



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
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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

Section 1.4 - 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:

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

Where,  $C_{S,c}$  = corrected chlorophyll *a* concentration ( $\mu\text{g/L}$ ) 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