TURNER DESIGNS
Reliable Instruments for an Unreliable World

USING THE TURNER DESIGNS MODEL 10 ANALOG, THE 10-AU DIGITAL, OR THE TD-700 FLUOROMETER WITH EPA METHOD 445.0:

"In Vitro Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Phytoplankton by Fluorescence"[1]
by Elizabeth J. Arar & Gary B. Collins

The United States Environmental Protection Agency (EPA) has recently published a chlorophyll method, Method 445.0, rev 1.2. Method 445.0 describes the use of a Turner Designs Model 10 Series Fluorometer (Section 6.1).[2] This fluorometer has been redesigned to make it easier to use. It is now called the Turner Designs Model 10-AU Fluorometer. The Model 10-AU is digital and is capable of performing calculations formerly done by the user. The TD-700, Turner Design's newest fluorometer, is also capable of performing these calculations.

In addition, there is a new method[3] for measuring chlorophyll *a* in the presence of chlorophyll *b* and pheopigments, which does NOT require the acidification step of conventional fluorescence techniques. Conventional fluorescence methods for measuring chlorophyll *a* require samples to be measured twice; once before acidification and once afterwards. Under the most extreme ratio of chlorophyll *a*/chlorophyll *b* likely to occur in nature (1:1 molar), conventional acidification techniques result in approximately a 60% underestimate of chlorophyll *a*. In these conditions the new method yields only a 10% overestimate of true chlorophyll *a*. It requires only a single fluorescence reading and is sensitive enough for estimates of euphotic zone chlorophyll *a* in all marine and freshwater ecosystems.

CONFIGURING THE TURNER DESIGNS MODEL 10-AU DIGITAL OR THE MODEL 10 ANALOG FLUOROMETER FOR METHOD 445.0. Your fluorometer should be equipped with the following Turner Designs optical kit (or equivalent):

Optical Kit:	PN: 10-037 or 10-037R
Lamp:	10-045 Daylight White Lamp
Excitation filter:	10-050 or 10-050R color specification 5-60
Emission filter:	10-051 or 10-051R color specification 2-64
Reference filter:	10-032 1 neutral density (1 ND), or the 10-035 2 neutral density (2 ND), or the 10-052 color specification 3-66

CONFIGURING THE TURNER DESIGNS MODEL TD-700 FLUOROMETER FOR METHOD 445.0. Your fluorometer should be equipped with the following Turner Designs optical filter kit (or equivalent):

Optical Kit:	PN: 7000-961
Excitation filter:	10-050R color specification 5-60
Emission filter:	10-051R color specification 2-64
Lamp:	10-045 Daylight White Lamp

USING YOUR TURNER DESIGNS FLUOROMETER WITH METHOD 445.0.
Section 10.1 (Calibration and Standardization) of Method 445.0: If you are using a digital fluorometer such as the 10-AU or the TD-700, you no longer need to calculate F_s. Follow the calibration instructions in your fluorometer user's manual and the instrument will give you direct readout of the concentration of the standard and samples without the need for compensation for the various sensitivity settings. If you are using the model 10 analog, you must perform the calculations in this section.

In Section 12.0 (Data Analysis and Calculations), when the Model 10-AU and the TD-700 are properly calibrated with a known standard, F_s always equals 1 (in the formulas in Section 12.1). The Model 10-AU and the TD-700 do the range and sensitivity setting calculations for you, so it is not necessary to calculate F_s. If you are using the model 10 analog, you must perform the calculations in this section.

CONFIGURING THE TURNER DESIGNS MODEL 10-AU DIGITAL OR THE MODEL 10 ANALOG FLUOROMETER FOR THE NEW CHLOROPHYLL *a* METHOD:**

Optical Kit:	PN: 10-040 or 10-040R
Excitation filter:	10-113 (436 nm)
Emission filter:	10-115 (680 nm)
Reference filter:	10-032 1 neutral density (1 ND)
Lamp:	10-089 Blue Mercury Vapor Lamp

CONFIGURING THE TURNER DESIGNS MODEL TD-700 FLUOROMETER FOR THE NEW CHLOROPHYLL *a* METHOD.**

Optical Kit:	PN: 7000-962
Lamp:	10-089 Blue Mercury Vapor Lamp
Excitation filter:	10-113 (436 nm)
Emission filter:	10-115 (680 nm)

****The new chlorophyll *a* method requires that your fluorometer be equipped with a special optical filter kit, which will read chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Your fluorometer should be equipped with the above Turner Designs optical filter kit (or equivalent).

USING YOUR TURNER DESIGNS FLUOROMETER WITH THE NEW CHLOROPHYLL *a* METHOD.
For this procedure, follow the instructions in the Turner Designs procedure (P/N 998-9000), "Measuring Extracted Chlorophyll *a* Free from the Errors Associated with Chlorophyll *b* and Pheopigments." Procedures in Method 445.0 apply generally, **EXCEPT** you must **NOT** acidify your samples as set forth in Method 445.0, section 11.2.2. It is not necessary, as the special optical filter set up is designed to read ONLY chlorophyll *a* and NOT chlorophyll *b* and the pheopigments. It is not necessary to perform calculations set forth in Section 12.1 of Method 445.0. When properly calibrated with a known concentration of pure chlorophyll *a*, the sample reading without acidification represents the actual proportion of chlorophyll *a* relative to the standard.

References

1. To obtain copies of the complete EPA standard methods book, *Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples*, call the EPA in Cincinnati, Ohio at (513) 569-7562. The book contains Method 445.0 and several other useful procedures. Ask for item EPA/600/R-92/121. You can also download EPA methods directly from the EPA website at www.epa.gov.

2. A note about optics. For chlorophyll studies according to Method 445.0, the Turner Designs Model 10-AU comes equipped with an excitation filter equivalent to the CS 5-60 excitation filter and an emission filter equivalent to the CS 2-64 emission filter (see Section 6.1 of Method 445.0). We supply the F4T5D daylight white lamp.

3. The method was developed by Dr. Nicholas A. Welschmeyer of Moss Landing Marine Laboratories, Moss Landing, California. A paper by Dr. Welschmeyer, Fluorometric Analysis of Chlorophyll *a* in the presence of Chlorophyll *b* and Pheopigments, can be found in *Limnology and Oceanography* (1994) 39: 1985-1992.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL EXPOSURE RESEARCH LABORATORY
CINCINNATI, OH 45268

Method 445.0

In Vitro Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Algae by Fluorescence

OFFICE OF
RESEARCH AND DEVELOPMENT

Revision 1.2

ERRATA SHEET

Section1.4 - References numbered 5-8 should be numbered 6-8.

Section 12.2 - Equation for calculating the "corrected" concentration of chlorophyll *a* in the whole water sample is as follows:

$$C_{s,c} = \frac{C_{E,c} \text{ X extract volume (L) X DF}}{\text{Sample volume (L)}}$$

Where, $C_{s,c}$ = corrected chlorophyll *a* concentration (µg/L) in the whole water sample
extract volume = volume (L) of extract prepared before dilution

Section 13.4.1 - The following has been added to the second paragraph.

The reported p-EDLs reflect between-lab variability and extraction variability. There was, however, a major flaw in the study design. Even though the concentrations used could be easily determined by fluorometry after appropriate dilution, we did not specify to the labs the dilution factor to be used. Because of that, each lab diluted at their discretion. Dilution factors ranged from 10-2000. The "observed" concentration by each fluorometer was not the reported concentration used in the multi-lab statistical analysis. Since p-EDLs are based on an estimate of variance (standard deviation) of the reported concentration in the extract, the p-EDL for fluorometry is not reflective of the concentration actually observed by the instrument. Since all the participants used different dilution factors there was no way to correct the determined p-EDLs for the fluorometric techniques. It is safe to say that the statistically determined p-EDLs are at least 1000 times too high. Still, fluorometry yielded the lowest p-EDLs. The p-EDLs for the other methods are valid.

Table 4 - The following has been added to footnote 5.

This is due to a flaw in the study design and not due to any inherent limitations of fluorometry. Please see Section 13.4.1 for a discussion of the determination of p-EDLs. Single-lab EDLs may be 1000 times lower than the p-EDLs reported here.

Tables 4-9 - The following footnote has been added.

Reported concentrations (ppm) are for the 10 mL extraction volume and not the concentrations in the whole water sample. Using the notation of Section 12 of the method, this would be $C_{E,c}$.

Table 9 - Eighth column, last value should be 0.675

Method 445.0

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Elizabeth J. Arar

and

Gary B. Collins

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National Exposure Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

pure chlorophyll *a* of known concentration, are used to calculate the concentration of chlorophyll *a* and pheophytin *a* in the sample extract. The concentration in the natural water sample is reported in µg/L.

3.0 Definitions

3.1 Estimated Detection Limit (EDL) -- The minimum concentration of an analyte that yields a fluorescence 3X the fluorescence of blank filters which have been extracted according to this method.

3.2 Linear Dynamic Range (LDR) -- The absolute quantity or concentration range over which the instrument response to an analyte is linear.

3.3 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. For this method the background is a solution of 90% acetone.

3.4 Stock Standard Solution (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.5 Primary Dilution Standard Solution (PDS) -- A solution of the analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.6 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions containing the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.7 Response Factor (RF) -- The ratio of the response of the instrument to a known amount of analyte.

3.8 Laboratory Reagent Blank (LRB) -- An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus.

3.9 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.

3.10 Quality Control Sample (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. Ideally, the QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11 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.

4.0 Interferences

4.1 Any substance extracted from the filter or acquired from laboratory contamination that fluoresces in the red region of the spectrum may interfere in the accurate measurement of both chlorophyll *a* and pheophytin *a*.

4.2 The relative amounts of chlorophyll *a*, *b* and *c* vary with the taxonomic composition of the phytoplankton. Chlorophylls *b* and *c* may significantly interfere with chlorophyll *a* measurements depending on the amount present. Due to the spectral overlap of chlorophyll *b* with pheophytin *a* and chlorophyll *a*, underestimation of chlorophyll *a* occurs accompanied by overestimation of pheophytin *a* when chlorophyll *b* is present in the sample. The degree of interference depends upon the ratio of *a*:*b*. This laboratory found that at a ratio of 5:1, using the acidification procedure to correct for pheophytin *a*, chlorophyll *a* was underestimated by approximately 5%. Loftis and Carpenter[10] reported an underestimation of 16% when the *a*:*b* ratio was 2.5:1. A ratio of 1:1 is the highest ratio likely to occur in nature. They also reported overestimation of chlorophyll *a* in the presence of chlorophyll *c* of as much as 10% when the *a*:*c* ratio was 1:1 (the theoretical maximum likely to occur in nature). The presence of chlorophyll *c* also causes the under-

6.11.5 Pasteur type pipets or medicine droppers.

6.11.6 Disposable glass cuvettes for the fluorometer.

6.11.7 Filtration apparatus consisting of 1 or 2-L filtration flask, 47-mm fritted glass disk base and a glass filter tower.

6.11.8 Centrifuge tubes, polypropylene or glass, 15-mL capacity with nonpigmented screw-caps.

6.11.9 Polyethylene squirt bottles.

7.0 Reagents and Standards

7.1 Acetone, HPLC grade, (CASRN 67-64-1).

7.2 Hydrochloric acid (HCl), concentrated (sp. gr. 1.19), (CASRN 7647-01-0).

7.3 Chlorophyll *a* free of chlorophyll *b*. May be obtained from a commercial supplier such as Sigma Chemical (St. Louis, MO). Turner Designs (Sunnyvale, CA) supplies ready-made standards.

7.4 **Water** -- ASTM Type I water (ASTM D1193) is required. Suitable water may be obtained by passing distilled water through a mixed bed of anion and cation exchange resins.

7.5 ***0.1 N HCl Solution*** -- Add 8.5 mL of concentrated HCl to approximately 500 mL water and dilute to 1 L.

7.6 ***Aqueous Acetone Solution*** -- 90% acetone /10% water. Carefully measure 100 mL of water into the 1-L graduated cylinder. Transfer to a 1-L flask or storage bottle. Measure 900 mL of acetone into the graduated cylinder and transfer to the flask or bottle containing the water. Mix, label and store.

7.7 ***Chlorophyll Stock Standard Solution*** (SSS) -- Chlorophyll *a* from a commercial supplier will be shipped in an amber glass ampoule which has been flame sealed. This dry standard should be stored at -20 or -70°C in the dark and the SSS prepared just prior to use. Tap the ampoule until all the dried chlorophyll is in the bottom of the ampoule. In subdued light, carefully break the tip off the ampoule. Transfer the entire contents of the ampoule into a 50-mL volumetric flask. Dilute to volume with 90% acetone, label the flask and wrap with aluminum foil to protect from light. The concentration of the solution must be determined spectrophotometrically using a multiwavelength spectrophotometer.[10] When stored in a light and airtight container at freezer temperatures, the SSS is stable for at least six months. The concentration of all dilutions of the SSS must be determined spectrophotometrically each time they are made.

7.8 ***Laboratory Reagent Blank*** (LRB) -- A blank filter which is extracted and analyzed just as a sample filter. The LRB should be the last filter extracted of a sample set. It is used to assess possible contamination of the reagents or apparatus.

7.9 ***Chlorophyll a Primary Dilution Standard Solution*** (PDS) -- Add 1 mL of the SSS (Sect. 7.8) to a clean 100-mL flask and dilute to volume with the aqueous acetone solution (Sect. 7.7). If exactly 1 mg of pure chlorophyll *a* was used to prepare the SSS, the concentration of the PDS is 200 µg/L. Prepare fresh just prior to use.

7.10 ***Quality Control Sample*** (QCS) -- Since there are no commercially available QCSs, dilutions of a stock standard of a different lot number from that used to prepare calibration solutions may be used.

8.0 Sample Collection, Preservation and Storage

8.1 ***Water Sample Collection*** -- Water may be obtained by a pump or grab sampler. Data quality objectives will determine the depth at which samples are taken. Healthy phytoplankton, however, are generally obtained from the photic zone (depth at which the illumination level is 1% of surface illumination). Enough water should be collected to concentrate phytoplankton on at least three filters so that precision can be assessed. Filtration volume size will depend on the particulate load of the water. Four liters may be required for open ocean water where phytoplankton density is usually low, whereas 1 L or less is generally sufficient for lake, bay or estuary water. All apparatus should be clean and acid-free. Filtering should be performed in subdued light as soon as possible after sampling since algal poulations, thus chlorophyll *a* concentration, can change in relatively short periods of time. Aboard ship filtration is highly recommended.

Assemble the filtration apparatus and attach the vacuum source with vacuum gauge and regulator. Vacuum filtration should not exceed 6 in. Hg (20 kPa). Higher

Twenty to thirty natural samples should be obtained using the procedure outlined in Sect. 8.1 of this method. Sets of 10 or more samples should be extracted and analyzed according to Sect. 11.2. The percent relative standard deviation (%RSD) of uncorrected values of chlorophyll *a* should not exceed 15% for samples that are approximately 10X the IDL. RSD for pheophytin *a* might typically range from 10 to 50%.

9.2.7 Corrected Chl *a* -- Multilaboratory testing of this method revealed that many analysts do not adequately mix the acidified sample when determining corrected chl a. The problem manifests itself by highly erratic pheo-*a* results, high %RSDs for corrected chl *a* and poor agreement between corrected and uncorrected chl *a*. To determine if a new analyst is performing the acidification step properly, perform the following QC procedure:

Prepare 100 mL of a 50 ppb chl *a* solution in 90% acetone. The new analyst should analyze 5-10 separate aliquots, using separate cuvettes, according to instructions in Section 11.2. Process the results according to Section 12 and calculate separate means and %RSDs for corrected and uncorrected chl *a*. If the means differ by more than 10%, then the stock chl *a* has probably degraded and fresh stock should be prepared. The %RSD for corrected chl *a* should not exceed 5%. If the %RSD exceeds 5%, repeat the procedure until the %RSD 5%.

9.3 Assessing Laboratory Performance (Mandatory)

9.3.1 Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one blank filter with each sample batch. The LRB should be the last filter extracted. LRB data are used to assess contamination from the laboratory environment. LRB values that exceed the IDL indicate contamination from the laboratory environment. When LRB values constitute 10% or more of the analyte level determined for a sample, fresh samples or field duplicates must be analyzed after the contamination has been corrected and acceptable LRB values have been obtained.

10.0 Calibration and Standardization

10.1 ***Calibration*** -- Calibration should be performed bimonthly or when there has been an adjustment made to the instrument, such as replacement of lamp, filters or photomultiplier. Prepare 0.2, 2, 5, 20 and 200 µg chl *a*/L calibration standards from the PDS (Sect. 7.11). Allow the instrument to warm up for at least 15 min. Measure the fluorescence of each standard at sensitivity settings that provide midscale readings. Obtain response factors for chlorophyll *a* for each sensitivity setting as follows:

$$F_s = C_a/R_s$$

where:

F_s = response factor for sensitivity setting, S.

R_s = fluorometer reading for sensitivity setting, S.

C_a = concentration of chlorophyll *a*.

NOTE: If you are using special narrow bandpass filters for chl *a* determination, **DO NOT** acidify. Use the "uncorrected" chl *a* calculation described in Section 12.1.

If pheophytin *a* determinations will be made, it will be necessary to obtain before-to-after acidification response ratios of the chlorophyll *a* calibration standards as follows: (1) measure the fluorescence of the standard, (2) remove the cuvette from the fluorometer, (3) acidify the solution to .003 N HCl[6] with the 0.1 N HCl solution, (4) use a pasteur type pipet to thoroughly mix the sample by aspirating and dispensing the sample into the cuvette, keeping the pipet tip below the surface of the liquid to avoid aerating the sample, (5) wait 90 sec and measure the fluorescence of the standard solution again. Addition of the acid may be made using a medicine dropper. It will be necessary to know how many drops are equal to 1 mL of acid. For a cuvette that holds 5 mL of extraction solution, it will be necessary to add 0.15 mL of 0.1 N HCl to reach a final acid concentration of 0.003N in the 5 mL. Calculate the ratio, r, as follows:

$$r = R_b/R_a$$

where:

R_b = fluorescence of pure chlorophyll *a* standard solution before acidification.

R_a = fluorescence of pure chlorophyll *a* standard solution after acidification.

Calculate the "uncorrected" concentration of chlorophyll *a* in the whole water sample as follows:

$$C_{S,u} = \frac{C_{E,u} \times \text{extract volume (L)} \times DF}{\text{sample volume (L)}}$$

where $C_{S,u}$ = uncorrected chlorophyll *a* concentration (µg/L) in the whole water sample,

extract volume = volume (L) of extraction prepared before any dilutions,

DF = dilution factor,

sample volume = volume (L) of whole water sample.

12.2 For "corrected chlorophyll *a*", calculate the chlorophyll *a* concentration in the extract as :

$C_{E,c} = F_s (r/r-1) (R_b - R_a)$

where:

$C_{E,c}$ = corrected chlorophyll *a* concentration (µg/L) in the extract solution analyzed,

F_s = response factor for the sensitivity setting S,

r = the before-to-after acidification ratio of a pure chlorophyll *a* solution (Sect. 10.1),

R_b = fluorescence of sample extract before acidification, and

R_a = fluorescence of sample extract after acidification.

Calculate the "corrected" concentration of chlorophyll *a* in the whole water sample as follows:

$$C_{S,c} = \frac{C_{E,u} \times \text{extract volume (L)} \times DF}{\text{sample volume (L)}}$$

where $C_{S,c}$ = corrected chlorophyll *a* concetration (µg/L) in the whole water sample,

extract volume = volume (L) of extract prepared before dilution,

12.3 Calculate the pheophytin *a* concentration as follows:

$$P_E = F_s (r/r-1) (rR_a - R_b)$$

$$P_S = \frac{P_E \times \text{extract volume (L)} \times DF}{\text{sample volume (L)}}$$

where P_E = pheophytin *a* concentration (µg/L) in the sample extract; and

P_s = pheophytin *a* concentration (µg/L) in the whole water sample.

12.4 LRB and QCS data should be reported with each sample data set.

13.0 Method Performance

13.1 The single lab EDL for the instrument used in the evaluation of this method was 0.05 µg/L for chlorophyll *a* and 0.06 µg/L pheophytin *a*.

13.2 The precision (%RSD) for chlorophyll *a* in mostly blue-green and green phytoplankton natural samples which were steeped for 2 h vs 24 h is reported in Table 1. Although the means were the same, precision was better for samples which were allowed to steep for 24 h prior to analysis. Since pheophytin *a* was found in the samples, the chlorophyll *a* values are "corrected" (Sect. 12.2). Table 2 contains precision data for pheophytin *a*. A statistical analysis of the pheophytin *a* data indicated a significant difference in the mean values at the 0.05 significance level. The cause of the lower pheophytin *a* values in samples extracted for 24 h is not known.

13.3 Three QCS ampoules obtained from the USEPA were analyzed and compared to the reported confidence limits in Table 3. **NOTE:** The USEPA no longer provides these QCSs.

13.4 **Multilaboratory Testing** - A multilaboratory validation and comparison study of EPA Methods 445.0, 446.0 and 447.0 for chlorophyll *a* was conducted in 1996 by Research Triangle Institute, Research Triangle Park, N.C. (EPA Contract No. 68-C5-0011). There were 21 volunteer participants in the fluorometric methods

environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation (e.g., Sect. 11.1.1). When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202)872-4477.

15.0 Waste Management

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult *The Waste Management Manual for Laboratory Personnel*, available from the American Chemical Society at the address listed in the Sect. 14.2.

16.0 References

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17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. COMPARISON OF PRECISION OF TWO EXTRACTION PERIODS

CORRECTED CHLOROPHYLL *a*

	Sample A[1]		Sample B[2]	
	2 h[3]	24 h[3]	2 h[3]	24 h[3]
Mean Concentration (µg/L)	49.6	52.9	78.6	78.8
Standard Deviation (µg/L)	4.89	2.64	6.21	2.77
Relative Standard Deviation (%)	9.9	5.0	7.9	3.5

1 Values reported are the mean measured concentrations (n=6) of chlorophyll *a* in the natural water based on a 100-mL filtration volume.

2 Values reported are the mean measured concentrations (n=9) of the extraction solution. Sample filtration volume was 300 mL.

3 The length of time that the filters steeped after they were macerated.

TABLE 3. ANALYSES OF USEPA QC SAMPLES

ANALYTE	REFERENCE VALUE	CONFIDENCE LIMITS
Chlorophyll *a*	2.1 µg/L	0.5 to 3.7 µg/L
Pheophytin *a*	0.3 µg/L	-0.2 to 0.8 µg/L

ANALYTE	MEAN MEASURED VALUE	% Relative Standard[1] Deviation
Chlorophyll *a*	2.8 µg/L	1.5
Pheophytin *a*	0.3 µg/L	33

[1] N = 3

TABLE 5. POOLED PRECISION FOR DUNALIELLA TERTIOLECTI SAMPLES

Method[1]	mLs of culture filtered	N[2]	Mean (mg chla/L)	Std. Dev.	%RSD
Fl-Mod	5	7	0.163	0.037	22.8
	10	7	0.298	0.080	26.7
	50	7	1.684	0.385	22.9
	100	7	3.311	0.656	19.8
Fl-Std	5	8	0.185	0.056	30.4
	10	8	0.341	0.083	24.4
	50	8	1.560	0.311	19.9
	100	8	3.171	0.662	20.9

(1) Fl-Mod = fluorometric method using special interference filters.

Fl-Std = conventional fluorometric method with pheophytin *a* correction.

(2) N = number of volunteer labs whose data was used.

TABLE 7. POOLED PRECISION FOR PHAEODACTYLUM TRICORNUTUM SAMPLES

Method[1]	mLs of culture filtered	N[2]	Mean (mg chla/L)	Std. Dev.	%RSD
Fl-Mod	5	7	0.221	0.040	18.0
	10	7	0.462	0.094	20.3
	50	7	2.108	0.491	23.3
	100	7	3.568	1.186	33.2
Fl-Std	5	8	0.214	0.053	24.8
	10	8	0.493	0.091	18.4
	50	8	2.251	0.635	28.2
	100	8	4.173	0.929	22.3

(1) Fl-Mod = fluorometric method using special interference filters.

Fl-Std = conventional fluorometric method with pheophytin *a* correction.

(2) N = number of volunteer labs whose data was used.

NOTE: The phaeodactylum extract contained significant amounts of chlorophyll *c* and chlorophyllide *a* which interferes in chlorophyll *a* measurement in the fluorometric method, therefore, the concentration of chlorophyll *a* is overestimated compared to the HPLC method which separates the three pigments. The FL-Mod interference filters minimize this interference more so than the conventional filters.