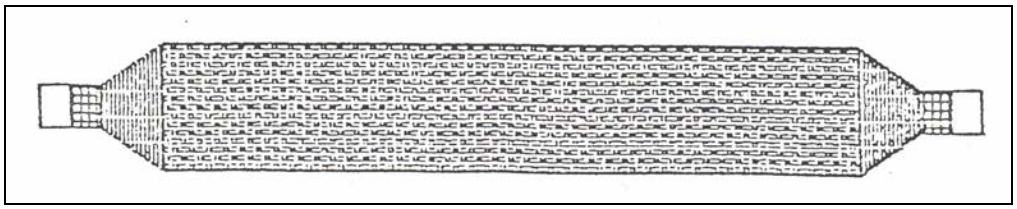
17.4.4. Reduction Tube

17.4.4.1. Pack about ¾" of quartz wool into the bottom of the tube from the opposite end.

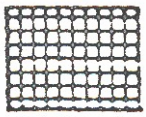
17.4.4.2. Fill the tube with copper wire while gently tapping to tightly settle the copper and avoid channeling.

17.4.4.3. Pack another plug of quartz wool into the tube against the copper.

17.4.4.4. Insert a rolled-up piece of silver gauze into each small diameter tube end.



| 1| 2 | 3 | 2 | 1|



#1 - Silver gauze

#2 - Quartz wool

#3 - Copper wire

17.4.5. Carbon Dioxide Trap and Gas Scrubbers (3)

17.4.5.1. These three tubes are identically packed even though the Scrubbers are a larger diameter. Pack a ¼" plug of quartz wool into one end of the tube.

17.4.5.2. Add 3½" Ascarite (Colorcarb) while gently tapping the tube.

17.4.5.3. Add ¼" plug of quartz wool.

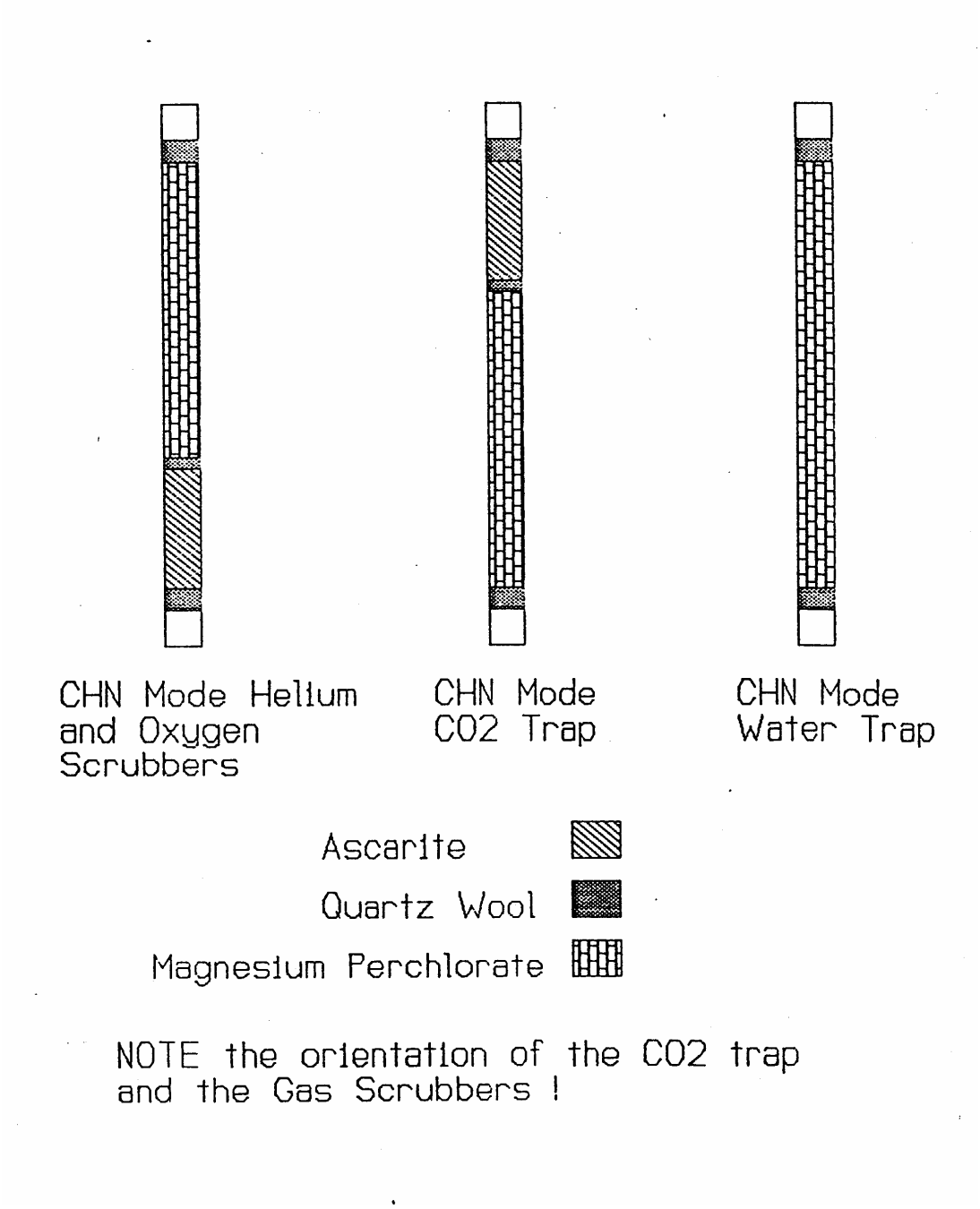
17.4.5.4. Add 1½" magnesium Perchlorate while gently tapping the tube.

17.4.5.5. Add ¼" plug of quartz wool.

17.4.5.6. There should be about ¼" of free space at each end of the tube.

17.4.5.7. Gas scrubbers should be loosely packed to allow for the high gas flows associated with the 440.

17.4.5.8. Note the orientation (in the instrument) of the helium and oxygen scrubbers versus the CO_2 scrubber. The orientation is reversed for the CO_2 scrubber.



17.4.6. Helium Scrubber Replacement

17.4.6.1. Close the inlet helium gas valve and back off the regulator valve. [HIT RETURN WHEN DONE]

17.4.6.2. At this point the helium tank can also be replaced by removing the regulator and installing a new tank.

17.4.6.3. To replace the helium scrubber carefully loosen the tube nut with a 440 tube nut wrench, loosen the wing nuts and lift the top assembly gently until the scrubber can be removed.

17.4.6.4. Repack the scrubber as described in 18.6.5.

17.4.6.5. Check the o-rings and effluent filters at this time. Make sure any quartz wool fibers, which could prevent a good seal, are removed from the outside of the scrubber before inserting.

17.4.6.6. Replace the tube, bring the top assembly down and tighten the wing nuts. Tighten the lower nut ONLY. Very carefully open the in-line valve and increase the helium gas pressure to 5 psig. [HIT RETURN WHEN DONE]

17.4.6.7. Wait one minute. A tone will sound. A clock on the screen counts down the time. When the tone sounds, the screen displays the message: "I'm finished purging the helium scrubber." [HIT RETURN TO ACKNOWLEDGE]

17.4.6.8. Tighten the top nut on the helium scrubber. Increase the pressure to normal. [HIT RETURN WHEN DONE]

17.4.6.9. Wait 5 minutes. This serves to purge the gas lines. Once the 5 minutes have passed, the Tube Replacement Menu for the chosen analysis mode will be displayed.

17.4.6.10. The instrument should be conditioned after replacing the halogen scrubber by running two blanks before proceeding to a sample run.

17.4.6.11. If the helium tank has been replaced, purge the regulator 5 times and run a helium blank profile to verify good gas.

17.4.7. Oxygen Scrubber Replacement

17.4.7.1. Close the inlet oxygen gas valve and back off the regulator valve. [HIT RETURN WHEN DONE]

17.4.7.2. At this point the oxygen tank can also be replaced by removing the regulator and installing a new tank.

17.4.7.3. To replace the oxygen scrubber carefully loosen the tube nut with a 440 tube nut wrench, loosen the wing nuts and lift the top assembly gently until the scrubber can be removed.

17.4.7.4. Repack the scrubber as described in 18.6.5.

17.4.7.5. Check the o-rings and effluent filters at this time. Make sure any quartz wool fibers, which could prevent a good seal, are removed from the outside of the scrubber before inserting.

17.4.7.6. Replace the tube, bring the top assembly down and tighten the wing nuts. Tighten the lower nut **ONLY**. Very carefully open the in-line valve and increase the oxygen gas pressure to 5 psig. [HIT RETURN WHEN DONE].

17.4.7.7. Wait one minute. A tone will sound. A clock on the screen counts down the time. When the tone sounds, the screen displays the message "I'm finished purging the oxygen scrubber. [HIT RETURN TO ACKNOWLEDGE]

17.4.7.8. Tighten the top nut on the oxygen scrubber. Increase the pressure to normal. [HIT RETURN WHEN DONE]

17.4.7.9. The instrument should be conditioned after replacing the oxygen scrubber by running two blanks before proceeding to a sample run.

17.4.7.10. The procedure for replacing the oxygen scrubber is identical to that of the helium scrubber. The only difference is the omission of the 5 minute purge.

17.4.8. Carbon Dioxide Trap Replacement

17.4.8.1. Replace the carbon dioxide trap. Tighten the lower nut only. [HIT RETURN WHEN DONE]

17.4.8.2. Be sure to orient the trap correctly, with the Ascarite portion toward the top.

17.4.8.3. Check the o-rings and re-grease lightly, also check the effluent filters at this time.

17.4.8.4. Wait 1 minute. A tone will sound. A clock counts down the time on the screen and then displays "I'm finished purging the carbon dioxide trap". [HIT RETURN TO ACKNOWLEDGE]

17.4.8.5. Tighten the top nut on the carbon dioxide trap. Increase the pressure to normal. [HIT RETURN WHEN DONE] (Ignore the instructions regarding pressure).

17.4.8.6. When completed, the Tube Replacement Menu for the CHN analysis mode will be displayed.

17.5. Important Factors for Proper 440 Operation

17.5.1. Pack the scrubber tubes loosely.

17.5.2. Vibrate or tap down the combustion tube packing chemicals while packing to assure a fairly tight tube. DO NOT over-tighten.

17.5.3. Oxygen pressure should be at ≈ 20 psig.

17.5.4. Helium pressure should be at ≈ 18 psig and the fill time (FT) for a run should be between 20 and 40 seconds.

17.5.5. When greasing o-rings or gaskets, it is recommended to use Krytox (R) by Dupont.

17.5.6. The furnace temperatures reach set temperature very quickly. Do not set the furnaces to anything but the temperature for analysis.

17.5.7. Never set the combustion temperature above 1100 °C.

17.5.8. Never set the reduction temperature above 900 °C.

17.5.9. All valves are “Normally Closed” type.

Standard Operating Procedures

Chlorophyll a
Standard Method 10200H (Spectrophometric, Shimadzu UV-2401PC)

1.0 SCOPE AND APPLICATION

1.1 This method is applicable to the determination of chlorophyll *a*, *b*, *c* and pheophytin a in fresh and marine waters.

1.2 The concentrations are reported in SI units of mg/m^3.

2.0 SUMMARY OF METHOD

The chlorophyll and related compounds are extracted from the algae collected on glass fiber filters with aqueous 90% acetone solution. Light absorption of the extract is measured at selected wavelengths and the concentrations of the pigments of interest are calculated using the equations as in 10.0.

3.0 INTERFERENCES

3.1 Pheophytin *a* is a common degradation product of chlorophyll *a*. Pheophytin *a* is similar in structure to chlorophyll *a*, but lacks the magnesium atom (Mg) in the porphyrin ring. The magnesium can be removed from chlorophyll in the presence of acid.

3.1.1 Field Samplers can prevent this degradation by the addition of magnesium carbonate to the plankton sample prior to filtration.

3.1.2 When a solution of pure chlorophyll *a* is converted to pheophytin *a* by acidification, the absorption peak is reduced to approximately 60% of its original value and shifts from 664 to 665 nm. For pure chlorophyll this before/after acidification absorption peak ratio (OD_{664}/OD_{665}) is 1.7. Solutions of pure pheophytin show no reduction at OD_{665} upon acidification and have 664/665 ratio of 1.0. The *acid rati*o should fall between 1.0 and 1.7. If it is not within this range, the data are not valid and will be discarded. Sample submitter is immediately notified if more than 10% of the data will be rejected.

$$\frac{(OD_{664} - OD_{750})b}{(OD_{665} - OD_{750})a} = \text{acid ratio}$$

b = before acidification
a = after acidification

3.2 Chlorophyll solutions degrade rapidly in strong light. Work with these solutions should be carried out in subdued light, and all vessels, tubes, etc. containing the pigment should be covered with aluminum foil.

3.2.1 Naturally occurring, structurally related chlorophyll precursors and degradation products, such as the chlorophyllides, pheophytins and pheophorbides, commonly occur in pigment extracts and may absorb light in the same region of the spectrum as the chlorophylls.

3.2.2 Ground samples should be covered by aluminum foil and steeped in the refrigerator not less than 15 minutes or more than 24 hours. After clarification decant the extract directly into the cuvette for analysis or a screw cap tube and put in the freezer. The extract can be stored for one year.

4.0 HEALTH AND SAFETY

4.1 Good laboratory practices should be followed during reagent preparation and instrument operation. Use of gloves and eye protection is recommended when preparing solutions.

4.2 Each chemical should be regarded as a potential health hazard. A reference file of MSDS is available in lab.

4.3 Inhalation of acetone should be minimized by performing all operations in a well-ventilated hood.

5.0 EQUIPMENT AND SUPPLIES

5.1 Equipment

5.1.1 Shimadzu UV-2401PC spectrophotometer, Beckman Instruments Inc.

5.1.1 Tissue Grinder – Con-Torque power-unit, Cat. # 7265, Eberbach Corp.

5.1.2 Centrifuge – Megafuge 2.0, Heraeus Instrument.

5.1.3 Magnetic Stirrer

5.2 Supplies

5.2.1 Centrifuge tubes – 15 ml graduated conical polypropylene tubes with screw caps, Catalog No. 05-538-43D, Fisher Scientific.

5.2.2 Cuvettes – Quartz, 1 cm and 5 cm path, catalog no. 104-OS 10mm and 104-OS 10mm, Hellma USA.

5.2.3 Repipet Dispensers – 5 and 10 ml volume, Fisher catalog no. 13-687-54 and 13-687-55.

5.2.4 Transfer Pipets – Disposable Polyethylene, Fisher Catalog no. 13-711-9AM

5.2.5 Cuvette Stirrers – Fisher catalog no. 14-386-22

6.0 REAGENTS AND STANDARDS

6.1 Acetone –Spectranalyzed, catalog no. A19-4, Fisher Scientific

6.2 Acetone solution, 90% – Add 900 ml of acetone to 100 ml of distilled water and mix well. The final volume will be less than 1 liter.

6.3 Hydrochloric Acid, 1N – Certified, catalog no. SA48-4, Fisher Scientific

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.1 Samples are collected and filtered in the field. Filters sealed in aluminum foil are cooled to – 4^{o}C on ice and transported to the laboratory by collection staff or courier service.

7.2 All samples must be delivered to the laboratory as soon as possible after collection.

7.3 The holding time between sample collection and extraction is 28 days at – 20^{o}C.

7.4 The acetone extracts can be stored in the dark at – 20^{o}C for one year (generally analyzed within 28 days) without appreciable chlorophyll degradation.

8.0 QUALITY CONTROL

8.1 90% acetone solution is measured as blank controls for every 10 samples

8.2 A reference sample, dried Chlorophyll a from spinach, ordered from Sigma Chemical Co., catalog no. C5753, is analyzed once every three months.

8.2.1 Prepare 10 mg/L solution by dissolving the 1 mg of dried chlorophyll a spinach in a small amount of 90% acetone in a 100ml

volumetric flask and bring to volume with 90% acetone. Prepare 1 mg/L and 0.1 mg/L solutions by serial dilutions.

8.2.2 Analyze the 10 mg/L, 1 mg/L, and 0.1 mg/L solutions as regular samples on the spectrophotometer using a 1 cm cell cuvette and adding 1 drop of 1 N HCl.

8.2.3 Enter all readings in the linear regression calculations to establish the curve and obtain concentration values.

8.3 Re-analyzed sample with concentration exceeding the calibrated range by reading the sample in cuvette with 1 cm. path.

8.4 Data acceptance criteria are listed on the data review checklist. (Appendix A).

9.0 PROCEDURE

9.1 Log in Samples in Sample Receiving Book

9.1.1 Open the envelope containing the samples. Check the name of the survey written on the plastic bag of the sample against that listed on the Analysis Request Sheet. If this information is not the same, contact the collector immediately for verification.

9.1.2 Arrange samples in numerical sequence as they appear on the Analysis Request Sheet and paper clip them together. Replace the samples in the storage envelope.

9.1.3 One lab number is assigned only to samples collected in the same month with same study code. Using the hand stamp (duplicate setting), stamp the Analysis Request Sheet and the envelope (Post-it pad if envelope is plastic). Return envelope to the freezer.

9.1.4 Enter the lab number and sample information on the Analysis Request Sheet in the computer log book.

9.1 Grinding

9.2.1 Remove samples from freezer and warm up for 10 minutes at room temperature.

9.2.2 Number centrifuge tubes in numerical sequence. After every ten samples, insert a tube filled with 90% acetone for use as a sample blank.

9.2.3 Check and fill the two repipetors with 90% acetone.

9.2.4 Place the filter pad in the tissue grinder. With a pair of forceps stick the filter 2/3 down to the side of the grinding tube.

9.2.5 Turn on the switch of the tissue homogenizer.

9.2.6 Add in 4 ml of the 90% acetone to wet the filter. Move tube up and down with the filter tightly against the pestle. Macerate until all of the pad is completely ground up (looks like mush with no pieces of intact pad). A chlorophyll result is only as good as the way it is ground. To avoid spillage, hold tube firmly and move tube slowly.

9.2.7 Add in 4 ml of the 90% acetone to wash down thoroughly the paper pulp and continue to macerate for 30 second.

9.2.8 Pour the homogenate into a 15 ml conical centrifuge tube.

9.2.9 Rinse the pestle and grinding tube three times each with 2 ml of 90% acetone and add all the rinse into the 15 ml centrifuge tube. The final volume should be close to 14.5 ml.

9.2.10 Cap the tubes to prevent evaporation of the acetone and store the samples overnight at 4°C in refrigerator. Cover the tubes with aluminum foil to avoid light exposure.

9.2.7 Ground samples, prior to centrifugation, are not allowed to stay more than 24 hours.

9.3 Centrifugation

9.3.1 Take out the overnight samples from refrigerator. Clarify the samples by centrifuging the capped tubes for 30 minutes at 3000 rpm.

9.3.2 Number another set of centrifuge tubes in the same numerical sequence.

9.3.3 Carefully pour the clear liquid into a clean numbered centrifuge tube for reading and throw away the old centrifuge tube with filter paper residue.

9.3.4 If it is not possible to read the samples immediately, keep the tubes in the – 20°C freezer for at most one year(generally read within 28 days). Centrifuge samples 10–15 minutes before reading.

9.4 Log in samples on UV-2401 computer

9.4.1 Turn on the computer, printer and Shimadzu UV-2401PC.

9.4.2 Click on the Greenland icon to open to the Status of chlorophyll sample processing.

9.4.3 Click Log Samples on the top bar and use this form to enter information from the top part of a chlorophyll field data sheet.

9.4.4 Select the correct fiscal year. (Even if the highlighted FY box has the correct year, the box still needs to be clicked once.) Survey names, submitter intials, study code and category code all may be picked from list. The box for Additional information or comment may leave unfilled.

9.4.5 Click Add Record when finish entering all information.

9.4.6 Click Enter Sample Information from the top bar and use this form to enter information from the sample pad part of a chlorophyll field data sheet. Enter the starting lab # and the ending lab #. Check the information at the bottom line for correct batch information.

9.4.7 The first Sample ID column will be automatically assigned with a run number. Sample station and layer code may be picked from list. Information same as the previous row may be autofilled by press Ctrl and ‘ at the same time. Use the arrow button at the bottom line to move between samples.

9.4.8 Click Print Worksheet on the top bar to view the sample list and select print worksheet. Enter first lab number and last lab number and hit print to print out the worksheets. (Feed the tray 1 with the exact number of papers needed.)

9.5 Sample Measurements

9.5.1 Centrifuge down the sample again at 3000 rpm for 15 minutes.

9.5.2 Click the UVProbe tab on the task bar orr minimize the Greenland and double click on the UVProbe. With no cuvette in the light path, click Connect to connect the computer to the spectrophotometer. The instrument will perform the initialization. Click ok when it is completed.

9.5.3 Click File. Double click the file in the list named: ChloroMethod_1.pmd.

9.5.4 File properties window opens and displays a path and a suggested name for the photometric file. Click OK to accept the suggested file name.

9.5.5 Fill up both cuvettes with 90% acetone and click Baseline on the photometer button bar. Enter or accept the range from 780 to 300 nm. Click OK to start the scanning.

9.5.6 Replace the sample curvette with blank from sample preparation. Enter blank as sample information and start the blank measurement. Press Read Unk or F9 to start the measurement.

9.5.7 Enter the first Sample ID number assigned to each sample on the worksheet followed by BX for a before acidification reading of the sample. Click Read Unk or F9 to start the measurement.

9.5.8 Enter the same sample ID number followed by AX and press enter. Add in 3 drops of acid and start a one min timer. Use a cuvette stirrer to mix the sample for about 30 seconds. Press Read Unk or F9 at the end of the one min timing to start the reading after acidification.

9.5.9 Measure a blank after every 10 samples.

9.5.10 Re-do the baseline after about every 10 samples or when the 750 nm or blank readings are getting higher by topping off the reference cuvette with 90% acetone and fill in the sample cuvette with fresh 90% acetone and click on Baseline button. Always read a blank after a baseline scan.

9.5.11 At the end of the run or at the end of the day, save the run into 2 files. First file is saved by clicking save and the second file is saved as a text file by clicking save as, then enter day-month-year and choose ASCII.txt to save.

9.5.12 Click Disconnect.

9.5.13 Close the UVProbe window. Shut down computer. Turn off the spectrophotometer.

9.6 Data Report

9.6.1 Click Import Readings and follow the instructions to import Shimadzu readings and merge readings with sample information.

9.6.2 Click Log Check to verify Log-In information. Enter read date and check date and intials.

9.6.3 Click Samples Checked to verify sample information. Check the correct box and initial.

9.6.4 Click Print Reports. Enter study code, year, month or lab number before printing the DNR (legal size) or Lab report (letter size).

10.0 DATA ANALYSIS AND CALCULATIONS

10.1 The chlorophyll a and pheophytin a concentrations in samples are calculated as follows:
First, 750 nm OD value is subtracted from the reading before and after acidification. Then, corrected values are used in the following equations:

$$\text{Chlorophyll a, } {}^{mg}\!/_{m^3} = \frac{26.7\,(OD_{664}b - OD_{665}a)}{V2 \times L} \times V1$$

$$\text{Pheophytin a, } {}^{mg}\!/_{m^3} = \frac{26.7\,[1.7\,(OD_{665}a) - (OD_{664}b)]}{V2 \times L} \times V1$$

Where V1 = volume of extract in liters
V2 = volume of sample in liters
L = light path length or width of cell in cm

$OD_{664}b$, $OD_{665}a$ = optical density of 90% acetone extract before and after acidification, respectively. These calculations are done using a dBase program. In sample reports, both calculated results are printed in the right most column for each sample line.

10.2 For reference samples, chlorophyll a concentrations are calculated using linear regression as in 8.2.3.

10.3 Calculate the relative percent difference for the duplicated samples as follows:

$$\%\,RPD = \frac{\text{difference between the duplicates}}{\text{average of the duplicates}} \times 100$$

11.0 DATA AND RECORDS MANAGEMENT

11.1 Copy results for DNR and MDE samples for each month to two separate floppy disks.

11.2 Instrument maintenance log is located near the instrument.

11.3 The Division shall retain all pertinent laboratory records for each sample for a period of five years. At the conclusion of this period, clients shall be given the option to take custody of their sample records. The Division shall not be responsible for retrieving, copying, or transferring laboratory records exceeding the five years custody period.

12.0 WASTE MANAGEMENT

The solvent, acetone, is poured into a labeled waste container. Safety officer will arrange periodic pick-up and disposal.

13.0 REFERENCES

13.1 United States Environmental Protection Agency, *Environmental Monitoring and Support Laboratory, Chlorophyll – Spectrophotometric, March, 1991*

13.2 American Public Health Association, *Standard Methods for the Examination of Water and Wastewater, Method Number 10200H, 21st Edition, 2005*

13.3 Chesapeake Bay Program, *Greenland: A Tool For Processing Chlorophyll Samples, 2005*

13.4 DEC, Maryland State Department of Health and Mental Hygiene, *Quality Assurance Plan*, May, 2007.

APPENDICES

Appendix A – Data Review Checklist

Standard Operating Procedures

Chlorophyll a
Standard Method 10200H (Spectrophometric, Beckman DU-650)

1.0 SCOPE AND APPLICATION

1.1 This method is applicable to the determination of chlorophyll *a*, *b*, *c* and pheophytin *a* in fresh and marine waters.

1.2 The concentrations are reported in SI units of mg/m^3.

2.0 SUMMARY OF METHOD

The chlorophyll and related compounds are extracted from the algae collected on glass fiber filters with aqueous 90% acetone solution. Light absorption of the extract is measured at selected wavelengths and the concentrations of the pigments of interest are calculated using the equations as in 10.0.

3.0 INTERFERENCES

3.1 Pheophytin *a* is a common degradation product of chlorophyll *a*. Pheophytin *a* is similar in structure to chlorophyll *a*, but lacks the magnesium atom (Mg) in the porphyrin ring. The magnesium can be removed from chlorophyll in the presence of acid.

3.1.1 Field Samplers can prevent this degradation by the addition of magnesium carbonate to the plankton sample prior to filtration.

3.1.2 When a solution of pure chlorophyll *a* is converted to pheophytin *a* by acidification, the absorption peak is reduced to approximately 60% of its original value and shifts from 664 to 665 nm. For pure chlorophyll this before/after acidification absorption peak ratio (OD_{664}/OD_{665}) is 1.7. Solutions of pure pheophytin show no reduction at OD_{665} upon acidification and have 664/665 ratio of 1.0. The *acid rati*o should fall between 1.0 and 1.7. If it is not within this range, the data are not valid and will be discarded. Sample submitter is immediately notified if more than 10% of the data will be rejected.

$$\frac{(OD_{664} - OD_{750})b}{(OD_{665} - OD_{750})a} = \text{acid ratio}$$

b = before acidification
a = after acidification

3.2 Chlorophyll solutions degrade rapidly in strong light. Work with these solutions should be carried out in subdued light, and all vessels, tubes, etc. containing the pigment should be covered with aluminum foil.

3.2.1 Naturally occurring, structurally related chlorophyll precursors and degradation products, such as the chlorophyllides, pheophytins and pheophorbides, commonly occur in pigment extracts and may absorb light in the same region of the spectrum as the chlorophylls.

3.2.2 Ground samples should be covered by aluminum foil and steeped in the refrigerator not less than 15 minutes or more than 24 hours. After clarification decant the extract directly into the cuvette for analysis or a screw cap tube and put in the freezer. The extract can be stored for one year.

4.0 HEALTH AND SAFETY

4.1 Good laboratory practices should be followed during reagent preparation and instrument operation. Use of gloves and eye protection is recommended when preparing solutions.

4.2 Each chemical should be regarded as a potential health hazard. A reference file of MSDS is available in lab.

4.3 Inhalation of acetone should be minimized by performing all operations in a well-ventilated hood.

5.0 EQUIPMENT AND SUPPLIES

5.1 Equipment

5.1.1 Beckman DU-65 and DU-650 UV/V/S spectrophotometers, Beckman Instruments Inc.

5.1.1 Tissue Grinder – Con-Torque power-unit, Cat. # 7265, Eberbach Corp.

5.1.2 Centrifuge – Megafuge 2.0, Heraeus Instrument.

5.1.3 Magnetic Stirrer

5.2 Supplies

5.2.1 Centrifuge tubes - 15 ml graduated conical polypropylene tubes with screw caps, Catalog No. 05-538-43D, Fisher Scientific.

5.2.2 Cuvettes with 1 cm, 2 cm, 5 cm path length, catalog nos. 14-385-932 C-E, Fisher Scientific.

5.2.3 Repipet Dispensers – 5 and 10 ml volume, Fisher catalog no. 13-687-54 and 13-687-55.

6.0 REAGENTS AND STANDARDS

6.1 Acetone –Spectranalyzed, catalog no. A19-4, Fisher Scientific

6.2 Acetone solution, 90% – Add 900 ml of acetone to 100 ml of distilled water and mix well. The final volume will be less than 1 liter.

6.3 Hydrochloric Acid, 1N – Certified, catalog no. SA48-4, Fisher Scientific

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.1 Samples are collected and filtered in the field. Filters sealed in aluminum foil are cooled to – 4^{o}C on ice and transported to the laboratory by collection staff or courier service.

7.2 All samples must be delivered to the laboratory as soon as possible after collection.

7.3 The holding time between sample collection and extraction is 28 days at – 20^{o}C .

7.4 The acetone extracts can be stored in the dark at – 20^{o}C for one year (generally analyzed within 28 days) without appreciable chlorophyll degradation.

8.0 QUALITY CONTROL

8.1 90% acetone solution is measured as blank controls for every 10 samples

8.2 A reference sample, dried Chlorophyll a from spinach, ordered from Sigma Chemical Co., catalog no. C5753, is analyzed once every three months.

8.2.1 Prepare 10 mg/L solution by dissolving the 1 mg of dried chlorophyll a spinach in a small amount of 90% acetone in a 100ml volumetric flask and bring to volume with 90% acetone. Prepare 1 mg/L and 0.1 mg/L solutions by serial dilutions.

8.2.2 Analyze the 10 mg/L, 1 mg/L, and 0.1 mg/L solutions as regular samples on the spectrophotometer using a 1 cm cell cuvette and adding 1 drop of HCl.

8.2.3 Calculate chlorophyll a concentration following the steps listed below (equation to calculate chlorophyll a from 10.1 is entered in Quatro Pro):

8.2.3.1 Go to Main menu, press 9, <enter>

8.2.3.2 Type 'cd qpro', <enter>

8.2.3.3 Type 'q', <enter>

8.2.3.4 Using the mouse, click on File.

8.2.3.5 Click on Open.

8.2.3.6 Click on Judith 6.WQ1.

8.2.3.7 Enter data points from lab report.

8.2.3.8 Click on Print.

8.2.3.9 Click on Print to Fit; data will be printed.

8.2.3.10 Click on File/ Save/ Replace. Click on File/ Exit.

8.2.3.11 Type 'cd..' , then 'db' to get back to main chlorophyll menu.

9.0 PROCEDURE

9.1 Log in Samples in Sample Receiving Book

9.1.1 Open the envelope containing the samples. Check the name of the survey written on the plastic bag of the sample against that listed on the Analysis Request Sheet. If this information is not the same, contact the collector immediately for verification.

9.1.2 Arrange samples in numerical sequence as they appear on the Analysis Request Sheet and paper clip them together. Replace the samples in the storage envelope.

9.1.3 One lab number is assigned only to samples collected in the same month with same study code. Using the hand stamp (duplicate

setting), stamp the Analysis Request Sheet and the envelope (Post-it pad if envelope is plastic). Return envelope to the freezer.

9.1.4 Enter the lab number and sample information on the Analysis Request Sheet in the computer log book.

9.2 Grinding

9.2.1 Remove samples from freezer and warm up for 10 minutes at room temperature.

9.2.2 Number centrifuge tubes in numerical sequence. After every ten samples, insert a tube filled with 90% acetone for use as a sample blank.

9.2.3 Check and fill the two repipetors with 90% acetone.

9.2.4 Place the filter pad in the tissue grinder. With a pair of forceps stick the filter 2/3 down to the side of the grinding tube.

9.2.5 Turn on the switch of the tissue homogenizer.

9.2.6 Add in 4 ml of the 90% acetone to wet the filter. Move tube up and down with the filter tightly against the pestle. Macerate until all of the pad is completely ground up (looks like mush with no pieces of intact pad). A chlorophyll result is only as good as the way it is ground. To avoid spillage, hold tube firmly and move tube slowly.

9.2.7 Add in 4 ml of the 90% acetone to wash down thoroughly the paper pulp and continue to macerate for 30 second.

9.2.8 Pour the homogenate into a 15 ml conical centrifuge tube.

9.2.9 Rinse the pestle and grinding tube three times each with 2 ml of 90% acetone and add all the rinse into the 15 ml centrifuge tube. The final volume should be close to 14.5 ml.

9.2.10 Cap the tubes to prevent evaporation of the acetone and store the samples overnight at 4°C in refrigerator. Cover the tubes with aluminum foil to avoid light exposure.

9.2.7 Ground samples, prior to centrifugation, are not allowed to stay more than 24 hours.

9.3 Centrifugation

9.3.1 Take out the overnight samples from refrigerator. Clarify the samples by centrifuging the capped tubes for 30 minutes at 3000 rpm.

9.3.2 Number another set of centrifuge tubes in the same numerical sequence.

9.3.3 Carefully pour the clear liquid into a clean numbered centrifuge tube for reading and throw away the old centrifuge tube with filter paper residue.

9.3.4 If it is not possible to read the samples immediately, keep the tubes in the – 20^{o}C freezer for at most one year(generally read within 28 days). Centrifuge samples 10–15 minutes before reading.

9.4 Log -in Samples on the Chlorophyll Measurement Computer

9.4.1 Turn on the computer and wait for Main Menu to appear.

9.4.2 Select "1" – Log in Samples.

.

9.4.3 Type the information on the Analysis Sheet into the computer. If a mistake is made while logging the individual sample, it can be corrected on the same line. When data entry goes to another line (a new record) , the prior record cannot be corrected until data for all other records (samples) are entered.

9.4.4 Press [Enter] key down when all sample data have been logged in this survey. The computer screen will automatically change to another prompt.

9.4.5 When asked "Are you aware of any entry errors you made for this sample that are not already corrected in the individual filter lines? (Y or N?)", answer 'Y' if corrections are needed, and give the number. The computer screen will automatically change to vertical (dBase Edit Screen) format and allow you to edit. When finished, press [CTRL-W] to return to horizontal (dBase Browse Screen) format.

9.5 Log in samples on any other computer that has dBase

9.5.1 Turn on computer. If a menu comes up, exit on DOS.

9.5.2 Put the disk labeled CHLOROPH in Drive A.

9.5.2.1 Type :CHLOROPH, [Enter] then the screen will show the menu of chlorophyll.

9.5.2.2 When all data have been logged, put this disk into the Drive A of the computer. At the Main Menu, select "8" – Miscellaneous Utility option. Select "8" - Merge the information of the disk to the computer.

9.5.3 Use :UPDATE disk to log-in some surveys which contain a long list of samples but for which most of the demographic information remains unchanged.

9.5.3.1 When menu comes hit 9, screen will show C:> Type A:UPDATE <Enter>.

9.5.3.2 The screen will show a list of surveys options.

9.5.3.3 Press the number that stands for the survey you want. The computer will retrieve the respective demographics for the samples. Type in the information that is changed or lacking. Keep the information that remains the same. (This program saves a lot of time and effort.)

9.5.3.4 When one lab number is logged in, it must be merged into the computer. If it is not merged and second survey is logged in, the second one replaces the first. Thus the former logged data are lost when a second survey is logged-in by using UPDATE disk. But this not true with the more general program like CHLOROPH.

9.6 Reading the samples

9.6.1 Turn on the spectrophotometer (by pressing the vis key), computer and the two printers. Let the spectrophotometer warm up for 30 minutes.

9.6.2 Select "3" at the Main Menu; read samples that have been logged in.

9.6.3 The spectrophotometer should be calibrated with a blank before scanning any samples. That is, fill the 5 cm cuvette with 90% acetone solution (the blank solution kept when grinding the samples). Place the cuvette into the spectrophotometer cell holder in the cell compartment. Press [Enter]; the printer attached to

spectrophotometer will print out the result, which should also appear on the screen of the computer. Write down the line number on the "blank" column of the Work Sheet. Accept or reject (than rescan) the data presented on the computer screen.

9.6.4 Rinse the 5 cm cuvette three times with distilled water. Then rinse with 90% acetone solution. Transfer sample extract into the rinsed cuvette using disposable pipette. If schleren (wavy lines when

looking through cell) appear, shake cell well until solution is homogeneous. Place cuvette into the spectrophotometer cell holder in the cell compartment. Type in the tube number and press [Enter]. Computer will count 30 seconds to allow particle settling in the sample, then the computer will command the spectrophotometer to begin to scan the required wavelengths. When the scan is complete, the data will appear on the computer screen, and the results will be printed out by the printer.

9.6.4.1 If data is satisfactory (absorption at 750 nm is less than 0.007 for DNR samples and less than 0.01 for MDE samples), press [Enter]. Write down the line number in the Work Sheet "line number" column.

9.6.4.2 If the absorption reading at 750 nm is unsatisfactory, press any key, then [Enter], then select from options presented. Write down the line numbering the Work Sheet "line number" column with an indication that the readings are not to be used.

9.6.5 Remove cuvette from spectrophotometer cell compartment. Remove stoppers and add 3 drops of 1N HCl into mouth of the cuvette. As soon as the acid is added, press [Enter] to start the reaction timer. Stopper, then shake well but quickly to mix for about 20 seconds. Place cuvette back into cell compartment and close lid securely. Computer timer will allow 90 seconds for the acid to react with the sample before starting the wavelength scan. When the screen shows the result, write down the line number on Work Sheet "line number" column and acid ratio in the "comments" column. If the ratio is out of the range, there will be a warning sound.

9.6.6 If the absorbance of the extract is greater than 0.8 at 664 nm (the computer will give a warning sound), transfer the sample to 2 cm or 1 cm cuvette and re-analyze the sample.

9.6.7 If absorbance at 750 nm appears to be a negative value (less than -0.000), for more than two successive scans, spectrophotometer drift has become excessive. To correct this drift, recalibrate the spectrophotometer with 90% acetone solution as in 9.6.3.

9.6.8 Recalibrate the spectrophotometer every ten samples with an extraction solvent "acetone solution blank" as in 9.6.3

9.7 Verification/Validation

9.7.1 Select "4" on the Main Menu. Approve completed work before printing.

9.7.2 Write the lab no., sample no., and tube no. from the worksheet onto the spectrophotometer printer output sheet.

9.7.3 Carefully check every item from the Analysis Request Sheet with screen data for the respective sample. If any item is incorrect, at the computer prompt indicate N and the dBase Edit screen will appear and let you edit. When editing is complete, press [CTRL-W] to return to a data screen.

9.8 Reports

9.8.1 To print reports for completed work, select "5" from Main Menu. Enter one lab number at a time and print reports; it will print one copy to send out, one copy in-house for reference, for each lab number entered.

9.8.2 If printing additional copy, choose "8" on menu, miscellaneous utility program.

10.0 DATA ANALYSIS AND CALCULATIONS

10.1 The chlorophyll a and pheophytin a concentrations in samples are calculated as follows:

First, 750 nm OD value is subtracted from the reading before and after acidification. Then, corrected values are used in the following equations:

$$\text{Chlorophyll a, } {}^{mg}/_{m^3} = \frac{26.7\ (OD_{664}b - OD_{665}a)}{V2 \times L} \times V1$$

$$\text{Pheophytin a, } {}^{mg}/_{m^3} = \frac{26.7\ [1.7\ (OD_{665}a) - (OD_{664}b)]}{V2 \times L} \times V1$$

Where V1 = volume of extract in liters
V2 = volume of sample in liters
L = light path length or width of cell in cm

$OD_{664}b$, $OD_{665}a$ = optical density of 90% acetone extract before and after acidification, respectively. These calculations are done using a dBase program. In sample reports, both calculated results are printed in the right most column for each sample line.

10.2 For reference samples, chlorophyll a concentrations are calculated using Quatro Pro as in 8.2.3.

10.3 Calculate the relative percent difference for the duplicated samples as follows:

$$\%\,\mathrm{RPD} = \frac{\text{difference between the duplicates}}{\text{average of the duplicates}} \times 100$$

11.0 DATA AND RECORDS MANAGEMENT

11.1 Copy results for DNR and MDE samples for each month to two separate floppy disks.

11.2 Instrument maintenance log is located near the instrument.

11.3 The Division shall retain all pertinent laboratory records for each sample for a period of five years. At the conclusion of this period, clients shall be given the option to take custody of their sample records. The Division shall not be responsible for retrieving, copying, or transferring laboratory records exceeding the five years custody period.

12.0 WASTE MANAGEMENT

The solvent, acetone, is poured into a labeled waste container. Safety officer will arrange periodic pick-up and disposal.

13.0 REFERENCES

13.1 United States Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Chlorophyll – Spectrophotometric, March, 1991

13.2 American Public Health Association, Standard Methods for the Examination of Water and Wastewater, Method Number 10200H, 21st Edition, 2005

13.3 Chesapeake Bay Program, *Greenland: A Tool For Processing Chlorophyll Samples, 2005*

13.4 DEC, Maryland State Department of Health and Mental Hygiene, *Quality Assurance Plan*, May, 2007.

APPENDICES

Appendix A – Data Review Checklist

APPENDIX IX

MARYLAND DEPARTMENT OF NATURAL RESOURCES
CHESAPEAKE BAY
WATER QUALITY MONITORING PROGRAM

SPLIT SAMPLE PROGRAM

The following summary is a summary of the split sample program excerpted from the *Recommended Guidelines for Sampling and Analysis in the Chesapeake Bay Monitoring Program*. (EPA 1996). Information about the Split Sample and Blind Sample Programs program is available on-line at the EPA Chesapeake Bay Program web site: http://www.chesapeakebay.net/qualityassurance_splitsample.aspx.

Background and Objectives

The Chesapeake Bay Coordinated Split Sample Program (CSSP) was established in June 1989 by recommendation of AMQAW [the Chesapeake Bay Program Analytical Methods and Quality Assurance Workgroup], to the Monitoring Subcommittee. The major objective of this program is to establish a measure of comparability between sampling and analytical operations for water quality monitoring basin-wide. A secondary objective is to evaluate the in-matrix dilution of standard U.S. Environmental Protection Agency (EPA) reference materials. These standard reference materials are analyzed in appropriate matrix, fresh to saline, and concentration level to match the sample. All laboratories participating in basin-wide data collection programs are also required to participate in the CSSP. For additional information on the program, please consult *Chesapeake Bay Coordinated Split Sample Program Implementation Guidelines Revision3* (EPA 1991).

Summary of Criteria

(1) The Participant will participate in the applicable component(s) of the CSSP.

(2) The Standard Operating Procedures (SOPs) that are developed and used should be in accordance with the Chesapeake Bay Coordinated Split Sample Program Implementation Guidelines CBP/TRS 58/91, May 1991 plus any revisions specified by the CBP Quality Assurance Officer.

(3) For each of the Virginia and Maryland CSSP stations and on a quarterly basis, the Participant will receive and analyze four sub-samples. Since 1998, Maryland DNR has performed the sample split at one station and depth, (usually the surface sample at station CB4.4C).

Four sub samples will be collected for each participating laboratory. Samples to be analyzed at Virginia Labs will be delivered to Port Royal, VA, the afternoon of the day they are collected and processed the following morning. In order to

treat all of the samples uniformly, the MD DNR field team will also wait until the next morning to process their split samples.

Laboratories currently participating in the program are: University of Maryland Chesapeake Biological Laboratory Nutrient Analytical Services Laboratory (CBL), Virginia Institute of Marine Science (VIMS), Old Dominion University College of Sciences Water Quality Laboratory (ODU), Virginia Division of Consolidated Laboratory Services (VADCLS), Hampton Roads Sanitation District Central Environmental Laboratory (HRSD) and Morgan State University (MSU).

Treating each sub-sample as a discrete sample, participating laboratories are generally required to perform only those analyses which they routinely perform in support of basin-wide data collection program. One of the three sub-samples should be used to generate laboratory duplicates and a laboratory spike. These quality control (QC) samples should be analyzed concurrently with the associated CSSP sub-samples.

(4) The routine submission of split sample data is the responsibility of each laboratory and its in-house data management organization.

(5) To supplement the analyses of the three sub-samples and the respective QC sample, EPA standard reference materials provides a strong measure of comparability between all laboratories and within one laboratory's analytical system over time. Quarterly analysis of Standard Reference Materials (SRMs) is the most independent evaluation of laboratory performance available at this time. It is a critical element of any diagnostic efforts associated with the CSSP.

REFERENCES:

U.S. Environmental Protection Agency (EPA). 1996. *Recommended Guidelines for Sampling and Analysis in the Chesapeake Bay Monitoring Program*. Chesapeake Bay Program, August 1996. CBP/TRS 148/96; EPA 903-R-96-006. http://archive.chesapeakebay.net/pubs/quality_assurance/doc-EPA903-R-96-006.pdf

U.S. Environmental Protection Agency (EPA). 1991. *Chesapeake Bay Coordinated Split Sample Program Implementation Guidelines* Chesapeake Bay Program, May 1991. CBP/TRS 58/91 is available as document 903R91006 via the EPA online publication server: http://nepis.epa.gov/EPA/html/Pubs/pubtitleOther.htm.

Split Sample Custody Log Example:

MAIN BAY SPLIT SAMPLE CUSTODY LOG

BOTTLE NUMBERS: C1,C2,C3,C4

LOCATION: CB4.4

COLLECTED FOR: CBL

COLLECTION DETAILS: DATE:_____ TIME:_____ DEPTH: 0.5M SALINITY:_____PPT

COMMENTS:(unusual conditions, problems, floating algae, rain, etc.)

==========

SPLITTING DETAILS: COMPOSITE SUB SPLIT BY:
COMPOSITE CONTAINER sequential bottles
FILLED BY: submersible pump @ 0.5 m into a 30 gallon Nalgen container

Splitting Seq. Bottle #	Splitting Seq. Bottle #	Splitting Seq. Bottle #
bottle 1---A1 EXTRA VA	bottle 9- - -C2 CBL	bottle 17- - -E3 MSU
bottle 2---B1 ODU	bottle 10- - -D2 VADCLS	bottle 18- - -F3 VIMS
bottle 3---C1 CBL	bottle 11- - -E2 MSU	bottle 19- - -A4 EXTRA VA
bottle 4---D1 VADCLS	bottle 12- - -F2 VIMS	bottle 20- - -B4 ODU
bottle 5---E1 MSU	bottle 13- - -A3 EXTRA VA	bottle 21- - -C4 CBL
bottle 6---F1 VIMS	bottle 14- - -B3 ODU	bottle 22- - -D4 VADCLS
bottle 7---A2 EXTRA VA	bottle 15- - -C3 CBL	bottle 23- - -E4 MSU
bottle 8---B2 ODU	bottle 16- - -D3 VADCLS	bottle 24- - -F4 VIMS

==========

TRANSFER SEQUENCE:	DATE	TIME	BY WHOM?	TEMP. OF SAMPLE (circle one)
Composite collected & split	_____	_____	DNR/_____	ambient
Subsample picked up	_____	_____	_____	0^0C 4^0C ambient
Subsamples delivered to lab	_____	_____	_____	0^0C 4^0C ambient

==========

FIELD PROCESSING INFORMATION

BOTTLE #	FIELD PROCESSING DONE	DATE/TIME	BY WHOM?
C1	processed sample #		
C2	processed sample #		
C3	processed sample #		
C4	processed sample #		

NOTE: PLEASE SEND A COPY OF THIS COMPLETED FORM TO:, ,
Maryland Dept of Natural Resources, TEA/D-2, 580 Taylor Avenue, Annapolis MD, 21401,
(410) 260-8629.

APPENDIX X

MARYLAND DEPARTMENT OF NATURAL RESOURCES
CHESAPEAKE BAY
WATER QUALITY MONITORING PROGRAM

DATA STATUS FORM
DOCUMENTATION AND PROCEDURES

The Data Status Form is used for all monthly water quality data for all monitoring projects. The form is designed to facilitate data management by tracking data management activities and identifying potential problems for remedy early in the process. Upon receiving the data sheets or files from the data source agencies (e.g., the Field Office and the laboratories), the data clerk initiates a Data Status Form, which then accompanies the data sheets/files. When all of data have been processed for that month, the Data Status Form is stored with the data sheets and other computer generated information at the DNR Tawes Office Building in Annapolis.

This sheet was developed in 1986 and updated in 1995. Note that many of the columns on the form are no longer actively used. The necessary information in the sheet is described in the following paragraphs. An example Data Status Form is attached for reference.

I. COMPLETE THE FOLLOWING WHEN THE FORM IS ISSUED:

1. DATE INITIATED (UPPER RIGHT HAND CORNER)

Indicate the date when the form is issued. In general, issue the form upon receiving the first group of data sheets and/or data files from data source agencies for a given month.

2. DATA SET NAME

Enter the data set name with the project abbreviation, data sampling month, year, and data type (e.g., TJAN98FD for tributary field data for January 1998). Refer to the detailed description of naming conventions at the end of this appendix.

3. RECEIVED

Upon receiving the first group of data sheets and/or data files, enter the date and initial in this field.

II. COMPLETE THE FOLLOWING UPON FINISHING THE DATA MANAGEMENT PROCESS

1. DATA REVIEWED

Once all monthly data sheets and/or data files have been received and the data have been reviewed, enter completion date. Initial in this field.

2. CROSS REFERENCE

If the completed cross reference sheets are included with the incoming data sheets, enter the date and initial in this field. If, for some reason, cross reference sheets are not included, the Quality Assurance Officer would be notified, and s/he would contact the field office.

3. XEROXING

Before sending field data sheets to the data entry service agency, copy data sheets and send originals to the data entry service agency. Enter the completion date and initial in this field.

4. DATA ENTRY – SEND

Enter the date data sheets are sent to data entry service in this field. Initial.

5. DATA ENTRY – RETURNED

Enter the date that data sheets and data diskette are received from data entry service in this field. Initial.

6. INITIAL DATA CHECK

7. DATA VERIFICATION

8. TEMPORARY MERGE(S)

BIO CHECK (#9 - #13)

9. VERIFICATION(S)

10. EDIT(S) IDENTIFIED

11. DATA CORRECTION(S)

12. TEMPORARY MERGE(S)

13. BIOLOGIST SIGN OFF

14. FINAL DATA CORRECTION

15. MERGE COMPLETED

16. GENERATE MS ACCESS DATA SET

17. GENERATE EPA MS ACCESS DATA (This field generally is left blank–this step is included under "PRODUCE CBP DATA TRN FILE.")

18. SUBMISSION DOCUMENT (This field generally is left blank–this step is included under “PRODUCE CBP DATA TRN FILE.”)

19. SUBMISSION LETTER (This field generally is left blank–this step is included under “PRODUCE CBP DATA TRN FILE.”)

20. FINAL SIGN-OFF

Upon verifying all of the above data management processes and ensuring that all corrections have been made, finalize the data set in the state data base system by running permanent merge process in the EZMERGE system, enter the completion date and initial in this field.

21. SUBMISSION TO CBP (PRODUCE CBP DATA TRN FILE)

Upon successfully creating data submission file, report, and document for the monthly data submission process, enter the completion date in this field and initial.

22. CBP ACCEPTS / SIGN OFF

After receiving the checklist and ACCEPTS/SIGN OFF form from CBP and upon completing all the necessary data verification actions (e.g., double checking errors), put the completion date in this field and initial.

NOTE: Any special comments can be entered in the COMMENTS column during the data management activities.

CONVENTIONS FOR NAMING THE DATA SET

An eight-character text string is used for this data set name. This section contains the naming conventions for data set names for all monitoring projects. Any new sampling monitoring and data collection projects must follow these conventions.

1. CHESAPEAKE BAY MAINSTEM MONITORING PROJECT

   Data Set Name: MMMYYDDD

   Description: The data set name contains the data sampling month, year, and data type only. The first three characters of the data set name (MMM) stand for the sampling month. The next two characters (YY) of the data set name are the last two digits of the sampling year. The last three characters of data set name (DDD) stand for sample collection type. The following types are available for this project:

DATA TYPE	DATA DESCRIPTION
FLD	Field Data
LAB	Laboratory Data
CHL	Chlorophyll Data

   Example of Mainstem Data Set Name: For field data sheets for January 1998 data, the data set name is 'JAN98FLD'.

2. MARYLAND TRIBUTARY MONITORING PROJECT

   Data Set Name: TMMMYYDD

   Description: The data set name begins with the project initial 'T', followed by the data sampling month (MMM), year (YY), and data type (DD). The last two characters of the data set name (DD) stand for data type. The following types are available for this project:

DATA TYPE	DATA DESCRIPTION
FD	Field Data
LB	Laboratory Data
CH	Chlorophyll Data

   Example of a Tributary Data Set Name: For field data sheets for January 1998 data, the data set name is 'TJAN98FD'.

3. MARYLAND PATUXENT RIVER INTENSIVE SURVEY (PART OF MARYLAND TRIBUTARY MONITORING PROJECT)

   Data Set Name: PTMMMYYD

Description: The data set name begins with the project initials 'PT', followed by the data sampling month (MMM), year (YY), and data type (D). The last character of data set name (D) stands for data type. The following types are available for this project:

DATA TYPE	DATA DESCRIPTION
F	Field Data
L	Laboratory Data

[Note: Chlorophyll data for the Patuxent is included in the tributary data set.]

Example of a Patuxent Data Set Name: For field data sheets for January 1998 data, the data set name is 'PTJAN98F'.

Example of Monitoring Data Status Form

MONITORING DATA STATUS

MEDIA:^ WQ PROGRAM:^ MD LOCATION:^Main

RECEIVED DATE: 03/12/2007 REPORT DATE: 03/14/2007
STAFF NAME: T. Huber LAST ENTRY: 03/14/2007
DATA SHORT NAME: December 2006 Mainstem fixed-station water quality data

DATA SUBMISSION INFORMATION

SUBMITTER NAME: A. V. Allred, Jr DATA START DATE: 12/01/2006
ORGANIZATION:^ MD DNR DATA END DATE: 12/31/2006
FILE,TAPE,DISK:^ On-line QAT
DATA FORMAT:^ ACCESS Database
IN FILE LOCATION: c:\qat\BayDec06.mdb
COMMENTS:

DATA PROCESSING INFORMATION

STATUS:^ Pending Submitter Action
OUT FILE LOCATION: GAR.wq_1
ACTIVITY LOG: (Keep daily records of activity)

03/12/2007 09:55:57 - Uploaded
03/12/2007 21:00:01 - Begin Checks
03/12/2007 21:00:51 - Passed Checks
03/08/2007, Sign off to R. Batiuk for MD DNR

The MD DNR dataset containing December 2006 mainstem water quality data was processed through DUQAT and added to the database. Please call or e-mail me if you have any questions.

The data set checklist has been reviewed by the data originator and the dataset is (check one):

X Accepted entirely as is
_____ Accepted with limited modifications (noted below)
_____ Rejected (reason noted below)

Anthon V. Allred, Jr. **March 15, 2007**
Signature of data originator Date

G:\Monitoring\FRESH-G\ADMIN\GRANTS\WQSIGNOF\MD\MAINSTEM\MDMAIN2006/BAYDEC06.RTF

APPENDIX XI

MARYLAND DEPARTMENT OF NATURAL RESOURCES
CHESAPEAKE BAY
WATER QUALITY MONITORING PROGRAM

OUTLINE OF CODES FOR FIELD AND LABORATORY DATA SHEETS

This file contains the computer codes for water quality data that will be used for field and laboratory data sheets. The computer codes are listed with their corresponding descriptions.

OUTLINE OF CODES

FIELD DATA SHEETS

Submitter Codes
Data Category Codes
Study Codes
Sample Method Codes
Tide State Codes
Weather Codes
Percentage Cloud Cover Codes
Dissolved Oxygen Method Codes
'Value Corrected' Codes
Sample Layer Codes
Wind Direction Codes

LABORATORY DATA SHEETS

COMPUTER CODES FOR NUTRIENT PARAMETER ANALYSES SHEET

Submitter Codes
Data Category Codes
Sample Method Codes
Sample Layer Codes
Study Codes
Parameter Codes
Analytical Problem Codes
Detection Limit Codes
Method Codes

COMPUTER CODES FOR CHLOROPHYLL PARAMETER ANALYSES SHEET

Submitter Codes
Data Category Codes
Sample Layer Codes
Study Codes
Analytical Problem Codes

DETAILED DESCRIPTION OF CODES

BECAUSE THE NEW DATA BASE SYSTEM IS DEVELOPING AND WILL REPLACE THE CURRENT DATA BASE SYSTEM IN THE NEAR FUTURE, MOST OF CURRENT COMPUTER CODES WILL BE DROPPED AFTER THE COMPLETION OF NEW DATA BASE SYSTEM. THE FOLLOWING CODES LIST THE MOST COMMON USED COMPUTER CODES ONLY.

FIELD DATA SHEETS

Submitter Codes:

CODE	DATA COLLECTION AGENCY	ANALYTICAL LAB
28	CBL/FIELD	CHEMICAL – CBL/LAB CHLOROPHYLL – DHMH TURBIDITY - DHMH
60	DNR/TEA	CHEMICAL – CBL AND WESTERN MD LAB CHLOROPHYLL - DHMH
79	DNR/TEA	CHEMICAL – CBL CHLOROPHYLL - DHMH

Data Category Codes: These codes are designed to indicate the type of data collected

CODE	DESCRIPTION
AA	PRIMARY MONITORING SAMPLE - LAND
AB	PRIMARY MONITORING SAMPLE – BOAT
IN	WATER QUALITY INTENSIVE SURVEY DATA
NR	NON-POINT SOURCE/RUN-OFF SAMPLING DATA
MB	CHESAPEAKE BAY MONITORING WATER QUALITY SAMPLE -- MAIN BAY
MN	AUTOMATED MONITORING STUDY
TB	CHESAPEAKE BAY MONITORING WATER QUALITY SAMPLE -- MARYLAND TRIBUTARY
ST	SEDIMENT DATA SAMPLE
WQ	WATER QUALITY SAMPLE, UNSPECIFIED PROGRAM

Study Codes

CODE	DESCRIPTION
01	CHESAPEAKE BAY MONITORING PROGRAM – MAIN BAY
02	CHESAPEAKE BAY MONITORING PROGRAM - TRIBUTARY
04	CORE/TREND MONITORING PROGRAM
06	POTOMAC COORDINATED MONITORING PROGRAM - COG
08	COASTAL BAYS PROGRAM
09	ROUTINE FISH WATER QUALITY
21	WATER QUALITY MAPPING (DATAFLOW)
22	CONTINUOUS MONITORING
97	ROUTINE PFIESTERIA WATER QUALITY
99	RAPID RESPONSE PFIESTERIA WATER QUALITY

Sample Method Codes

CODE	DESCRIPTION
1	GRAB SAMPLE
7	FIELD MEASUREMENTS ONLY

Tide State Codes

CODE	DESCRIPTION	COMMENT
E	EBB TIDE	STAGE OF WATER MOVEMENT FROM A HIGHER TO A LOWER LEVEL.
F	FLOOD TIDE	STAGE OF WATER MOVEMENT FROM A LOWER TO A HIGHER LEVEL.
L	LOWER SLACK TIDE	STAGE OF WATER WHERE THE LEVEL IS BELOW MEAN AND VELOCITY APPROACHES ZERO
H	HIGH SLACK TIDE	STAGE OF WATER WHERE THE LEVEL IS ABOVE MEAN AND VELOCITY APPROACHES ZERO
BLANK	NOT RECORDED	NOT APPLICABLE

Weather Codes

CODE	DESCRIPTION
10	NONE
11	DRIZZLE
12	RAIN
13	HEAVY RAIN
14	SQUALLY
15	FROZEN PRECIPITATION
16	MIXED RAIN AND SNOW
BLANK	NOT RECORDED, OR NOT APPLICABLE

Percentage Cloud Cover Codes

PERCENTAGE CLOUD COVER IS REPORTED AS VALUES FROM 0 –100 %

Dissolved Oxygen Method Codes

CODE	DESCRIPTION
W	WINKLER METHOD
M	YSI METER
H	HYDROLAB
BLANK	NOT RECORDED, OR NOT APPLICABLE

'Value Corrected' Codes: These codes are designed to specify if corrections have been made by the instrument calculation for the dissolved oxygen value.

CODE	DESCRIPTION
N	NO CORRECTION
T	TEMPERATURE CORRECTION ONLY
C	TEMPERATURE AND CONDUCTIVITY CORRECTION

Sample Layer Codes

CODE	DESCRIPTION
S	SURFACE SAMPLE
AP	ABOVE PYCNOCLINE
BP	BELOW PYCNOCLINE
B	BOTTOM SAMPLE
M	MID-DEPTH SAMPLE
BLANK	NOT RECORDED, OR NOT APPLICABLE

Wind Direction Codes

CODE	DESCRIPTION
E	FROM THE EAST (90 DEGREES)
ENE	FROM THE EAST NORTHEAST (67.5 DEGREES)
ESE	FROM THE EAST SOUTHEAST (112.5 DEGREES)
N	FROM THE NORTH (0 DEGREES)
NE	FROM THE NORTHEAST (45 DEGREES)
NNE	FROM THE NORTH NORTHEAST (22.5 DEGREES)
NNW	FROM THE NORTH NORTHWEST (337.5 DEGREES)
NW	FROM THE NORTHWEST (315 DEGREES)
S	FROM THE SOUTH (180 DEGREES)
SE	FROM THE SOUTHEAST (135 DEGREES)
SSE	FROM THE SOUTH SOUTHEAST (157.5 DEGREES)
SSW	FROM THE SOUTH SOUTHWEST (202.5 DEGREES)
SW	FROM THE SOUTHWEST (225 DEGREES)
W	FROM THE WEST (270 DEGREES)
WNW	FROM THE WEST NORTHWEST (292.5 DEGREES)
WSW	FROM THE WEST SOUTHWEST (247.5 DEGREES)
NR	NOT RECORDED, OR NOT APPLICABLE

LABORATORY DATA SHEETS

COMPUTER CODES FOR NUTRIENT PARAMETER ANALYSES SHEET

Submitter Codes: The codes are the same as for field data sheets

Data Category Codes: The codes are the same as for field data sheets

Sample Method Codes: The codes are the same as for field data sheets

Sample Layer Codes: The codes are the same as for field data sheets

Study Codes: The codes are the same as for field data sheets

Parameter Codes:

CODE	DESCRIPTION	UNIT
BIOSI	PARTICULATE BIOGENIC SILICA	mg/L
BOD5W	FIVE DAY BIOLOGICAL OXYGEN DEMAND	mg/L
CHLA	ACTIVE CHLOROPHYLL A	µg/L
DOC	DISSOLVED ORGANIC CARBON AS C	mg/L
DON	DISSOLVED ORGANIC NITROGEN AS N	mg/L
DOP	DISSOLVED ORGANIC PHOSPHORUS AS P	mg/L
TDS	DISSOLVED SOLIDS if on filtered water sample	mg/L
FCOL_M	FECAL COLIFORM	MPN/100ml
FE_M	TOTAL IRON	mg/L
NH4F	AMMONIA AS N (FILTERED)	mg/L
NH4W	AMMONIA AS N (WHOLE)	mg/L
NO2F	NITRITE AS N (FILTERED)	mg/L
NO2W	NITRITE AS N (WHOLE)	mg/L
NO23F	NITRITE + NITRATE AS N (FILTERED)	mg/L
NO23W	NITRITE + NITRATE AS N (WHOLE)	mg/L
NO3F	NITRATE AS N (FILTERED)	mg/L
NO3W	NITRATE AS N (WHOLE)	mg/L
PC	PARTICULATE ORGANIC CARBON AS C	mg/L
PHEO	MONOCHROMATIC PHEOPHYTIN A	µg/L
PIP	PARTICULATE INORGANIC PHOSPHORUS	mg/L
PN	PARTICULATE ORGANIC NITROGEN AS N	mg/L
PO4F	DISSOLVED ORTHOPHOSPHATE AS P (FILTERED)	mg/L
PO4W	DISSOLVED ORTHOPHOSPHATE AS P (WHOLE)	mg/L
PP	PARTICULATE PHOSPHORUS AS P	mg/L
SIF	REACTIVE SILICA AS SI (FILTERED)	mg/L
SIW	REACTIVE SILICA AS SI (WHOLE)	mg/L
SO4F	SULFATE (FILTERED)	mg/L
SO4W	SULFATE (WHOLE)	mg/L
TALK	TOTAL ALKALINITY	mg/L
TCOLI_M	TOTAL COLIFORM	MPN/100ml
TDN	TOTAL DISSOLVED NITROGEN AS N (FILTERED)	mg/L
TDP	TOTAL DISSOLVED PHOSPHORUS AS P (FILTERED)	mg/L
TKNF	TOTAL KJELDAHL NITROGEN AS N (FILTERED)	mg/L
TKNW	TOTAL KJELDAHL NITROGEN AS N (WHOLE)	mg/L
TN	TOTAL NITROGEN	mg/L
TOC	TOTAL ORGANIC CARBON	mg/L
TP	TOTAL PHOSPHORUS	mg/L
TSS	TOTAL SUSPENDED SOLIDS	mg/L
TURB_NTU	TURBIDITY	NTU

Analytical Problem Codes (APC):

TEA Maryland Department of Natural Resources Tidal Ecosystem Assessment
CBP Environmental Protection Agency Chesapeake Bay Program Office
CBL University Of Maryland Center for Environmental Science Chesapeake Biological Laboratory Nutrient Analytical Services Laboratory
DHMH Maryland Department of Health and Mental Hygiene

TEA Problem Code	CBP Problem Code	CBL Problem Code	DHMH Problem Code	Description
A	A	1	A	LABORATORY ACCIDENT
B	B		B	CHEMICAL MATRIX INTERFERENCE
BB	BB	19	BB	TORN FILTER PAD
C	C	12	C	INSTRUMENT FAILURE
CC				PAD UNFOLDED IN FOIL POUCH
D	D	2	D	INSUFFICIENT SAMPLE
DD	DD	15	DD	SAMPLE SIZE NOT REPORTED (ASSUMED)
DM	V			LAB SAMPLE DEPTH MISMATCH WITH FIELD SAMPLE DEPTH
E	E		E	SAMPLE RECEIVED AFTER HOLDING TIME
EE				FOIL POUCH VERY WET (SALTY) WHEN RECEIVED FROM FIELD; MEAN REPORTED
F	F			POST-CALIBRATION FAILURE LIKELY DUE TO EQUIPMENT DAMAGE AFTER SAMPLING; DATA APPEAR NORMAL
FF	FF	14	FF	POOR REPLICATION BETWEEN PADS, MEAN REPORTED
GG	GG		GG	SAMPLE ANALYZED AFTER HOLDING TIME
H	H	3	H	ANALYSIS RUN BY ANOTHER LAB
	I			SUSPECT VALUE HAS BEEN VERIFIED CORRECT
J	J		J	INCORRECT SAMPLE FRACTION FOR ANALYSIS
JJ	JJ		JJ	VOLUME FILTERED NOT RECORDED (ASSUMED)
K	X	4	K	SAMPLE FROZEN WHEN RECEIVED (RESULT QUESTIONABLE)
KK	KK		KK	PARAMETER NOT REQUIRED FOR STUDY
	L			LICOR CALIBRATION OFF BY >=10% PER YEAR. USE WITH CALC KD WHERE PROB OF LU, LS, LB EXIST IN RAW
	LB			LICOR CALIBRATION OFF BY >= 10% PER YEAR FOR BOTH AIR AND UPWARD FACING SENSORS
LL	LL	16	LL	SAMPLE MISLABELED
	LS			LICOR CALIBRATION OFF BY >= 10% PER YEAR FOR AIR SENSOR

Analytical Problem Codes (APC) continued:

TEA Problem Code	CBP Problem Code	CBL Problem Code	DHMH Problem Code	Description
	LU			LICOR CALIBRATION OFF BY >= 10% PER YEAR FOR UPWARD FACING SENSOR
M	X	5	M	SAMPLE RECEIVED WARM
MM	MM	17	MM	OVER 20% OF SAMPLE ADHERED TO POUCH AND OUTSIDE OF PAD
N		6		SAMPLE LOST
NN	NN	21	NN	PARTICULATES FOUND IN FILTERED SAMPLE
P	A	7	P	LOST RESULTS
PP	D	22	PP	ASSUMED SAMPLE VOLUME
QQ	QQ	23	QQ	PART EXCEEDS WHOLE VALUE, YET DIFFERENCE IS WITHIN ANALYTICAL PRECISION
R	R	8	R	SAMPLE CONTAMINATED
RR	RR	18		NO SAMPLE RECEIVED
S	A		S	SAMPLE CONTAINER BROKEN DURING ANALYSIS
SS	SS			SAMPLE REJECTED, HIGH SUSPENDED SEDIMENT CONCENTRATIONS
T	T		T	NO PHEOPHYTIN IN SAMPLE
U	U		U	MATRIX PROBLEM RESULTING OF THE INTERRELATIONSHIP BETWEEN VARIABLES SUCH AS PH AND AMMONIA
UU	V			ANALYSIS DISCONTINUED
V	V	9	X	SAMPLE RESULTS REJECTED DUE TO QUALITY CONTROL CRITERIA
VV	VV			STATION NOT SAMPLED DUE TO BAD FIELD CONDITIONS
W	RR		V	DUPLICATE RESULTS FOR ALL PARAMETERS
WW	WW			HIGH OPTICAL DENSITY (750 NM); ACTUAL VALUE REPORTED
X	X	10		SAMPLE NOT PRESERVED PROPERLY
XX	V			SAMPLING FOR THIS VARIABLE NOT INCLUDED IN THE MONITORING PROGRAM AT THIS TIME
Y	V	11	Y	ANALYZED IN DUPLICATE, RESULTS BELOW DETECTION LIMIT
Z	Z		Z	ANALYZED BY METHOD OF STANDARD ADDITIONS

Detection Limit Codes:

CODE	DESCRIPTION
BLANK	NORMAL
G	GREATER THAN THE UPPER METHOD DETECTION LIMIT (MDL)
L	LESS THAN THE LOWER METHOD DETECTION LIMIT (MDL) AND STORED LOWER DETECTION LIMIT
U	VALUE LESS THAN LOWER METHOD DETECTION LIMIT (MDL) AND STORED IN REAL VALUE

Method Codes:

CODE	METHOD TITLE	UNIT	METHOD
BOD5W	5-DAY BIOCHEMICAL OXYGEN DEMAND	mg/L	L01
CHLA	MONOCHROMATIC; SPECTROPHOTOMETRIC	µg/L	L01
DOC	COMBUSTION INFRARED METHOD	mg/L	L01
FE_M	TOTAL IRON; PHENANTHROLINE METHOD	mg/L	L01
NH4F	COLORIMETRIC; AUTOMATED PHENATE (INDOPHENOL)	mg/L	L01
NO23F	ENZYME CATALYZED NITRATE REDUCTION	mg/L	L03
NO2F	AUTOMATED; COLORIMETRIC; DIAZOTIZATION	mg/L	L01
NO3F	CALCULATED NO3F (SUBMITTED TO CBPO)	mg/L	C01
PC	PARTICULATE CARBON	mg/L	L01
PHEO	MONOCHROMATIC; SPECTROPHOTOMETRIC	µg/L	L01
PN	PARTICULATE NITROGEN	mg/L	L01
PO4F	ORTHOPHOSPHATE; AUTOMATED; ASCORBIC ACID	mg/L	L01
PP	PARTICULATE PHOSPHORUS; SEMI-AUTOMATED; DIRECT	mg/L	L01
SIF	COLORIMETRIC; AUTOMATED; MOLYBDENUM BLUE	mg/L	L01
SO4F	SULFATE; TURBIDIMETRIC METHOD	mg/L	L01
TALK	ALKALINITY; TITRIMETRIC; pH 4.5	mg/L	L01
TDN	ALKALINE PERSULFATE WET OXIDATION + ENZYME CATALYZED NITRATE REDUCTION	mg/L	L02
TDP	ALKALINE PERSULFATE WET OXIDATION + EPA365.1OR EPA 365	mg/L	L01
TDS	TOT. DISSOLVED SOLIDS; GRAVIMETRIC; DRIED AT 180 C	mg/L	L01
TKNF	SEMI-AUTOMATED BLOCK DIGESTOR; COLORIMETRIC; NITRO	mg/L	L02
TKNW	SEMI-AUTOMATED BLOCK DIGESTOR; COLORIMETRIC; NITRO	mg/L	L02
TSS	GRAVIMETRIC; DRIED AT 103-105 C	mg/L	L01
TURB_NTU	NEPHELOMETRIC	NTU	L01

COMPUTER CODES FOR CHLOROPHYLL PARAMETER ANALYSIS SHEET

Submitter Codes: The codes are the same as field data sheets

Data Category Codes: The codes are the same as field data sheets

Sample Layer Codes: The codes are the same as field data sheets

Study Codes: The codes are the same as field data sheets

Analytical Problem Codes: The codes are the same as laboratory data sheets

APPENDIX XII

MARYLAND DEPARTMENT OF NATURAL RESOURCES
CHESAPEAKE BAY
WATER QUALITY MONITORING PROGRAM

DATA ENTRY REQUEST FORM DOCUMENTATION AND PROCEDURES

When submitting a job for data entry service, a data entry request form must be completed with the following information. A sample data entry request form is attached to the end of this appendix for reference.

1. APPLICATION REQUEST ID OR JOB ID (upper right hand corner)

   Enter the application request ID number using application procedure ID information provide at the end of this appendix. An example of the application ID is 'A34210CB'.

2. TYPE OF JOB REQUEST

   Check one of the four boxes indicating the type of job request (i.e. SCHEDULE, TEST, SPECIAL, OR RERUN). Most commonly, "SCHEDULE" will be checked, because the job is usually a scheduled request.

3. ESTIMATED VOLUME

   This space can be used to enter the number of data sheets that will be keypunched but, in practice, it generally is not used.

4. REQUESTED BY

   Fill in the name of the person who is requesting the work to be keypunched.

5. REQUESTED COMPLETION DATE

   Indicate the date when the job must be completed. According to our current contract with a data entry service, at least three business days is a reasonable time frame for one month's set of data sheets.

6. REQUESTED COMPLETION TIME

   Indicate the time when the job must be completed.

7. AGENCY

Enter ‘MARYLAND DEPARTMENT OF NATURAL RESOURCES’ as the name of the agency issuing the job request.

8. CONTACT

Enter the name of the DNR contact responsible for the job request.

8. TELEPHONE NUMBER

Enter the telephone number of the contact person. Include the telephone extension where applicable.

9. CONTROL INFORMATION

a. DELIVER DOCUMENTS TO:

Enter the name and address of the agency requesting the job, i.e.,:

Maryland Department of Natural Resources
Tidewater Ecosystem Assessment
580 Taylor Avenue, D-2
Annapolis, MD 21401

b. DELIVER DVD TO:

Enter the name and address of the agency requesting the job, i.e.,:

Maryland Department of Natural Resources
Tidewater Ecosystem Assessment
580 Taylor Avenue, D-2
Annapolis, MD 21401

10. DATA SET NAME

Enter the name of the .ORG file. For example, for Mainstem May 2007 laboratory data, use the description ‘MAY07LAB.ORG’.

Note that the following fields generally do not need to be filled out:

AGENCY CONTROL NO
D.P.D. CONTROL NO
DATE/TIME STAMP
JRT No.

INFORMATION FOR APPLICATION PROCEDURE ID

This section contains the application procedure ID for the various types of data sheets used for the Chesapeake Bay Monitoring Program (e.g., Field Sheets, Laboratory Sheets, and Chlorophyll Sheets). This ID number is needed for Data Entry Request Forms. The data manager will issue a new application procedure ID as needed for new projects.

1. Field Data Sheets for the Chesapeake Bay Mainstem and Maryland Tributaries

   Application Procedure ID: A34202CB

2. Field Data Sheets for Patuxent River Intensive Survey

   Application Procedure ID: A34200CB

3. Laboratory Data Sheets for the Maryland Tributaries

   Application Procedure ID: A34204CB

4. Chlorophyll Data Sheets for the Chesapeake Bay Mainstem, Maryland Tributaries, and Patuxent River Intensive Survey

   Application Procedure ID: A34205CB

MARYLAND DEPARTMENT OF NATURAL RESOURCES
RESOURCES ASSESSMENT ADMINISTRATION

DATA ENTRY REQUEST FORM

AGENCY CONTROL NO : 0002 D.P.D. CONTROL NO : _____

DATE / TIME STAMP	
	AGENCY PREPARED
	AGENCY RELEASED
	D. P. D. RECEIVED
	D. P. D. RELEASED
	AGENCY RECEIVED

APPLICATION REQUEST ID (JOB ID)

A 34202 CB

☑ SCHEDULE ☐ TEST

☐ SPECIAL ☐ RERUN

EST. VOLUMN _____ RERUN OF JRT _____

REQUESTED BY : Renée V. Randall AGENCY : DNR

REQUESTED COMPLETION DATE: 3/17/06 CONTACT: Tyrone Lee

REQUESTED COMPLETION TIME : 12:00 P.M. TELEPHONE: (410) 260-8643

SPECIAL INSTRUCTIONS TO D.P.D.

CONTROL INFORMATION	JRT NO	DATA SET NAME
DELIVER DOCUMENTS TO :		FEB06FID Org
DNR		
Tawes State Office Bldg., D-2		
580 Taylor Ave.		
Annapolis, Md. 21401		
DELIVER MAGNETIC TAPE W/ TAPE REQUEST TO :		
DNR		
Tawes State Office Bldg., D-2		
580 Taylor Ave.		
Annapolis, Md. 21401		

REMARK

MARYLAND DEPARTMENT OF NATURAL RESOURCES
DATA STATUS FORM

Control No.: 0002

Date Initiated: 2/22/06

* D.M. function ** D.M. verify only

* Data Set Name: FEB06FLD

		Project Dates	Initials	Comments
*	Data Received	2/22/06	RVR	
*	Data Reviewed	2/22/06	RVR	
**	Cross Reference			
*	Xeroxing Field sheets			
**	Data Entry Field/Lab/ Patuxent — Sent	3/16/06	RVR	
	Data Entry Field/Lab/ Patuxent — Returned	3/23/06	RVR	
*	Initial Data Check			
*	Data Verification			
*	Temporary Merge(s)			

BIO CHECK		Check Records (Date/Initial) 1	2	3	4	Comments
B I O C H E C K	Verification(s)					
*	Edit(s) Identified					
*	Data Correction(s)					
*	Temporary Merge(s)					
	Biologist Sign Off					
*	Final Data Correction					
*	Merge Completed					
*	Generate FLC Data Set					
*	Generate EPA FLC Data					
*	Submission Document					
*	Submission Letter					
	Final Sign Off					
	Submission to CBP					
	CBP Data Check List(s)					
	CBP Accepts / Signoff					

Appendix XIII. Sample Verification Reports and Plots and Edit Form

Maryland Department of Natural Resources

Field Sheet

Station Name: CB5.3 | Project Code: MAIN | Sequence Number: 0600201

Sample Date	Arrival Time	Departure Time	Sample Number	Measured Depth	Air Temperature	Tide Code	Weather Yesterday	Weather Today	Cloud Cover (%)	Wave Height
2/7/2006	10:55	11:15	4	27.5	4	F	10	10		0.4

Wind Direction	Wind Min. Velocity	Wind Max. Velocity	Equipment Set Unit No.	Probe Number	Photometer Unit Number	Pycnocline Lower	Pycnocline Upper	Secchi	G/L
W	7	10	9V	9V		17.5	10.5	02.00	

Description :

Parameter List:

Rep	Sample depth	Water Temp	PH	DO	SPCOND	Salinity	Calc. Salinity	Rep Code	Sample depth	Layer Code	INSDEC	INSUW	EPAR_S	EPARU_Z
1	0.5	5.7	8.2	11.9	21700	13	12.86	1	0.5	S				
1	1	5.7	8.2	11.8	21700	13	12.86	1	1	M				
1	2	5.7	8.2	11.9	21700	13	12.86	1	2	M				
1	3	5.7	8.2	11.8	21700	13	12.86	1	3	M				
1	5	5.8	8.3	12	21700	13	12.86	1	5	M				
1	7	5.8	8.2	12	21800	13	12.93	1	7	M				
1	9	5.8	8.2	11.7	21800	13	12.93	1	9	AP				
1	10	5.8	8.2	11.5	21800	13.1	12.93	1	10	M				
1	11	5.8	8.1	10.7	24000	14.1	14.4	1	11	M				
1	12	5.9	8	9.8	28900	18	18.41	1	12	M				
1	13	5.9	7.9	9.6	30000	18.6	18.48	1	13	M				
1	14	5.9	8	9.6	30100	18.6	18.55	1	14	M				
1	15	5.9	7.9	9.5	30200	18.7	18.62	1	15	M				
1	16	5.9	7.9	9.5	30300	18.7	18.69	1	16	M				
1	17	5.9	7.9	9.5	30500	18.9	18.83	1	17	M				
1	18	6	7.9	9.5	31300	19.4	19.38	1	18	M				
1	19	6	8	9.5	31400	19.5	19.45	1	19	BP				
1	21	6.1	8	9.5	31000	19.7	19.17	1	21	M				

Tuesday, March 28, 2006 | Sample Agency | Sample Officer | Page 1 of 20

Maryland Department of Natural Resources

Chlorophyll Sheet — Chl Sequence No: 3-460

Project Code MAIN **SampleDate:** 2/7/2006

Parameters:

Station Name	SEQ	Rep #	Layer Code	Sample Depth	EXVOL_ML	APC CODE	LIPAT_CM	SAMVOL_L	OD630B	OD645B	OD647B	OD663B	OD664B	OD665A	OD750A	OD750B	PHEO	CHLA
CB5.2	3-460	1	S	0.5	14		5	0.50	0.038	0.035	0.039	0.135	0.136	0.085	0.005	0.005	000.748	007.626
CB5.2	3-460	2	S	0.5	14		5	0.50	0.037	0.035	0.039	0.132	0.132	0.082	0.005	0.005	000.583	007.476
CB5.3	3-460	1	S	0.5	14		5	0.50	0.056	0.052	0.059	0.210	0.210	0.128	0.006	0.005	000.359	012.410
CB5.3	3-460	1	AP	9	14		5	0.50	0.058	0.054	0.062	0.218	0.218	0.134	0.007	0.007	000.733	012.560
CB5.3	3-460	1	BP	19	14		5	0.40	0.042	0.039	0.045	0.152	0.152	0.098	0.005	0.005	002.075	010.093
CB5.3	3-460	1	B	26	14		5	0.40	0.047	0.045	0.051	0.169	0.169	0.115	0.008	0.006	003.532	010.466
LE2.3	3-460	1	S	0.5	14		5	0.50	0.052	0.048	0.054	0.192	0.192	0.117	0.006	0.006	000.404	011.214
LE2.3	3-460	1	AP	7	14		5	0.50	0.069	0.064	0.073	0.263	0.264	0.160	0.007	0.005	000.164	015.849
LE2.3	3-460	1	BP	13	14		5	0.40	0.059	0.054	0.062	0.227	0.227	0.136	0.005	0.005	000.131	017.008
LE2.3	3-460	1	B	19	14		5	0.40	0.121	0.106	0.120	0.432	0.434	0.272	0.007	0.006	004.205	030.465

Tuesday, March 28, 2006 — Analysis Agency: DNR/TEA — Analysis Officer — Page 1 of 9

Appendix XIII. Sample Verification Reports and Plots and Edit Form

Maryland Department of Natural Resources

Lab Sheet

Station Name	Project	Sample Date	Arrival Time	Sample Depth	Layer Code	Replicate Number	Sample Number	Sequence Number
CB5.3	MAIN	2/7/2006	10:55	0.5	S	1	4	200602070001

Sample Description :

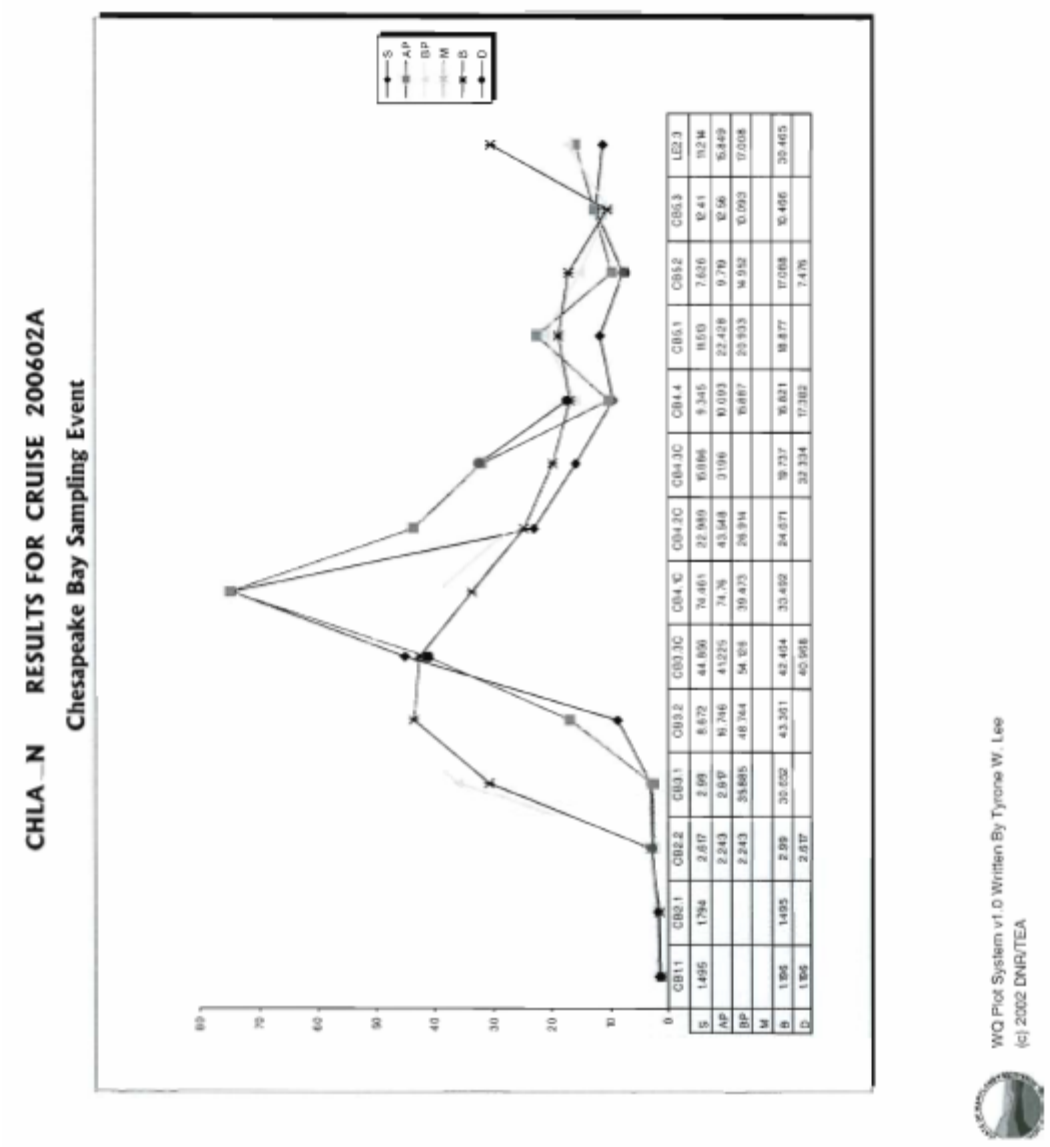
LAB Description :

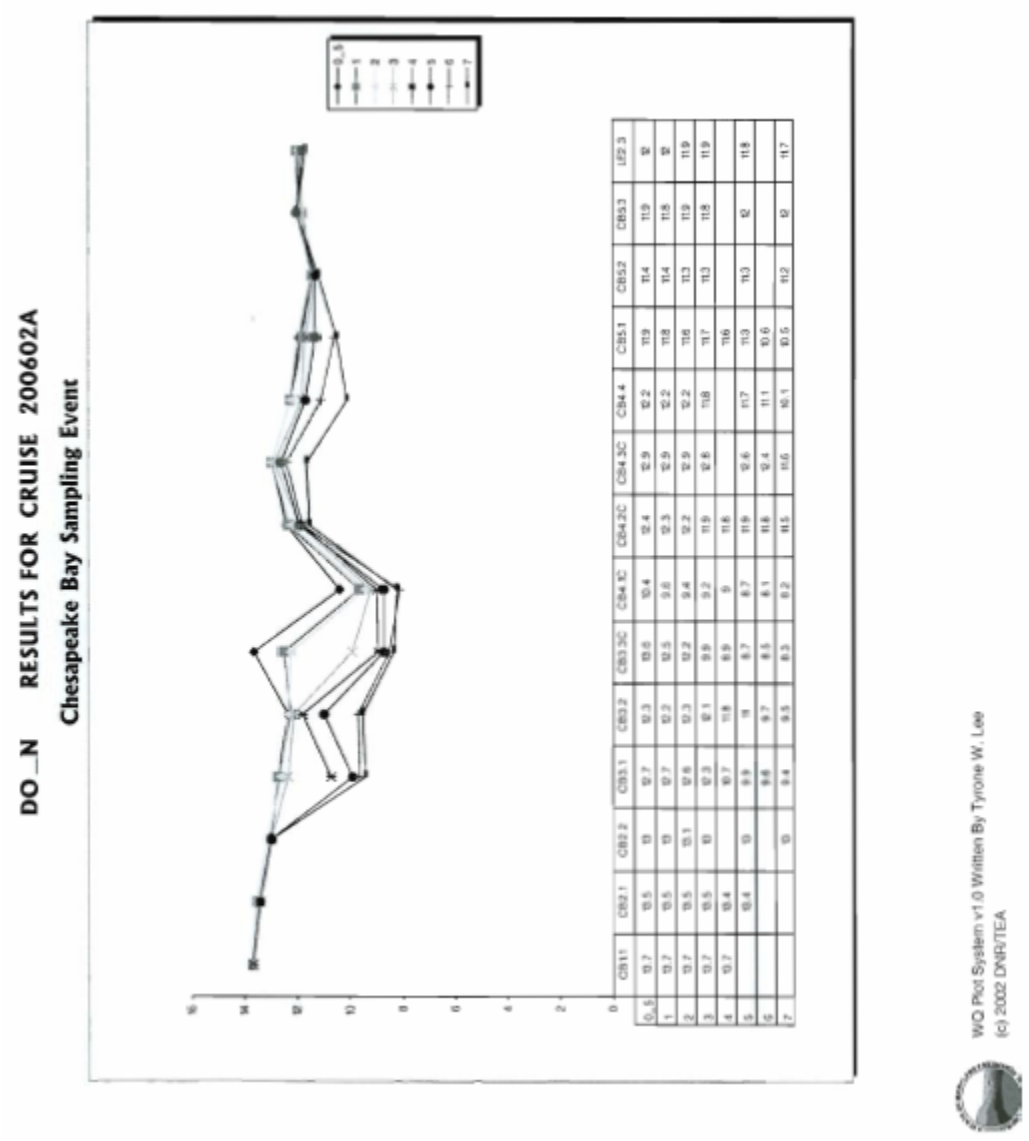
Parameters	Type	Method Code	APC Code	DL	Value	Visible	Enabled	Pseudo	Calculated
NH4	F	L01		<	0.003	☑	☑	☑	☐
NO2	F	L01			0.0063	☑	☑	☐	☐
NO23	F	L01			0.156	☑	☑	☐	☐
PC	N	L01			1.14	☑	☑	☐	☐
PN	N	L01			0.18	☑	☑	☐	☐
PO4	F	L01			0.002	☑	☑	☐	☐
PP	N	L01			0.0099	☑	☑	☐	☐
SI	F	L01			0.4	☑	☑	☐	☐
TDN	N	L01			0.5	☑	☑	☐	☐
TDP	N	L01			0.0096	☑	☑	☐	☐
TSS	N	L01			3.7	☑	☑	☐	☐

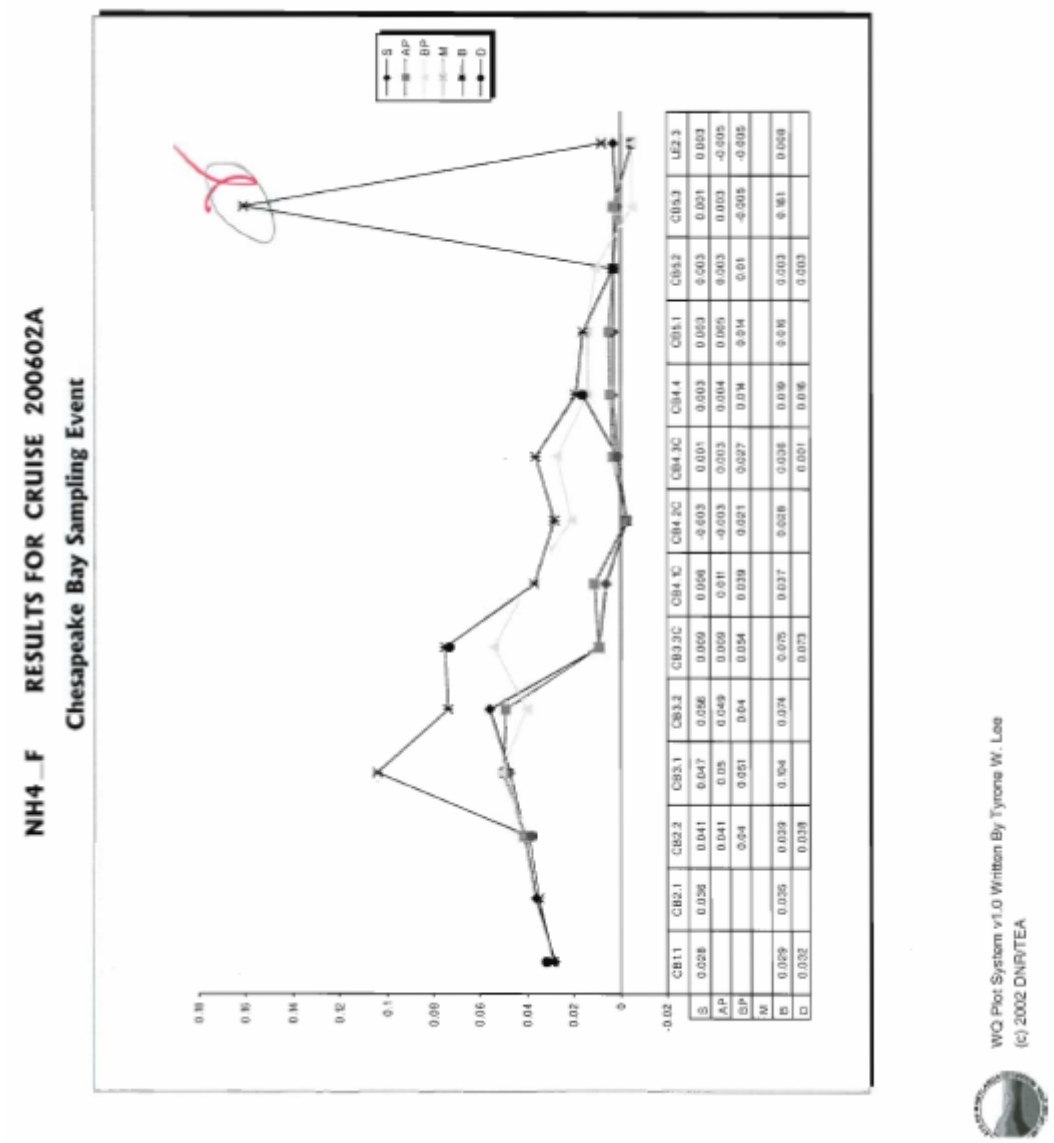
Actual Parameters	Type	Method Code	APC Code	DL	Value	Visible	Enabled	Pseudo	Calculated
NH4	F	L01		<	0.001	☐	☑	☐	☐

Tuesday, March 28, 2006 Analysis Agency DNR/TEA Analysis Officer DNR/SAD *Page 1 of 58*

Appendix XIII. Sample Verification Reports and Plots and Edit Form




DO_N RESULTS FOR CRUISE 200602A
Chesapeake Bay Sampling Event
WQ Plot System v1.0 Written By Tyrone W. Lee
(c) 2002 DNR/TEA


NH4_F RESULTS FOR CRUISE 200602A
Chesapeake Bay Sampling Event
S
AP
BP
M
B
D
WQ Plot System v1.0 Written By Tyrone W. Lee
(c) 2002 DNR/TEA

Appendix XIII. Sample Verification Reports and Plots and Edit Form

0600206	CB4.3C	MAIN	200602A	2/8/2006	0.5		S		1				EQ_NUM	
0600207	CB4.2C	MAIN	200602A	2/8/2006	0.5		S		1				EQ_NUM	
0600208	CB4.1C	MAIN	200602A	2/8/2006	0.5		S		1				EQ_NUM	
0600209	CB3.3C	MAIN	200602A	2/8/2006	0.5		S		1				EQ_NUM	
0600210	CB3.2	MAIN	200602A	2/8/2006	0.5		S		1				EQ_NUM	
0600211	CB3.1	MAIN	200602A	2/9/2006	0.5		S		1				EQ_NUM	
0600212	CB2.2	MAIN	200602A	2/9/2006	0.5		S		1				EQ_NUM	
0600213	CB2.1	MAIN	200602A	2/9/2006	0.5		S		1				EQ_NUM	
0600214	CB1.1	MAIN	200602A	2/9/2006	0.5		S		1				EQ_NUM	

Column Check: Parameter Values are falling outside of a reasonable range

Field Seq No	StationName	Project Code	SampleDate	Depth	Layer	Rep. No	Parameter	Value	Lower DL	Upper DL
0600201	CB5.3	MAIN	2/7/2006	0.5	S	1	WTEMP	5.7	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	1	M	1	WTEMP	5.7	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	2	M	1	WTEMP	5.7	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	3	M	1	WTEMP	5.7	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	5	M	1	WTEMP	5.6	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	7	M	1	WTEMP	5.6	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	9	AP	1	WTEMP	5.6	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	10	M	1	WTEMP	5.6	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	11	M	1	WTEMP	5.8	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	12	M	1	WTEMP	5.9	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	13	M	1	WTEMP	5.9	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	14	M	1	WTEMP	5.9	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	15	M	1	WTEMP	5.9	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	16	M	1	WTEMP	5.9	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	17	M	1	WTEMP	5.9	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	18	M	1	WTEMP	6	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	19	BP	1	WTEMP	6	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	21	M	1	WTEMP	6.1	-0.5	5
0600201	CB5.3	MAIN	2/7/2006	23	M	1	WTEMP	6.1	-0.5	5

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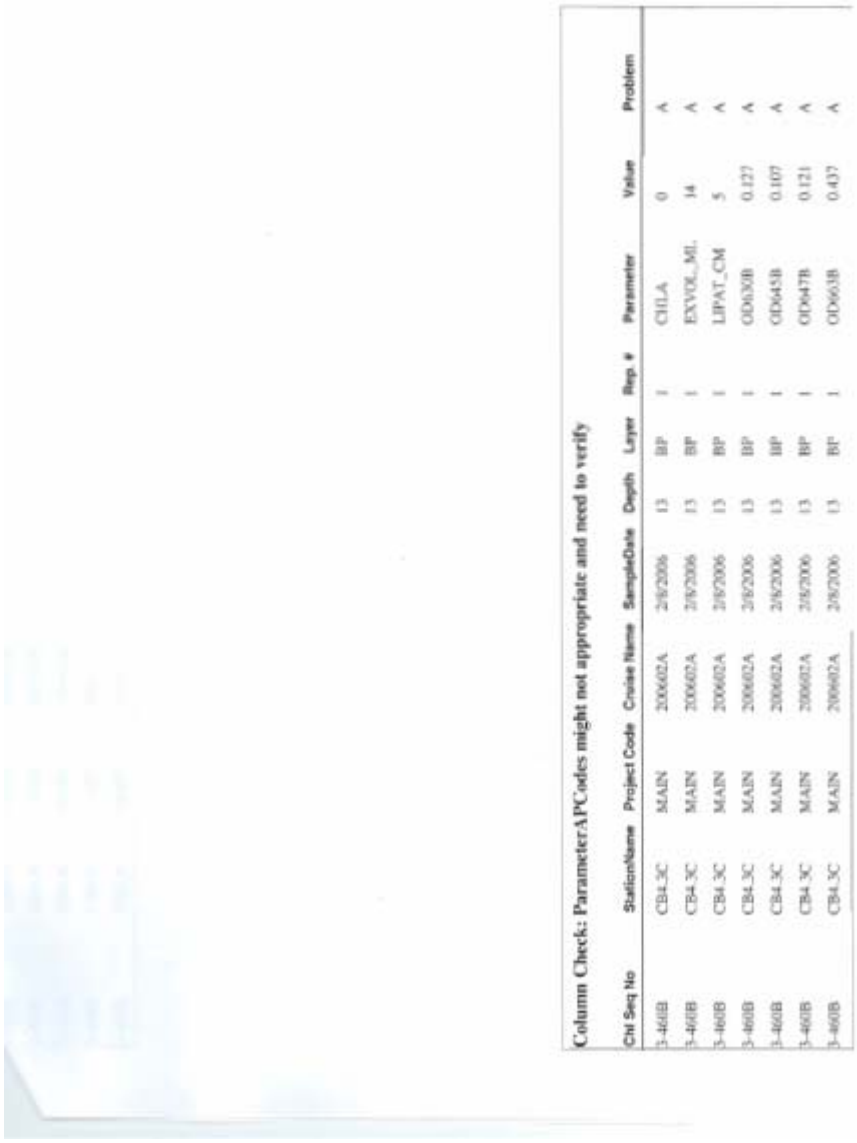
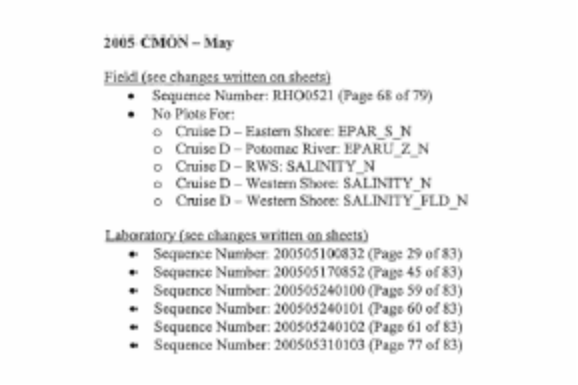
Appendix XIII. Sample Verification Reports and Plots and Edit Form



Column Check: Parameter:APCodes might not appropriate and need to verify

Chl Seq No	StationName	Project Code	Cruise Name	SampleDate	Depth	Layer	Rep #	Parameter	Value	Problem
3-460B	CB4.3C	MAIN	2006602A	2/8/2006	13	BP	1	CHLA	0	A
3-460B	CB4.3C	MAIN	2006602A	2/8/2006	13	BP	1	EXVOL_ML	14	A
3-460B	CB4.3C	MAIN	2006602A	2/8/2006	13	BP	1	LIPAT_CM	5	A
3-460B	CB4.3C	MAIN	2006602A	2/8/2006	13	BP	1	OD630B	0.127	A
3-460B	CB4.3C	MAIN	2006602A	2/8/2006	13	BP	1	OD645B	0.107	A
3-460B	CB4.3C	MAIN	2006602A	2/8/2006	13	BP	1	OD647B	0.121	A
3-460B	CB4.3C	MAIN	2006602A	2/8/2006	13	BP	1	OD663B	0.437	A

Appendix XIII. Sample Verification Reports and Plots and Edit Form



2005 CMON – May

Field (see changes written on sheets)

- Sequence Number: RHO0521 (Page 68 of 79)
- No Plots For:
  - Cruise D – Eastern Shore: EPAR_S_N
  - Cruise D – Potomac River: EPARU_Z_N
  - Cruise D – RWS: SALINITY_N
  - Cruise D – Western Shore: SALINITY_N
  - Cruise D – Western Shore: SALINITY_FLD_N

Laboratory (see changes written on sheets)

- Sequence Number: 200505100832 (Page 29 of 83)
- Sequence Number: 200505170852 (Page 45 of 83)
- Sequence Number: 200505240100 (Page 59 of 83)
- Sequence Number: 200505240101 (Page 60 of 83)
- Sequence Number: 200505240102 (Page 61 of 83)
- Sequence Number: 200505310103 (Page 77 of 83)

APPENDIX XIV

CHESAPEAKE BAY MONITORING PROGRAM
PROCEDURE MODIFICATION TRACKING FORM

PMTF # _____ ☐ **APPROVED** ☐ **DENIED**

This form is used to request approval for modifications and to document approved modifications made to Chesapeake Bay Program Office procedures or methods. It is not a substitute for timely contact with the CBPO Quality Assurance Officer or his/her designee, who may be reached at 1-800-968-7229. A detailed method description including the proposed modification must be attached to this form prior to submittal to CBPO.

DATE SUBMITTED		DATE APPROVED	
REQUESTOR NAME		ORGANIZATION	
NEWLY PROPOSED MODIFICATION [ ]	FIELD-APPROVED MODIFICATION [ ]	APPROVED BY: DATE:	
TYPE OF PROCEDURE / METHOD	SAMPLING [ ] FIELD MEASUREMENT [ ]	ANALYTICAL [ ] OTHER SPECIFY:	REPORTING [ ]
DURATION	PERMANENT [ ] TEMPORARY [ ]	EFFECTIVE DATE: START DATE: END DATE:	
PROCEDURE/METHOD DESCRIPTION			
MODIFICATION DESCRIPTION			
JUSTIFICATION FOR MODIFICATION			
ANALYTICAL PARAMETERS THAT MAY BE AFFECTED BY THIS CHANGE			
AFFECTED QA PLAN(S) (TITLE, REVISION, & DATE)			
AFFECTED CRUISE(S)			
PMTF COMPLETED BY	NAME:		DATE:

STATE APPROVAL: NAME _____ TITLE _____

SIGNATURE _____ DATE _____

CBPO APPROVAL: NAME _____ TITLE _____

SIGNATURE _____ DATE _____

APPENDIX VX

MARYLAND DEPARTMENT OF NATURAL RESOURCES
CHESAPEAKE BAY WATER QUALITY MONITORING PROGRAM

Log of Significant Changes

Date Initiated	Procedural Changes
See Tables 1, 2, 3 and 4 following the Log	**NOTE** Changes in Measured Parameters and in Detection Limits are detailed in the following tables: • Table 1 - Tributary Detection Limit • Table 2 - Patuxent Detection Limits • Table 3 - Potomac Detection Limits • Table 4 - LE2.3 and Mainstem Detection Limits.
April 1, 1989	Dropped station XCG8613 on the Patuxent
July 1, 1990	Nutrient analysis switched from State lab at Department of Health and Mental Hygiene (DHMH) to University of Maryland Chesapeake Biological Laboratory for Patuxent samples
October 1, 1990	Switch to filtering samples for PO4, NH4, NO23, NO2 in Potomac instead of analyzing whole water sample
December 10, 1990	A data quality assurance issue titled "Adjusting Maryland Department of Health and Mental Hygiene (MDHMH) total phosphorus (TP) and total dissolved phosphorus (TDP) data," was entered into the Data Analysis Issues Tracking System on this date. MDHMH was not using calibration data or blank data in calculating TP and TDP from 1984 through 1989. Most of the data affected by this problem were re-calibrated and re-submitted to the Chesapeake Bay Program. Samples analyzed in 1984 were not re-calculated. Some samples analyzed between 1985 and 1990 were also not re-calibrated due to missing blank data and other problems. As a result, there may be a mix of uncorrected and corrected TP and TDP data in the data base.
January 28, 1992	A report titled "Adjusting helix Kjeldahl nitrogen results: Maryland Chesapeake Bay mainstem water quality monitoring program, 1984-1985" was produced by Computer Sciences Corporation under contract to the U.S. Environmental Protection Agency, contract number 68-WO-0043. The report examined the effects of helix digestion on Kjeldahl nitrogen, which is biased low relative to other digestion methods, and presented adjustment equations.

Date Initiated	Procedural Changes
January 1996	TOC and DOC was dropped from Mainstem sampling
May 1, 1998	Nutrient analysis switched from State lab at Department of Health and Mental Hygiene (DHMH) to University of Maryland Chesapeake Biological Laboratory for Potomac and Minor Tributary samples
March 2003	Addition of 10 new long-term stations previously part of the *Pfiesteria* special project sampling
January 2007	Sampling for Silica was dropped from the tributary monitoring program for all stations except for the 13 Phytoplankton monitoring stations during January-June
January 2008	Second monthly cruises on the Patuxent were dropped for April, June, September and October

Tributary Detection Limits

Calculated Values | Not measured

Time Period	1/1/85-5/31/86	6/1/86-12/31/88	1/1/89-4/30/90	5/1/90-6/30/94	7/1/94-7/11/95	7/12/95-4/30/98	5/1/98-12/31/99	1/1/00-12/31/03	1/1/04-12/31/05	1/1/06-12/31/06	1/1/07-12/31/07	1/1/08-12/31/08
parameter /lab	DHMH	DHMH	DHMH	DHMH	DHMH	DHMH	CBL	CBL	CBL	CBL	CBL	CBL
DOC	1	1	0.8	0.5	0.5	0.5	0.24	0.24	0.15	0.15	0.24	0.24
NH4	0.02	0.008	0.008	0.008	0.008	0.008	0.003	0.003	0.003	0.003	0.003	0.003
NO2	0.002	0.002	0.002	0.002	0.002	0.002	0.0002	0.0002	0.0002	0.0002	0.0006	0.0006
NO23	0.02	0.02	0.02	0.02	0.02	0.002	0.0002	0.0007	0.0007	0.0007	0.0007	0.0007
PC							0.0633	0.0633	0.0633	0.0759	0.0633	0.0633
PN							0.0105	0.0105	0.0105	0.0105	0.0105	0.0105
PO4	0.01	0.004	0.004	0.004	0.004	0.004	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006
PP							0.0012	0.0024	0.0024	0.0024	0.0054	0.0021
SI	0.1	0.1	0.1	0.1	0.1	0.1	0.01	0.01	0.01	0.01	0.08	0.01
TDN							0.02	0.02	0.02	0.02	0.02	0.05
TDP	0.01	0.01	0.01	0.01	0.01	0.01	0.001	0.001	0.001	0.0015	0.0015	0.0015
TKNF	0.1	0.1	0.1	0.1	0.1	0.1						
TKNW	0.1	0.1	0.1	0.1	0.1	0.1						
TOC	1	1	0.8	0.5	0.5	0.5						
TP	0.01	0.01	0.01	0.01	0.01	0.01						
TSS	1	1	1	1	1	1	1.5	2.4	2.4	2.4	2.4	2.4
VSS										0.9	0.9	0.9

Table 1 Tributary Detection Limits

Patuxent Detection Limits

Calculated Values | Not measured

Time Period	1/1/85-5/31/86	6/1/86-12/31/88	1/1/89-4/30/90	5/1/90-6/30/90	7/1/90-12/31/99	1/1/00-12/31/03	1/1/04-12/31/05	1/1/06-12/31/06	1/1/07-12/31/07	1/1/08-12/31/08
parameter/lab	DHMH	DHMH	DHMH	DHMH	CBL	CBL	CBL	CBL	CBL	CBL
DOC	1	1	0.8	0.5	0.24	0.24	0.15	0.15	0.24	0.24
NH4	0.02	0.008	0.008	0.008	0.003	0.003	0.003	0.003	0.003	0.003
NO2	0.002	0.002	0.002	0.002	0.0002	0.0002	0.0002	0.0002	0.0006	0.0006
NO23	0.02	0.02	0.02	0.02	0.0002	0.0007	0.0007	0.0007	0.0007	0.0007
PC					0.0633	0.0633	0.0633	0.0759	0.0633	0.0633
PN					0.0105	0.0105	0.0105	0.0105	0.0105	0.0105
PO4	0.01	0.004	0.004	0.004	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006
PP					0.0012	0.0024	0.0024	0.0024	0.0054	0.0021
SI	0.1	0.1	0.1	0.1	0.01	0.01	0.01	0.01	0.08	0.01
TDN					0.02	0.02	0.02	0.02	0.02	0.05
TDP	0.01	0.01	0.01	0.01	0.001	0.001	0.001	0.0015	0.0015	0.0015
TKNF	0.1	0.1	0.1	0.1						
TKNW	0.1	0.1	0.1	0.1						
TOC	1	1	0.8	0.5						
TP	0.01	0.01	0.01	0.01						
TSS	1	1	1	1	1.5	2.4	2.4	2.4	2.4	2.4
VSS								0.9	0.9	0.9

Table 2 Patuxent Detection Limits

Potomac Detection Limits

Calculated Values Not measured

Time Period	1/1/85-5/31/86	6/1/86-12/31/88	1/1/89-4/30/90	5/1/90-6/30/94	7/1/94-7/11/95	7/12/95-4/30/98	5/1/98-12/31/99	1/1/00-12/31/03	1/1/04-12/31/05	1/1/06-12/31/06	1/1/07-12/31/07	1/1/08-12/31/08
parameter /lab	DHMH	DHMH	DHMH	DHMH	DHMH	DHMH	CBL	CBL	CBL	CBL	CBL	CBL
DOC	1	1	0.8	0.5	0.5	0.5	0.24	0.24	0.15	0.15	0.24	0.24
NH4	0.02	0.008	0.008	0.008	0.008	0.008	0.003	0.003	0.003	0.003	0.003	0.003
NO2	0.002	0.002	0.002	0.002	0.002	0.002	0.0002	0.0002	0.0002	0.0002	0.0006	0.0006
NO23	0.02	0.02	0.02	0.02	0.02	0.002	0.0002	0.0007	0.0007	0.0007	0.0007	0.0007
PC							0.0633	0.0633	0.0633	0.0759	0.0633	0.0633
PN							0.0105	0.0105	0.0105	0.0105	0.0105	0.0105
PO4	0.01	0.004	0.004	0.004	0.004	0.004	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006
PP	0	0	0	0	0	0	0.0012	0.0024	0.0024	0.0024	0.0054	0.0021
SI	0.1	0.1	0.1	0.1	0.1	0.1	0.01	0.01	0.01	0.01	0.08	0.01
TDN	0.08	0.12	0.12	0.12	0.12	0.102	0.02	0.02	0.02	0.02	0.02	0.05
TDP	0.01	0.01	0.01	0.01	0.01	0.01	0.001	0.001	0.001	0.0015	0.0015	0.0015
TKNF	0.1	0.1	0.1	0.1	0.1	0.1						
TKNW	0.1	0.1	0.1	0.1	0.1	0.1						
TOC	1	1	0.8	0.5	0.5	0.5						
TP	0.01	0.01	0.01	0.01	0.01	0.01						
TSS	1	1	1	1	1	1	1.5	2.4	2.4	2.4	2.4	2.4
VSS										0.9	0.9	0.9

Note: PO4, NH4, NO23, NO2 whole water samples prior to October 1990; filtered samples after October 1990

Table 3 Potomac Detection Limits

LE2.3 and Mainstem Detection Limits

Calculated Values | Not measured

parameter	1/1/85-2/28/85	3/1/85-5/15/85	5/16/85-9/30/86	10/1/86-9/31/87	10/1/87-9/19/88	9/20/88--12/31/99	1/1/00-12/31/03	1/1/04-12/31/05	1/1/06-12/31/06	1/1/07-12/31/07	1/1/08-12/31/08
	CBL	CBL	CBL	CBL	CBL	CBL	CBL	CBL	CBL	CBL	CBL
DOC	1	1	0.5	0.5	0.5	0.24*					
NH4	0.04	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
NO2	0.01	0.0005	0.0005	0.0005	0.00015	0.0002	0.0002	0.0002	0.0002	0.0006	0.0006
NO23	0.04	0.0009	0.0009	0.0009	0.00015	0.0002	0.0007	0.0007	0.0007	0.0007	0.0007
PC			0.001		0.001	0.0633	0.0633	0.0633	0.0759	0.0633	0.0633
PN			0.001		0.001	0.0105	0.0105	0.0105	0.0105	0.0105	0.0105
PO4	0.007	0.0016	0.0016	0.0016	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006
PP			0.0013		0.0012	0.0012	0.0024	0.0024	0.0024	0.0054	0.0021
SI	0.1	0.012	0.012	0.012	0.01	0.01	0.01	0.01	0.01	0.08	0.01
TDN			0.03		0.02	0.02	0.02	0.02	0.02	0.02	0.05
TDP	0.012	0.005	0.005	0.012	0.001	0.001	0.001	0.001	0.0015	0.0015	0.0015
TKNF	0.375	0.375		0.2							
TKNW	0.443	0.443		0.2							
TOC	1	1		1							
TP	0.012	0.005		0.012							
TSS	4	4	1	1	1.98	1.5	2.4	2.4	2.4	2.4	2.4
VSS						1.98	1.98	1.98	0.9	0.9	0.9

DOC was discontinued at these stations as of 1/1/1996.

Table 4 LE2.3 and Mainstem Detection Limits

NOTE: Due to logistical considerations, sample for the Tributaries station LE2.3 are collected during Mainstem cruises.