DISSOLVED ORGANIC CARBON: DATA VARIABILITY AND PROCEDURAL RECOMMENDATIONS REPORT FOR AMQAW

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Revision 1
July 1996

DRAFT
Revision 1
July 9, 1996

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ABBREVIATIONS

AMQAW = Analytical Methods & Quality Assurance Workgroup

DOC = Dissolved organic carbon

DIC = Dissolved Inorganic Carbon

CBMP = Chesapeake Bay Monitoring Program

OI = Oceanographic Instruments ampule method

HTC = High Temperature Combustion

H_3PO_4 = Phosphoric Acid

HCL = Hydrochloric Acid

SRM = Standard Reference Material

LABORATORIES NAMES:

CBL = Chesapeake Biological Laboratories, Solomons Island, MD

DEQ = Department of Environmental Quality, Richmond, VA

DHMH = Department of Health and Mental Hygiene, Baltimore, MD

ODU = Old Dominion University, Norfolk, VA

VIMS = Virginia Institute of Marine Science, Gloucester Point, VA

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SECTION I: CBMP DATA GENERATED 1984-1995

INTRODUCTION

The Chesapeake Bay Monitoring Program (CBMP) has been reporting dissolved organic carbon (DOC) data since the program was initiated in 1984. However, the laboratories involved in reporting the data used different instrumentation and methods. Ultimately, data was considered questionable when DOC samples which had been spilt between laboratories were found to show statistically significant differences (US Environmental Protection Agency, 1990-1991).

Beginning with the first Bay cruise in January 1996, DOC data will no longer be reported; however, AMQAW requested that the DOC data generated since the beginning of the program be scrutinized and that 1.) a factor be found, if possible, to improve the comparability of the data, 2.) the affect of salinity on DOC measurements, in general, be assessed, 3.) a DOC procedure be recommended for future DOC work so that future DOC data will be in closer agreement.

TABLE I: SUMMARY OF PAST AND PRESENT CBMP METHODOLOGIES

Laboratories were asked to check their records and indicate what instrument and method was used to analyze past DOC CBMP samples and for what time period such that data could be divided into sections.

LABORATORY	INSTRUMENT	DATES USED	BAY CRUISES REPORTED	METHOD USED
VIMS	Shimadzu TOC-500	16 Dec 1991 to 5 May 1994	Bay 152 to Bay 198	High Temperature Combustion
	Shimadzu TOC-5000	23 May 1994 to Dec 1995	Bay 199 to Bay 231	High Temperature Combustion
CBL*	OI, Model 524 Ampule Method	From beginning to Jan 1987		Persulfate Oxidation
	OI, Model 700	Feb 1987 to Dec 1995	?? to Bay 231	Persulfate Oxidation
DHMH	Astro 1850	1985		UV Light, ~~(High Temperature Combustion)~~ (is with Persulfate Oxidation
	Shimadzu TOC-5000	12 April 1994 to Present		High Temperature Combustion
ODU**				

*CBL does own a Shimadzu Instrument, but all of the submitted CBMP data was generated by the OI instruments.
**No information provided.

CURRENT DATA REDUCTION METHODS:

As of this writing, AMQAW member laboratories are not uniform in their method of DOC data reduction. Our belief is that this, in addition to some instrumentation differences and sample handling procedures may be the main contributing factor in observed "differences" reflected in the CBMP's DOC Split Sample Reports. The DOC Split Sample Reports (1994-1995) are given in Appendix A of this document. The following table, generated from a questionnaire which was distributed to AMQAW member laboratories, underlines the need to address this matter in detail and arrive upon agreement.

TABLE II.: SUMMARY OF CURRENT DATA REDUCTION SPECIFICATIONS

	CBL	DHMH	ODU*	VIMS
How many carbon standards used?	5 or 6	4		4
Carbon standards are prepared in:	Nanopure Water (Rev. Osmosis)	Distilled Water		Distilled, deionized water
Compensate for DOC in water used for standard preparation?	Yes. (Shim: Subtract H_20 blk from stds. About 0.2 mg/L)	No.		Yes. (Increase std values +0.25 mg /L.)
Do you include a zero standard in standard curve?	No.	Yes.		No.
Do you force the linear regression through zero?	OI: No. Shim: Yes.	Yes.		No.
Do you include SRM's? Prepared in reagent grade water? Prepared in synthetic seawater?	Yes. Yes. No.	No. ----- -----		Yes. Yes. Yes.
When calculating your SRM, do you take the carbon content of the diluent solution into account?	No--reagent blk takes DOC in prep water into account.	-----		Yes--Each SRM std has a blank which was prepared at the same time.
Spike Recovery for Bay Samples? In-house Spike Recovery?	80-120% 90-110%	 90--110%		80-120% 81-113%

Drift Check? [Include mid-range standard every 10th sample in analysis.]	No.	No.		Yes.
Do you make adjustments for "Drift?"	No.	No.		Yes--Rarely Necessary.
Baseline Check?: [Include reagent grade or acidified reagent grade water as every 10th sample in analysis.]				Yes.
Adjust data due to baseline shifts?				Yes.
Include at least a high/low standard At beginning of each run? At end of each run?	 Yes. Yes.	 No. Yes.		 No. Yes.
Adjust data due to "significant diff" between begin/ending std curve?	No.			Yes.

*No information provided.

USE OF SHIMADZU INSTRUMENTATION

For those laboratories which have a Shimadzu Instrument, differences also exist in how the analyses are performed in each laboratory. The following table, generated from a questionnaire which was distributed to AMQAW member laboratories, underlines the need to arrive upon a Standard Operating Procedure should the DOC analysis be reinstated into the CBMP in the future.

TABLE III.: SHIMADZU INSTRUMENT PARAMETERS

Instrument's Set up and Abilities:	CBL	DHMH	ODU*	VIMS
Cooling Coil? Cooling Coil modification?	Yes. -----	Yes. -----		5/94 - 2/96 Feb '96+
Cupric oxide used on top of catalyst?	Yes. (8g)	No.		No.
Autosampler?	Yes.	Yes.		Yes.
Instr. capable of drawing Std. Curve & returning a concentration value for each sample?	No.	Yes.		Yes.

Standard Curve typically used (for Bay samples).		0 - 10 mg/L		1 - 8 mg/L
Established UCL (for Bay samples)?	No.	No.		0.26 mg/L
MDL?	0.12 mg/L	0.448 mg/L		0.2 mg/L
How many injections made per sample?	3 (max 5)	3 (max 5)		3 (max 5)
How many injections are averaged for final result?	3 averaged	3 averaged		3 averaged

*No information provided.

SECTION II: CBMP COMPARISON STUDIES

INSTRUMENT COMPARISON, SALLEY AND CURLING, 1992 AND 1995

In response to 1988 reports of a new DOC instrument which was reporting higher DOC concentrations in ocean water samples than previously reported and the intense interest in the scientific community regarding DOC measurements as reflected by the January 1993 Marine Chemistry issue which was solely devoted to DOC measurements, AMQAW requested that Betty Salley and Kevin Curling conduct a study and prepare a report investigating the instrument variability and salinity interference specifically as they apply to CBMP DOC results.

Salley and Curling (1995) incorporated a study of salinity's effect on DOC measurements in the instrument comparison study by sending prepared carbon samples of different salinities to each laboratory. The salinity results will be discussed later. In regards to instrumentation the report concluded that:

> "Comparison of the dissolved organic carbon results from the five methodologies/instruments used for the Chesapeake Monitoring Program demonstrates that the consensus was good between all instruments and excellent between three instruments. There was no need to develop a correction factor between instruments. Any problems with the two instruments [Astro (Model 1850) and Dohrmann (Model 180)] which yielded the greatest variability was probably due to the age of the instruments and their detectors."

In an earlier study, Salley et al. (1992) made a six month comparison of two DOC instruments, the Oceanographic Instruments (OI) ampule method and the Shimadzu TOC analyzer. This study concluded that the Shimadzu TOC analyzer typically produced results 0.5 mg carbon/liter greater than the OI analyzer and suggested that data users wishing to adjust either data set could do so by employing the following equation:

> SHIM = 0.563 + 0.976 (OI)
>
> where SHIM is the DOC concentration in mg/l using the Shimadzu analyzer and OI is the DOC concentration in mg/l measure with the Oceanographic Instruments ampule method."

After reviewing Salley and Curling (1995) findings, we concur with the conclusions that the accuracy of the data generated during this period was as accurate as feasibly possible for the time period and the instrumentation then available and should be used without alteration. Caution should be used in making comparisons between data obtained on different instrumentation; however, sight specific trends can be considered valid. In the DOC Subgroup Report, Marine Chemistry, Volume 41, 1993, it is stated "In the particular cases of time series and flux studies, the stability of the analysis is often more critical than accuracy, as changes are characteristically more important than the absolute value." Should one wish to make a comparison of DOC results obtained specifically from the OI (ampule method) analyzer and the Shimadzu TOC-500, the equation given by Salley et al. (1992) should be employed; otherwise, sight specific trends may be considered valid.

THE INFLUENCE OF SALINITY ON DOC MEASUREMENTS, SALLEY AND CURLING, 1995

Salley and Curling (1995) CBMP DOC instrument comparison study also examined the influence of salinity on DOC measurements made by five DOC instruments/methodologies. The report concluded that:

> "Salinity may present a bias in wet oxidation methods, but the instruments in this study were modified in their reagent concentration and reaction time to compensate for salinity. The motion linearity of the curves with the adjusted means for examination of salinity influence is probably an artifact of the addition measurements in that range. It could be argued from these curves that salinity does affect DOC measurements, but if so, the influence is so slight that it can be ignored."

In addition, an earlier study by Salley et al. (1992) made a six month comparison of two instruments, the Oceanographic Instruments ampule method and the Shimadzu TOC analyzer, which found little variation over salinity ranges of 12 to 27 ppt.

Thus, previous DOC measurements should not have been significantly influenced by salinity regardless of the instrument on which the DOC determination was made. Since the laboratories participating in the CBMP no longer use wet oxidation methods but now employ high temperature combustion instrumentation in which no noticeable saline interference has been detected, future DOC variations should not be attributed to salinity interference.

SECTION III: SUMMARY OF STUDIES PERFORMED BY J. SHARP ET AL.

DOC SUBGROUP REPORT AND THE PROCEDURES SUBGROUP REPORT, MARINE CHEMISTRY 41 (1993)

The DOC subgroup prepared a report (1993) to answer the following three questions and give recommendations pertaining to each of the questions.

1) What are the requirements for the accuracy and precision of DOC analysis?

2) What are the appropriate procedures for obtaining (a) analytical blanks, (b) standards, (c) reference DOC materials, and what protocols should be set up for the quality assurance?

3) Is there compelling evidence that high temperature combustion methods give DOC concentrations which are greater than those determined by previously employed wet chemical and photochemical oxidation methods? If so, can we adjust previously collected DOC data to any advantage?

The recommendations and general conclusions focused on the need to establish precision and accuracy and the accuracy of currently used techniques must be established using referee techniques. Quality assurance was addressed and focuses on the analytical blank determination which incorporated three components: reagents,which includes reagent water and system blank which may have contributions from sample contamination; steam induced pressure effects on the detector; and matrix effects combined with release of CO_2 from components of the system. An immediate need for reference DOC material for the oceanographic community and a uniform method of preservation of seawater for organic analysis were also identified as critical issues. The recommendation was to reevaluate current methods with close attention to analytical blanks to determine discrepancies. The final conclusion was that until the current problems were addressed, it was simply not possible to evaluate the accuracy or recoverability of past data sets.

The Procedures Subgroup reviewed alternatives to each step of sample handling prior to analysis and recommended the best options to maintain the integrity of the samples. The discussion included the examination of sampling apparatus such as niskin bottles. Consensus was that a niskin bottle with teflon coated springs, silicon o-rings, and nylon sampling spouts could be used to minimize contamination. Another option was an *in situ* pumping system but requires additional care to avoid ship's contamination during collection. The filtration step is another potential source of contamination. Although Glass fiber filters are adequate, they should be muffled at 450°C for 15 minutes prior to use. Caution should be employed so as to not alter the pore size and brittleness of the filter during this procedure. Filter apparatus is preferably glass which has been muffled at 550°C. If stainless steel or polycarbonate is used a strict cleaning protocol must be followed. This would consist of a detergent wash, distilled water rinse, 10% HCL rinse followed by an extensive distilled water rinse. Acid must be added to the sample prior to analysis to remove Dissolved Inorganic Carbon (DIC). Type and concentration of acid is established on analysis and analyzer. High purity grades of H_3PO_4 and HCL are the acids of choice. Sparging of the sample after acidification is necessary for DOC determination. The effectiveness of sparging is dependent on pH, gas flow, sample volume and frit pore size. These criteria must be optimized by the individual laboratories to ensure complete sparging of DIC. Lastly the report discusses sample storage. The Procedures Subgroup prefers on site analysis. If storage and transport are necessary muffled glass containers are preferred. Acidification and refrigeration is acceptable as is freezing without acidification.

Both Subgroup reports identify necessary work which must be completed in order to accurately access the methods and analytical procedures used for past analyses and to develop DOC protocols for the oceanographic community to adopt for DOC analysis. Precision and accuracy must be carefully assessed for each method. Comparisons

of past methods, blank corrections and instrumentation must be reevaluated to identify the best techniques to establish accurate analyses of DOC concentrations.

ANALYSES OF DISSOLVED ORGANIC CARBON IN SEAWATER: THE JGOFS EQPAC METHODS COMPARISON, MARINE CHEMISTRY 48 (1995)

In Jonathan Sharp et al's latest published study (1995) comparing DOC methods, five high temperature combustion (HTC) instruments and a wet chemical oxidation method were evaluated. It is important to note that four of the five HTC instruments were either homemade or extensively modified from the original configuration; and only the Shimadzu 5000 was used as received from the manufacturer. This study also introduces "blind" samples and analyses were performed in the home laboratories at the convenience of each analyst. This is opposed to previous studies (Sharp et al., 1993) whereby HTC analyses and the persulfate oxidation method were compared in a controlled exercise with analyses performed in one laboratory by the same analyst. The instrument modifications of this study included extensive conditioning of the catalyst (Benner and Strom, 1993) and incorporated all quartz combustion tubes in the vertical position. All used oxygen as the carrier gas and platinum catalyst. Each HTC instrument had a water removal system and used an infrared analyzer with integration of the signal.

The results of the comparison yielded similar results for three of the five HTC instruments and the persulfate analysis. Each of these also produced suitable blank values. For one of the other instruments, a modified Ionics system, the reference material did not agree well with the others and a modified Dohrmann gave erratic and varied results as compared to the other instruments. The three of the five HTC instruments and the wet chemical oxidation procedure produced standard curves with $r^2>0.99$ and precision +/- 5%. The homemade instrument, the modified Dohrmann and the Shimadzu results had average precision better than +/- 2.5%.

Conclusions drawn by J. Sharp are as follows:

1) This exercise indicates that it is possible to achieve comparable analyses of DOC in seawater when a strict protocol is used for sample acquisition, careful assessment is made of the instrument blank, and a uniform reference is available for calibration.

2) The success of the laboratory comparison reported here was facilitated by using a close to zero blank water and reference standards to evaluate instrument behavior. The blank water used here was later estimated by comparison to ultrapure waters in individual laboratories to contain a small amount of carbon, perhaps 4uM C. However, since this was uniformly subtracted from all the samples, it does not detract from the comparison. The exercise presented here suggests that standardization can be done with either deionized water or low carbon content seawater. However, this should be specifically checked on each individual instrument. The two analyses that did not have similar values for seawater samples also did not have low blanks nor good recovery of the reference standard.

3) It is necessary to modify some commercial instruments to prepare an oxidizing environment and halogen and water removal system that is sufficient to complete CO_2 production from the DOC and removal of interfering gases. It also appears to be important to have a sensitive, stable CO_2 detector and a good peak integration system to achieve the greatest possible precision and accuracy. Several of the commercial instruments were modified to achieve this; the Shimadzu instrument appeared to have a sufficiently sensitive CO_2 detector and integrator system without modification; we also saw this previously (Sharp et al., 1993a). In comparing the results from the first run of the modified Dohrmann

II analyzer with later runs of samples on that same instrument and to results of other instruments , the influence of the blank correction can be seen. In that first run, it is probable that the catalyst bed of the oxidation tube had not been adequately conditioned. In at least one recent paper (Martin and Fitzwater, 1992) the authors suspected that this error in blank correction and inadequately conditioned combustion tube were the reasons for presumed overestimation of DOC values in oceanic samples.

4) The sample storage bottles were sealed with teflon lined caps and quick frozen and stored so that liquid seawater did not have contact with teflon liners. In sample bottles that were stored with dilute acid after cleaning, but before sample collection, it appeared that slight contamination occurred. Also samples that thawed and had liquid seawater in extended contact with the cap liners or experienced slight leakage, appeared to have become contaminated. Samples were quick frozen and transported carefully for later analyses on land. It has been demonstrated here that this method of sample storage is adequate for keeping samples free of contaminants and also for preserving samples. It has been demonstrated elsewhere that successful sample preservation can also be achieved by acidifying and refrigerating samples (Hedges et al., 1993). Analysis of samples at sea (as verified with "At Sea" data) may be preferable since opportunities for contamination during shipment and storage are eliminated.

SECTION IV: CONCLUSIONS AND RECOMMENDATIONS

1994-1995 DATA

1. For 1984 to 1995 data, there is general agreement with the findings of Salley et al. (1992) and Salley and Curling (1995):

a. The variability of past DOC data was mainly due to data processing, blank correction and sample handling and that no factor can be found to bring all data sets into agreement at this time. Due to disposal of past instrumentation, no further studies may be performed on each instrument to identify the factors causing variability and whether these factors were consistent from run to run or were erratic and variable throughout a run possibly due to inadequate blank correction, incomplete data reduction procedures, or sample handling .

b. An equation has been developed specifically for comparison of DOC data sets generated on the OI (ampule method) analyzer used by ODU and the Shimadzu TOC-500 used by VIMS and can be employed for these data sets only.

c. The instruments used with the exception of the Astro (Model 1850) and Dohrmann (Model 180) may not be the cause of the variability demonstrated in the split sample data but rather the operating procedure, data reduction processes, and sample handling.

d. Salinity has not been found to significantly contribute to the observed DOC variations when high temperature combustion methods are used.

2. Blank correction be performed as recommended by Jonathan Sharp.

The overwhelming conclusion from Sharp's studies is that catalyst conditioning, the handling of blanks and standards in data reduction, and sample handling itself are the main factors which affect the variability of DOC data.

3. Coordinated Split Samples vs Chesapeake Bay Samples

The DOC data variability may be inherent of the split sample analyses and not necessarily indicative of the Chesapeake Bay water quality samples. The split samples are collected and handles differently the the bay water quality samples. There is a lag time for filtration for the split samples and immediate on board filtration of the bay samples. Storage prior to filtration is in a plastic cubitainer with the sample remaining unpreserved in the interim between sample collection and filtration. Muffled glass containers are preferrable for storage. Plastic may either absorb or desorb carbon. Further studies of handling procedures (Coordinated split samples vs Bay Water Quality Samples) may identify potential sources contributing to the variablity of DOC concentrations identrified by the results of the split sample program.

4. For generation of future DOC data, as referenced by AMQAW guidelines and J. Sharp et al.,(1993a and 1995), the following recommendations are proposed:

a. All mainstem laboratories use a high temperature combustion instrument equipped with a nondispersive infared detector such as the Shimadzu TOC-500 or TOC-5000.

b. All mainstem laboratories follow the procedure put forth in this document (Section V).

c. The procedure recommended in Section V be included in the AMQAW method Manual.

d. All mainstem laboratories follow the data reduction method put forth in this report, specifically, blank correction as recommended by J. Sharp.

e. Further study of sample handling procedures, especially with the split sample program, to identify and revise potential practices which may alter the true concentartion of DOCof the sample.

PROCEDURAL CHANGES AS OUTLINED BY THE PROCEDURES SUBGROUP REPORT

These specific procedural changes should be incorporated into the sample handling portion of the SOP.

1) Storage and transport of unfiltered water should be in a precleaned, premuffled glass container with teflon top. The sample should not be allowed to be in contact with the sample lid. The glass container should be rinsed three times with the sample prior to taking the final sample aliquot.

2) Glass fiber filters should be premuffled at a minimum of 450° C for several hours. Care should be taken not exceed the recommended temperature as this may alter the pore size and make the filter brittle.

3) Store filtered sample in precleaned 40 ml muffled Wheaton vial (muffled at 550° C for 2 hours). The teflon lids and septa should be precleaned by washing with detergent, distilled water rinse, 10% HCL rinse followed by extensive distilled water rinse. Sample may be acidified with 200ul of 20% HCL or quick frozen to -20°C.

RECOMMENDED UNIFORMITIES REGARDING DATA REDUCTION

In order to confirm that results are accurate and that the instrument is functioning normally, specific pieces of data require scrutiny. As with all data generated in an analytical manner, the main criteria for accessing the accuracy is a thorough review of the quality controls (duplicates, spikes, and standard reference materials) included with the samples. The following steps provide a uniform guide to DOC data reduction. When used by all laboratories involved in DOC data generation, comparability of results should be high.

1. STANDARD CURVE:

The validity of the standard curve is the foundation of the DOC analysis. In accordance with EPA, CBMP, and AMQAW QA/QC guidelines, an acceptable DOC standard curve must consist of a minimum of four standards and return a correlation coefficient of 0.99 or higher. All laboratories must adhere to these quality assurance guidelines. Blank correction must be employed in order to achieve comparability of results.

Possible sources of error:

A. Forcing curve through zero
B. No blank correction

Recommended Actions:

A. Perform intercept calculation.

B. Perform blank correction by averaging all replicate injections, subtracting the blank value and dividing by the slope of the standard curve, (Sharp et al. 1995).
C. Software packages such as Labtronics Aanalyze allows for blank correction, baseline drift and slope change.

2. SAMPLE DUPLICATES:

In accordance with EPA, CBMP, and AMQAW QA/QC guidelines, 10% of the samples analyzed should always be duplicated. That is, poured separately and analyzed a second time. The difference in the sample concentration values obtained must not exceed the laboratory's established control limit. Should the difference exceed the laboratory's control limit, a problem should be considered to exist and the analysis can not be considered valid until the problem source is found.

Possible sources of error:
A. Contaminated glassware/vials.
B. Instrument difficulties: frequently indicated by poor peak reproducibility.

Recommended Actions:
A. If an instrument problem has been identified, correct problem and reanalyze all samples.
B. Reanalyze any duplicates which fall outside of established control limit.

3. SAMPLE SPIKES:

In accordance with EPA, CBMP, and AMQAW QA/QC guidelines, 10% of the samples analyzed should always be "spiked." That is, a sample is poured in the sample tube and the sample immediately following is from the same source but has been "spiked" with a known concentration of carbon. The calculated percent recovery must not exceed the laboratory's established accuracies. Should the percent recovery fall outside that of the laboratory's established accuracies, a problem should be considered to exist and the analysis can not be considered valid until the problem source is found.

Calculation:

$$\text{Percent Recovery} = \frac{(\text{Concentration of Spiked Sample} - \text{Concentration of Sample})}{\text{Spike Concentration}} \times 100$$

Possible sources of error:
A. Baseline shift during analysis.
B. Slope change during analysis.
C. Instrument difficulties: frequently indicated by poor peak reproducibility.

Recommended Actions:
A. If a baseline shift or slope change can be identified, then recalculate spikes after adjusting for change. Reanalyze any spikes which fall outside of established control limit.
B. If an instrument problem has been identified, correct problem and reanalyze all samples.

4. METHOD BLANKS

In accordance with EPA, CBMP, and AMQAW QA/QC guidelines, a method blank should be analyzed every 10-20 CBP samples. A method blank is defined as a volume of reagent grade water that is carried through the entire analytical procedure. The purpose of a method blank is to determine the levels of contamination with the processing and analysis of the samples.

Possible sources of error:
A. Contaminated glassware, reagents, water.

B. Incomplete catalyst conditioning or instrument difficulties.

Recommended Actions:
A. Follow recommended procedures for glassware preparation.
B. Check water purification system.
C. Recondition catalyst.
D. Reanalyze samples.

5. IDENTIFICATION OF BASELINE SHIFT:

Baseline shifts can be monitored on the Shimadzu Instruments using acidified reagent grade water as first sample in each run as well as after every 10th sample. Baseline values are then compared to each other. Should baseline value differences exceed the laboratory's control limit, a problem should be considered to exist.

Possible sources of error:
A. If a value is outside of possibility, indicates contaminated vial or an instrument problems. This would be an isolated instance; all other baseline values are comparable within the laboratory's established control limits and all other QA/QC is solid.
B. Baseline value steadily increasing/decreasing such that beginning and end baseline values exceed established control limits. Slope change may also be involved and should be confirmed prior to data adjustment.

Recommended Actions:
A. Confirm sample value was isolated instance.
B. If slope change also involved, first correct for slope change then reevaluate corrected baseline values.
C. If slope not involved, identify where baseline changes and to what degree and correct all sample values by adding/subtracting appropriately.

6. IDENTIFICATION OF SLOPE CHANGE:

Slope changes occurring during DOC analysis are identified by comparison of the slopes of the beginning standard curve and that of at least two standards which are analyzed as samples at the termination of analysis. Incorporation of a mid-range standard within the analysis (every 10th sample is ideal) also assists in determination of where a significant slope change might have occurred during analysis.

To access if the slope is significantly different, select sample values of varying peak area (so as to check the values returned over the entire linear regression line) and divide the peak area by each slope. If the difference of the returned values are not within the established laboratory control limit, then the change in slope should be considered significant. Use the standard curve which returns the most accurate spike and mid range standard values.

EXAMPLE: Slope of initial std curve = 5287
Slope of end std curve = 5112
VIMS precision control limit = 0.26 (For 1-8 mg carbon/L standard curve)

Sample #1 peak area = 26693
26693/5287 = 5.05 26693/5112 = 5.22
Diff = 5.22- 5.05 = 0.17 which is still within the precision limit of 0.26; therefore, the change in slope is NOT significant.

Sample #2 peak area = 14586

14586/5287 = 2.76 14586/5112 = 2.85

Diff = 2.85 - 2.76 = 0.09 which is still within the precision limit of 0.26; therefore, the change in slope is NOT significant.

Sample #3 peak area = 21422

21422/5287 = 4.05 21422/5112 = 4.19

Diff = 4.19-4.05 = 0.14 which is still within the precision limit of 0.26; therefore, the change in slope is NOT significant.

7. STANDARD REFERENCE MATERIALS (SRM's):

Standard Reference Materials (SRM's) allow operator to know that instrument is functioning properly by returning known values. Both a saline and a freshwater base are used to show any variations which may occur due to the sample matrix. With an SRM containing 4.1 mg carbon/L. The following example illustrates how the % Recovery is calculated:

DI blank = 0.52 DI with 4.1 mg C/L = 4.48

Salt blank = 1.02 Salt with 4.1 mg C/L = 4.93

So $\frac{\text{(DI with 4.1 mg C/L - DI blank)}}{4.1} \times 100 = \%$ recovery

Thus $\frac{4.48 - 0.52}{4.1} \times 100 = 97\%$

Similarly for the saline matrix: Thus $\frac{4.93 - 1.02}{4.1} \times 100 = 95\%$

SECTION V: RECOMMENDED SOP FOR DOC DETERMINATION

1. Dissolved Carbon (DOC; TOC; TC; DTC)

1.1 Scope and Application:

1.1.1 This method can be used for Total Carbon, Total Organic Carbon, Total Dissolved Carbon and Dissolved Organic Carbon. Total Carbon is unfiltered; Total Organic Carbon is unfiltered with a pH less than 2. The dissolved constituents are filtered through a Whatman GF/F glass fiber filter or a Gelman A/E glass fiber filter.

1.2 Summary of Method:

1.2.1 A preset amount of sample is injected into the Total Carbon port and enters the pure air flow. The sample is carried through a platinum catalyst at 680°C. Carbon atoms are oxidized into carbon dioxide which is measured by the Nondispersive Infrared detector (NDIR).

1.3 AMQAW recommends that the Shimadzu TOC-5000 be connected to a personal computer equipped with a software program capable of collecting the information and reprocessing it. An example of a suitable program is Aanalyze for Windows produced by Labtronics, Inc.

1.4 Sample Handling: (Pgs 13-14 of Instrument Manual.)

1.4.1 Total Carbon (TC) sample is unfiltered and not acidified.

1.4.2 Total Organic Carbon (TOC) is unfiltered with the pH adjusted to less than 3.

1.4.3 Dissolved Total Carbon (DTC) is filtered and not acidified.

1.4.4 Dissolved Organic Carbon (DOC) is filtered with the pH adjusted to less than 3. In the case of the Shimadzu TOC-5000, DOC is referred to as NPOC (non-purgeable organic carbon). Samples are sparged with ultra pure air immediately prior to analysis to remove inorganic carbon.

1.4.5 Acidification to pH less than 3 accomplished by adding 5 drops of 6N ultra pure HCl to 40 mL of sample. Final pH is approximately 2.0. Acidified samples are refrigerated at 4°C until analysis. Unacidified samples frozen @ -20°C.

1.4.6 Upon sampling, the sample vials are flushed three time with the sample before final sample introduced into the vial. After introduction of the final sample aliquot, the sample vials should be sealed with teflon lined caps and quick frozen or acidified and refrigerated. The sample should be stored such that the seawater does not come in contact with the teflon liners as this may contaminate the sample (Sharp et al, 1994).

1.5 Glassware:

1.5.1 Carbon contamination is difficult to avoid but essential for accurate data acquisition. Glassware must be stringently cleaned. The recommended method is to wash with 10% HCl, rinse three times with DDI water, and place in muffle furnace at 550°C for two hours. Teflon septa and closures are cleaned separately with Micro (soak overnight), thorough DDI rinse, 10% HCl (soak overnight) , and thorough DDI rinse (Sharp et al, 1994).

1.5.2 All plastics (i.e. parafilm, bottles) should be **avoided** where possible as plastics may affect carbon content. (New plastic bottles actually absorb carbon.)

1.6 Reagents:

NOTE: All water used in reagents must be fresh distilled, deionized (DDI) water. Add 4 mL of 6 N HCL to 2.5 liters of DDI water to acidify to pH <3.0. Label, date, and initial all reagents.

1.6.1 Carbon Stock Solution - Dry 5 to 10 g overnight in 100°C oven. Dissolve 2.125 g of Potassium Hydrogen Phthalate ($HOCOC_6H_4COOK$) in a 1 L volumetric and dilute to mark with acidified (pH <3.0) DDI water. Store in a dark glass container at 4°C. Solution is stable for 6 months. 1 mL = 1 mg carbon.
NOTE: KPH has a FW = 204.23 g/mole & MW of Carbon = 12.011 g/mole.

1.6.2 Secondary Standard Solution - (spiking solution) Dilute 30 mL of stock solution to 100 mL in volumetric flask with acidified (pH <3.0) DDI water. Stable for 2 months when stored at 4°C. 1 mL = 0.3 mg carbon.

1.6.3 Standards - Use acidified (pH <3.0) DDI water to make the standard solutions. Stable for 2 months when stored at 4°C.

Conc.	mL of stock/500 mL	Conc.	mL of stock/200 mL
10 mg/L	5	40 mg/L	8
8 mg/L	4	20 mg/L	4
6 mg/L	3	15 mg/L	3
4 mg/L	2	10 mg/L	2
2 mg/L	1		
1 mg/L	0.5		

1.6.4 Carrier gas - Compressed ultra pure air

1.6.5 6N Ultra Pure Hydrochloric Acid (HCl) - Mix equal portions (250 mL + 250 mL) ultra pure concentrated HCl and DDI water.

1.7 Starting up a completely shut down instrument:

WARNING: Never have furnace on without air flowing through the combustion tube.

1.7.1 Plug instrument and autosampler into surge protector; turn on surge protector.

1.7.2 Turn on the autosampler and main instrument.

1.7.3 Open compressed air tank valve and adjust carrier gas flow to 150 mL/min on instrument gauge. Tank pressure must be >500 psi. If instrument will warm up **overnight**, set carrier gas flow to 30 mL/min (using knob on instrument).

1.7.4 IC Reagent vessel should fill with water and bubble. If not, there is a carrier gas "leak." **Do NOT turn on furnace until "leak" is fixed.**

1.7.5 Turn on furnace.

1.7.6 **WAIT** at least 1½ hours until furnace stabilizes at 680°C.

1.8 Sample preparation:

1.8.1 The autosampler has a maximum capacity of 78 samples. A sample table is written on a separate form to include 10% of duplicates and 10% spiked, and 10% blanks.

1.8.1.1 The first sample of each run should be an acidified DDI water blank (to establish baseline).

1.8.1.2 10 mL of sample is spiked with 100 µL Secondary Standard Solution. Foil is used when shaking the spike as plastics such as parafilm may affect carbon content. (Spike = 3 mg C/L.)

1.8.1.3 Drift and baseline are checked approximately every 10th sample. To check for drift use the 4 mg carbon/L standard (or one which closely approximates the samples). Acidified DDI water is used to monitor changes in the baseline.

1.8.2 Standard Reference Materials (SRM's) should be included in each run (i.e. 78 sample set). The SRM's include: DDI blank, DDI 4.1, Salt blank, and Salt 4.1.

1.8.3 EACH run should END with at least a two point standard curve (full curve is preferable for slope change identification), a baseline check, and the LAST sample is ALWAYS non-acidified DDI water in order to flush acid from the system.

1.9 Instrument preparations/Beginning a run:

1.9.1 Compressed air tank pressure must be >500 psi and carrier gas gauge (gauge on **instrument)** set

to 150 mL/min. If set at 30 mL/min (from overnight warm-up), increase to 150 psi and wait at least 30 minutes prior to beginning a run.
NOTE: Changing the carrier gas pressure WILL affect the baseline!

1.9.2 Humidifier water level should be between the two lines marked on the reservoir.
NOTE: Addition of water will create a "peak" on the baseline!

1.9.3 Add thermal paper, if needed.

1.9.4 [Maintenance/"Service: Mechanical Check"].

1.9.4.1 Check syringe for air bubbles and flush.

1.9.4.2 Clean around the TC injection needle. The sample should be delivered in a stream.

1.9.4.3 Move IC drain tubing by pressing key indicating "IC ON" to release the lock on the drain tubing. Manually move drain tubing.

1.9.4.4 To exit service screen, press key indicating "END."

1.9.5 Perform "Zero Point Detection" of sample syringe pump if syringe has been removed or if this will be first run since instrument was turned on. MUST HAVE A STANDARD OR WATER IN THE S1 POSITION!!!!

1.9.6 Empty the waste collection jug.

1.9.7 Monitor baseline. Observe that furnace temperature is 679 - 680°C. Dehumidifier temperature = 1.0 - 1.1°C.

1.9.7.1 Adjust baseline up or down, if necessary, using screwdriver in forward hole labeled "OPTICAL ZERO" located on the lid of the instrument

1.9.8 Load calibration curve settings. AMQAW recommended settings for BAY samples:
Standard Range: 1 to 8 mg/L
Min/Max Number of Injections: 3 out of 5
Injection Volume: 60 to 80 µL
Sparge Time: 6 minutes

1.9.8.1 A value of 0.25 mg C/L as added to each carbon standard as this has been estimated to be the inherent carbon "contamination" of the DDI water which is absent from all samples but present in the standards.

1.9.9 Load sample settings. AMQAW recommended settings for BAY samples:
Injection Volume: 60 to 80 µL
Min/Max Number of Injections: 3 out of 5
Standard Deviation: 200
Coefficient of Variance: 2.0
Sparge Time: 6 minutes

1.9.10 Load standards and samples into autosampler.

1.9.11 Access software program and set up to collect DOC data.

1.9.12 Start instrument.

1.10 Check after run has begun:

1.10.1 Fill rinse bucket on autosampler with DDI water.

1.10.2 Check syringe for bubbles a second time. If bubbles are noticed, ABORT the run.

1.10.3 After completion of standard curve, check linearity. If a correlation coefficient of <0.99 is obtained, then ABORT the run.

1.11 At conclusion of analysis, shut down procedure:

1.11.1 Stop data collection in software program.

1.11.2 Flush injection needle to remove acid/salt residue which may cause corrosion and/or clogging of sample injection needle.

1.11.3 If instrument will not be used overnight, but will be used the following day, set carrier gas flow at 30 mL/min and leave the furnace ON (680°C).

1.11.4 For long term shutdown of weekend or longer:

1.11.4.1 Turn off the furnace.

1.11.4.2 **Wait 30 minutes** for the furnace to begin to cool.

1.11.4.3 Turn carrier gas down to 30 mL/min (on instrument gauge).

1.11.4.4 When furnace temperature is 200°C (approximately 2 hours) or lower, turn off carrier gas at tank regulator. Turn off BOTH the DOC and the autosampler.

1.12 Shimadzu TOC-5000 Log

1.12.1 Record pressure of carrier gas tank and all maintenance performed in the log. This is to include catalyst and combustion tube changes.

1.12.2 Record unusual events involving the instrument or any trial attempts to expand its abilities or the knowledge of the operator.

1.12.3 Record when stock and standard solutions are made.

1.13 Typically used settings for the Shimadzu 5000:

1.13.1 [(#3) GENERAL CONDITIONS]

TC Catalyst	: Normal Sens
Syringe Size	: 250µl
Number of Washes	: 2
Unit of Conc	: 3 (mg/l)
Auto Ranging & Inj Vol	: 1 (Auto Change)
Auto Regeneration of IC	: 1 (ON)
Auto Print out	: 1 (data only) or 2 (data w/ peaks)
Furnace ON/OFF	: TOC [if ON]
Buzzer	: 1 (Used)
Injection Speed	: 1 (Std)
ESU	: 2 (Not Used)
Bubble Removal	: 1 (ON)
Syringe Wash	: 1 (STD)
Cell Length	: 1 (STD)
TOC or SSM	: 1 (TOC)
Printer Device	: 2 (Internal)
Page Length	: ******
Calibration Curve Form	: 2 (Least Squares)

1.13.2 [(#9) Sample Measurement (ASI)/ Conditions]

	TYPE	IS	FS	C1	C2	C3	F1	F2	F3
1	NPOC	1	78	1	**	**	**	**	**

	RG	VOL	W	INJ	MAX	SD	CV	SP	DIL
1	1	80	2	3	5	200	2.0	6	1

NOTE: All three of these numbers will change depending on the run conditions. **IS**= initial sample **FS**= final sample **C1**= std curve #1
VOL = 80 µL injection volume INJ = minimum of 3 injections
MAX = maximum of 5 injections SP = 6 minutes of sparging

1.13.3 ASI Conditions

RINSE	: RINSE
NO OF NEEDLE WASHES	: 2
FLOW LINE WASHES	: 4
CALIBRATE BEFORE	: 2 (EACH SMPL GROUP)
PRINT INFORMATION	: 1 (data only) or 2 (data w/ peaks)
AUTO ADDITION OF ACID	: 2 (OFF)
ACID VOLUME*	: 0 µL (if sample is unacidified acid must be added)
RINSE AFTER ADDITION	: 2 (NO RINSE)
KEY LOCK	: 2 (UNLOCK)
FINISH OR RUNNING	: 3 (NO CHANGE)

1.14 Catalyst replacement:

1.14.1 The catalyst will have to be replaced when the approximately 190,000 µL of sample/standards have been injected. At this time, problems such as tailing peaks begin to occur.

1.14.2 Combustion tube should only be removed when cool (100°C or less), not only to avoid severe burns but also to avoid cracking the combustion tube (from cooling too quickly).

1.14.3 Remove spent catalyst to a beaker or storage container. Use "poker" to remove catalyst adhered to the tube's sides. Be sure screens and quartz wool are removed as well.

1.14.4 If combustion tube is to be reused, clean with a brush and tap water. Rinse with 10% HCl and 3X with DDI water. Place in 100°C oven to dry for 1 hour.

1.14.5 Section 5.1.4 (pgs 43-45) and Figure 5.1 in Instrument Manual give a suitable description on how to change/fill the combustion tube. Note that one change has been made as per the recommendation of Shimadzu: the amount of catalyst should only be 125 mm high. The logic for this change is that the furnace heating area is exactly 130 mm high so if the catalyst is at 130 mm or higher, the result will be that the top of the catalyst may not reach 680°C and complete combustion of the sample may not occur, thus, yielding poor results.

1.14.6 Section 5.1.6 (pgs 45-47) and Figure 5.2 of Instrument Manual present a detailed list of how to reinstall the combustion tube. Be sure to apply a THIN coat of high vacuum silicon grease on the **OUTER** surface of the TC combustion tube neck.

1.14.7 RESET the Sample Volume Count (TC) back to zero whenever the catalyst has been changed. Experience has shown that new catalyst can handle approximately 190,000 µL before problems such as tailing begin to occur.

1.14.8 Conditioning of new catalyst:
Set up four standard curves to be run overnight. (Sparge time is always 6 minutes.)

EXAMPLE

First curve	: 10 injections of 0.5 N HCl (100 µL).
Second curve	: 10 injections of 0.5 N HCl (100 µL).
	10 injections of DDI water (100 µL).
Third curve	: Normal std curve (1 to 8 mg C/L) with
	3 out of 5 inj; 80 µL vol; 6 min sparge.
Fourth curve	: 10 injections of 0.5 N HCl (100 µL).
	10 injections of DDI water (100 µL).

1.15 Maintenance:

1.15.1 Record all maintenance performed on this instrument in the Shimadzu TOC-5000 Log.

1.15.2 Injection needle (TC/IC) should be replaced when it no longer delivers sample in a stream.

1.15.3 IC drain tubing should be changed if it begins to leak.

1.15.4 The teflon plunger in the sampling syringe should be changed if air bubbles are frequently encountered in the syringe, especially if they occur during a 78 sample set.

1.15.5 Every 3 months replace the water in the three "reservoirs." The IC reagent bottle should be contain 5 mL of 25% phosphoric acid and then be filled to the shoulder with DDI water. 4-5 pellets of NaOH must be added to the humidifier. The dehumidifier drain pot is filled with DDI water only.

1.15.6 Yearly replacement of the CO_2 absorber, halogen scrubber and membrane filter, rubber and teflon O-rings in slidable sample injection block, and the cigarette filter is recommended. Replacement of cigarette filter is not mentioned anywhere in manual. It is located in the rear of the instrument, inside the carrier gas connection.

1.16 Reference(s):

NOTE: The manuals (for instrument and autosampler) have more details regarding all of the above and trouble-shooting guides. They should be referred to frequently by anyone who is operating this instrument.

Shimadzu Instruction Manual for Total Organic Carbon Analyzer, Model TOC-5000.

Shimadzu Instruction Manual for Autosampler ASI-5000 for Total Organic Carbon Analyzer.

Shimadzu Instruction Manual for RS-232 Interface, TOC-5000/5050 System Use.

SECTION VI: REFERENCES

Measurement of Dissolved Organic Carbon And Nitrogen In Natural Waters. Proceedings of NSF/NOAA/DOE Workshop, Seattle, WA, USA 15-19 July, 1991. Edited by J.I. Hedges and C. Lee. Marine Chemistry, 41, Nos. 1-3, January 1993.

J.H. Sharp, R. Benner, L. Bennett, C.A. Carlson, R. Dow, and S.E. Fitzwater. 1993. Re-evaluation of high temperature combustion and chemical oxidation measurements of dissolved organic carbon in seawater. Limnol. Oceanogr., 38(8), pp. 1774-1782.

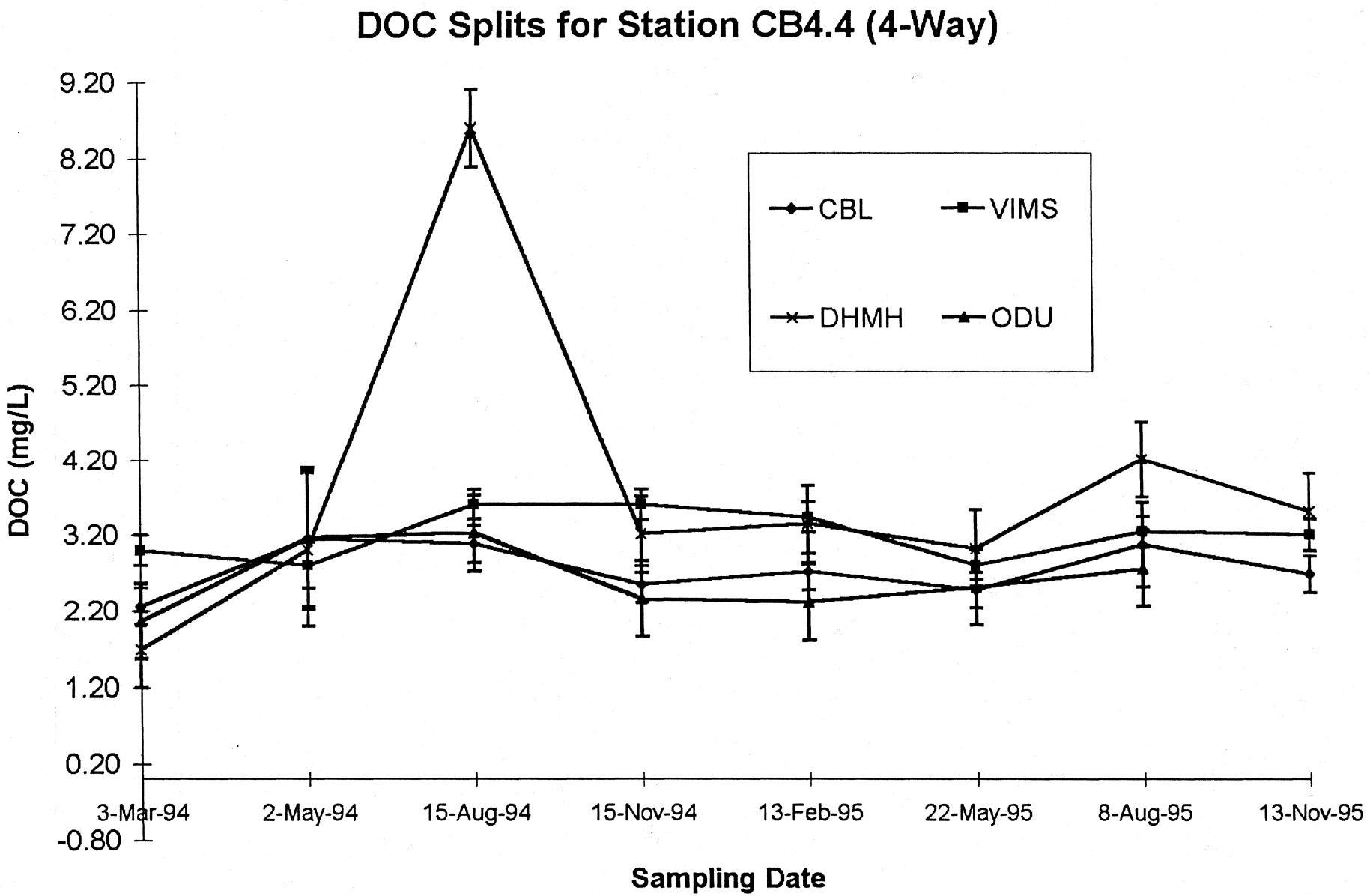
J.H. Sharp, R. Benner, L. Bennett, C.A. Carlson, S.E. Fitzwater, E.T. Peltzer, and L.M. Tupas. 1995. Analyses of dissolved organic carbon in seawater: the JGOFS EqPac methods comparison. Marine Chemistry, 48, pp. 91-108.

B.A. Salley and K. Curling. March 1995. Comparison Study of Five Instruments Measuring Dissolved Organic Carbon for the Chesapeake Bay Monitoring Program. Special Report No. 326, Virginia Institute of Marine Science, Gloucester Point, VA.

B.A. Salley, K. Curling, and B. Neilson. March 1992. A Comparison of Two Methods of Measuring Dissolved Organic Carbon. Special Report No. 128, Virginia Institute of Marine Science, Gloucester Point, VA.

US Environmental Protection Agency, 1990-1991. Chesapeake Bay Coordinated Split Sample Program Annual Report. Chesapeake Bay Program Office, Annapolis, Maryland.

VII: APPENDIX A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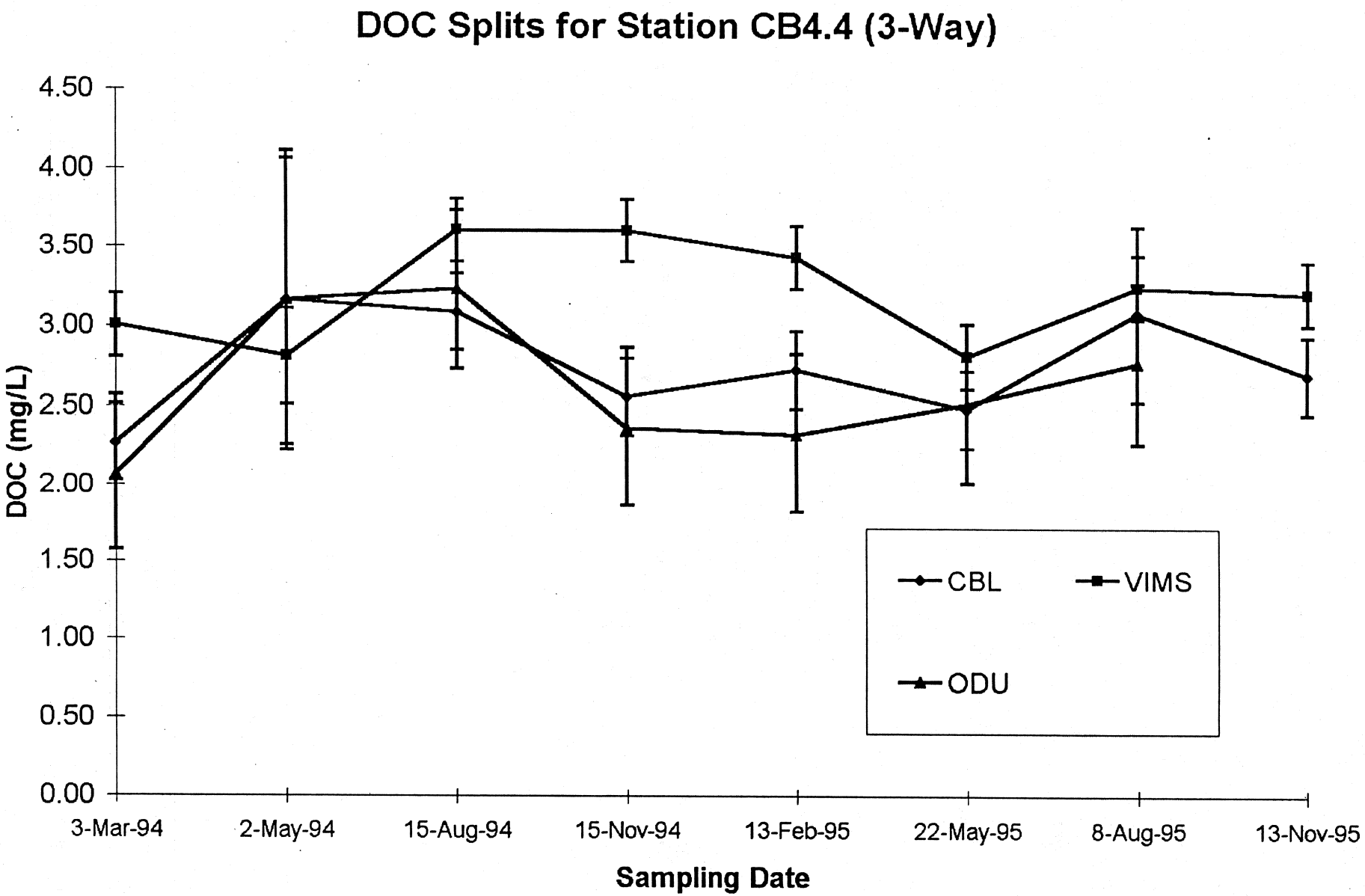
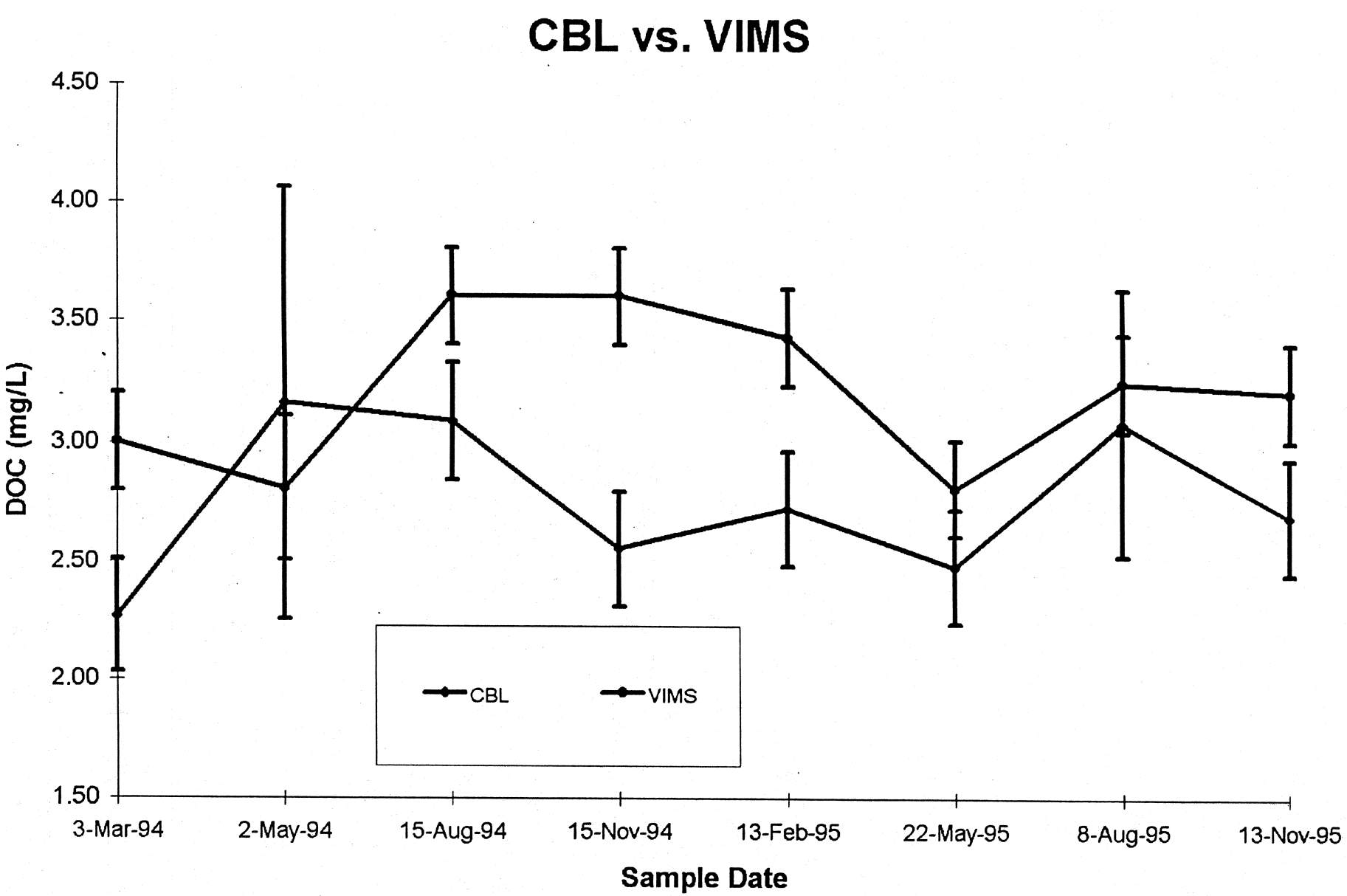
8.20
7.20
6.20
5.20
4.20
3.20
2.20
1.20
0.20
-0.80
DOC (mg/L)
CBL
VIMS
DHMH
ODU
3-Mar-94
2-May-94
15-Aug-94
15-Nov-94
13-Feb-95
22-May-95
8-Aug-95
13-Nov-95
Sampling Date


DOC Splits for Station CB4.4 (3-Way)
DOC (mg/L)
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
3-Mar-94
2-May-94
15-Aug-94
15-Nov-94
13-Feb-95
22-May-95
8-Aug-95
13-Nov-95
Sampling Date
CBL
VIMS
ODU


CBL vs. VIMS
4.50
4.00
3.50
3.00
2.50
2.00
1.50
DOC (mg/L)
CBL
VIMS
3-Mar-94
2-May-94
15-Aug-94
15-Nov-94
13-Feb-95
22-May-95
8-Aug-95
13-Nov-95
Sample Date

	MAINSTEM DOC SPLITS										
						Averages of the 3 replicates (the number that is graphed)					
	CBL	VIMS	ODU	DHMH			CBL	VIMS	ODU	DHMH	
3-Mar-94	2.31	3.21	2	1.68		3-Mar-94	2.27	3.00	2.07	1.70	
3-Mar-94	2.3	2.87	2.13	1.82		2-May-94	3.16	2.81	3.17	3.02	
3-Mar-94	2.21	2.93	2.08	1.61		15-Aug-94	3.09	3.60	3.23	8.60	
2-May-94	4.2	2.96	2.4	3.65		15-Nov-94	2.56	3.60	2.37	3.22	
2-May-94	2.66	2.47	4.22	3.56		13-Feb-95	2.73	3.43	2.32	3.35	
2-May-94	2.63	3	2.88	1.85		22-May-95	2.48	2.81	2.52	3.03	
15-Aug-94	3.04	3.66	3.47	8.22		8-Aug-95	3.08	3.24	2.77	4.21	
15-Aug-94	3.19	3.59	3.34	8.46		13-Nov-95	2.69	3.20		3.51	
15-Aug-94	3.04	3.56	2.88	9.12							
15-Nov-94	2.57	3.39	2.43	3.25							
15-Nov-94	2.63	3.79	2.29	3.19			Length of error bars for each lab				
15-Nov-94	2.48	3.62	2.38				CBL	VIMS	ODU	DHMH	
13-Feb-95	2.79	3.32	2.51	3.46		3-Mar-94	0.24	0.20	0.50	0.50	
13-Feb-95	2.55	3.51	2.25	3.28		2-May-94	0.90	0.30	0.94	1.01	
13-Feb-95	2.84	3.46	2.21	3.32		15-Aug-94	0.24	0.20	0.50	0.50	
22-May-95	2.57	2.79	2.7	3.06		15-Nov-94	0.24	0.20	0.50	0.50	
22-May-95	2.45	2.82	2.43	3.01		13-Feb-95	0.24	0.20	0.50	0.50	
22-May-95	2.43	2.82	2.42	3.03		22-May-95	0.24	0.20	0.50	0.50	
8-Aug-95	3.71	3.26	2.8	4.07		8-Aug-95	0.55	0.20	0.50	0.50	
8-Aug-95	2.79	3.27	2.75	4.11		13-Nov-95	0.24	0.20		0.50	
8-Aug-95	2.74	3.2	2.75	4.45							
13-Nov-95	2.78	3.26		3.53							
13-Nov-95	2.65	3.25		3.5							
13-Nov-95	2.65	3.1		3.51							

Rankings for Friedman ANOVA (3-Way)

	CBL	VIMS	ODU
3-Mar-94	6	9	1
3-Mar-94	5	7	3
3-Mar-94	4	8	2
2-May-94	8	6	1
2-May-94	4	2	9
2-May-94	3	7	5
15-Aug-94	2.5	9	6
15-Aug-94	4	8	5
15-Aug-94	2.5	7	1
15-Nov-94	5	7	3
15-Nov-94	6	9	1
15-Nov-94	4	8	2
13-Feb-95	5	7	3
13-Feb-95	4	9	2
13-Feb-95	6	8	1
22-May-95	5	7	6
22-May-95	4	8.5	2.5
22-May-95	2.5	8.5	1
8-Aug-95	9	7	5
8-Aug-95	4	8	2.5
8-Aug-95	1	6	2.5
13-Nov-95			
13-Nov-95			
13-Nov-95			
TOTALS	***94.5***	***156***	***64.5***

$$x^2 = [(12)/(BKn^2(nK + 1))]*[\Sigma R^2] - [3B(nK + 1)]$$

$$x^2 = [(12/(7)(3)(9)(10)]*[37426.5] - [(3)(7)(10)]$$

$$x^2 = [0.006349206]*[37426.5] -210$$

$\underline{x^2 = 27.63}$ **Anything greater than 9.21 is significant**

n=number of replicates
K=treatments(Labs)
B=blocks(sample dates)