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NATIONAL WATER QUALITY LABORATORY TECHNICAL MEMORANDUM 2002.02

22 May 2002

Distribution: E

Subject: Revision of the Procedure Used to Calculate Concentrations of Chlorophyll a and b in Phytoplankton and Periphyton by High-Performance Liquid Chromatography.

Effective Date: October 1, 2001

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Purpose and Background

The National Water Quality Laboratory (NWQL) analyzes Periphyton and Phytoplankton samples for chlorophyll a (chl a) and chlorophyll b (chl b) by high-performance liquid chromatography with fluorescence detection (Britton and Greeson, eds., 1977, revised 1987).

The appropriate Method Code, Parameter Codes and Lab Codes are as follows:

Name	Lab Code	Parameter Code	Method Code
Chl a, phytoplankton	586	70953	A
Chl b, phytoplankton	587	70954	A
Chl a, periphyton	588	70957	A
Chl b, periphyton	589	70958	A

The NWQL has analyzed samples by this method since 1978. Standard solutions for this method are prepared by dissolving purified chlorophyll a or b in a 90-percent acetone/water solution, measuring the spectrophotometric absorbance at specified wavelengths, and applying an extinction coefficient to calculate the concentration of chlorophyll in these standard solutions. The solvent used to prepare chlorophyll standards was changed to methanol in April 1988 because the acetone solution caused chromatographic problems. However, the extinction coefficient for chlorophyll in 90-percent acetone is different from the coefficient for chlorophyll in methanol. The difference in extinction coefficients was determined during a recent literature review.

The calculated concentration of the standard chlorophyll solutions has been in error since April 1988; this error has resulted in a consistent, predictable bias in the chlorophyll a and chlorophyll b results in the national data base. The correct extinction coefficients have been used since Oct. 1, 2001.

The only samples affected are those analyzed by the high-performance liquid chromatographic methods (Britton and Greeson, eds., 1987). About 14,000 samples for phytoplankton and 1,000 samples for periphyton are affected.

Samples analyzed by U.S. Environmental Protection Agency fluorometric method (Method 445.0) are not affected. The lab codes, parameter codes and method codes for the data that are not affected follow:

Name	Lab Code	Parameter Code	Method Code
Chl a, phytoplankton	2645	70953	B
Chl a, periphyton	2643	70957	B

The purposes of this memo are

1. To evaluate the magnitude and the timeframe of the bias: and
2. To discuss the effects on the national data base and to explain planned corrections.

Scope

Standard solutions are prepared by dissolving solid chlorophyll in an organic solvent. The absorbance of the solution is measured in a 1-cm cell at a wavelength of 664 nanometers (nm) for chl a and at 647 nm for chl b. The concentrations of the solutions are calculated by dividing the absorbance by the extinction coefficient.

The extinction coefficient for chl a in 90-percent acetone is 0.0877 liter/milligram centimeter (L/mg cm) at 664 nm (Jeffery and Humphrey, 1975) and for chl a in methanol is 0.07995 L/mg cm at 665 nm (Porra and others, 1989). The use of the wrong extinction coefficient produced chl a results that were biased low.

The extinction coefficient for chl b in 90-percent acetone is 0.0514 L/mg cm at 647 nm (Jeffery and Humphrey, 1975). The extinction coefficient in methanol is 0.04248 L/mg cm at 652 nm (Porra and others, 1989). There is also a substantial difference in absorbance readings of a standard chlorophyll b solution, at the two different wavelengths. The use of the wrong extinction coefficient, along with the absorbance difference, produced chl b results that were biased low.

Conclusions

The NWQL started using the correct extinction coefficients and wavelengths for samples taken on or after Oct. 1, 2001. The bias is substantial for both chlorophyll a and b, for samples taken between April 1988 and October 1, 2001. Trend data will show shifts upward for both phytoplankton and periphyton after Oct. 1, 2002, if the data base is not corrected. Therefore, the National Water Information System (NWIS) data base will be corrected.

Effects on Data Base

The chlorophyll a results will be corrected by multiplying appropriate data by a factor of 1.0969. The chlorophyll b results will be corrected by multiplying the appropriate data by 1.2855. The percent difference between the incorrect results and the corrected results will remain constant over the range of data-base results. However, the absolute difference between incorrect results and correct results will increase exponentially because a constant multiplier is being applied to results that span several orders of magnitude.

The correction will be limited to samples collected between April 12, 1988, and October 1, 2001, identified in NWIS with method code "A." Results for samples collected prior to April 12, 1998, but analyzed afterwards will not be corrected. Similarly, results for samples not identified with a method code, or identified with any other method code will not be altered. Censored values will not be changed.

Jonathon Scott, NWIS Phoenix group, has developed a procedure (Scott, 2002) to correct the data base, as described below:

1. Login as userid "nwis" on the local NWIS computer host.
2. Perform the following Unix commands. These steps should take less than five minutes to complete.

```
ftp nwdokokl.cr.usgs.gov
anonymous
userid
cd nawqwa
get do.chl
quit
chmod +x do.chl
./do.chl > chl.log
```

3. Scan the "chl.log" log file for problems. The program "do.chl" should have performed the correction for all local NWIS databases and created a report for each database where changes were made. Review the reports named "chlorophyll.DBnn.rpt" (where nn is a database number). A sample report is shown below.

Mar 27 2002 14:39 Chlorophyll Results Updated in Database 01 Page 1

```
-----
record_no    site_no      sample_start_dt  parameter_cd  remark_cd  result_va
-----
00003273    06037500    26-jul-2000 14:46:00      70957                128
98805112    06037500    28-jul-1988 04:57:00      70953                154
00003269    06037500    15-dec-1999 11:00:00      70954                153
00003278    06037500    20-jul-2000 09:46:00      70958                134
00003268    06037500    04-oct-1999 11:30:00      70953                 5.5
00003269    06037500    15-dec-1999 11:00:00      70953                13.1
00003272    06037500    05-jun-2000 12:00:00      70953                 0.9
00003274    06037500    26-jul-2000 15:01:00      70957                33.1
00003276    06037500    10-jul-2000 11:31:00      70957                 8.7
98905812    06037500    03-may-1989 09:55:00      70957                 1
00003268    06037500    04-oct-1999 11:30:00      70954                 0.2
00003270    06037500    24-feb-2000 11:15:00      70954                 2.2
00003273    06037500    26-jul-2000 14:46:00      70958                 24
00003274    06037500    26-jul-2000 15:01:00      70958                 4.6
00003277    06037500    13-jul-2000 11:01:00      70958                 1.2
00003283    06037500    02-nov-1999 08:00:00      70954                 E                24.4
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```

4. If you have any problems with this procedure, contact Jon Scott.

//signed//

Gregory B. Mohrman, Chief
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Branch of Analytical Services

References

- Britton, L. J., and Greason, P.E., eds., 1987, Methods for the collection and analysis of aquatic biological and microbiological samples: Techniques of Water-Resources Investigations of the U.S. Geological Survey, Book 5, Chapter A4.
- Jeffrey, S. W., and Humphrey, G. F., 1975, New spectrophotometric equations for determining chlorophyll a, b, c1 and c2 in higher plants, algae and natural phytoplankton: *Biochem. Physiol. Pflanz*, v. 167, p. 191-194.
- Porra, R. J., Thompson, W. A., and Kriedmann, P. E., 1989, Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: Verification of the concentrations of chlorophyll standards by atomic absorption spectroscopy: *Biochem. Biophys. Acta* v. 975, p. 384 - 394.
- Scott, J. C., 2002, Chlorophyll-Bias Correction Procedure: U.S. Geological Survey, accessed May 2, 2002, at URL <http://ok.water.usgs.gov/nawqa/phoenix/training/guidancechl.html> (Internal USGS web document)