



Designation: **D5412 – 93 (Reapproved 2011)<sup>ε1</sup> D5412 – 93 (Reapproved 2017)<sup>ε1</sup>**

## Standard Test Method for Quantification of Complex Polycyclic Aromatic Hydrocarbon Mixtures or Petroleum Oils in Water<sup>1</sup>

This standard is issued under the fixed designation D5412; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

<sup>ε1</sup> NOTE—~~Editorial corrections were made.~~ Warning statements were editorially corrected throughout in ~~March 2014.~~ December 2017.

### 1. Scope

1.1 This test method covers a means for quantifying or characterizing total polycyclic aromatic hydrocarbons (PAHs) by fluorescence spectroscopy (FI) for waterborne samples. The characterization step is for the purpose of finding an appropriate calibration standard with ~~similar~~ similar emission and synchronous fluorescence spectra.

1.2 This test method is applicable to PAHs resulting from petroleum oils, fuel oils, creosotes, or industrial organic mixtures. Samples can be weathered or unweathered, but either the same material or appropriately characterized site-specific PAH or petroleum oil calibration standards with similar fluorescence spectra should be chosen. The degree of spectral similarity needed will depend on the desired level of quantification and on the required data quality objectives.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate ~~safety~~ safety, health, and ~~health~~ environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

### 2. Referenced Documents

#### 2.1 ASTM Standards:<sup>2</sup>

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water

D3325 Practice for Preservation of Waterborne Oil Samples

D3326 Practice for Preparation of Samples for Identification of Waterborne Oils

D3415 Practice for Identification of Waterborne Oils

D3650 Test Method for Comparison of Waterborne Petroleum Oils By Fluorescence Analysis

D4489 Practices for Sampling of Waterborne Oils

D4657 Test Method for Polynuclear Aromatic Hydrocarbons in Water (Withdrawn 2005)<sup>3</sup>

E131 Terminology Relating to Molecular Spectroscopy

E169 Practices for General Techniques of Ultraviolet-Visible Quantitative Analysis

E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers

E388 Test Method for Wavelength Accuracy and Spectral Bandwidth of Fluorescence Spectrometers

E578 Test Method for Linearity of Fluorescence Measuring Systems

E579 Test Method for Limit of Detection of Fluorescence of Quinine Sulfate in Solution

<sup>1</sup> This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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<sup>2</sup> For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

<sup>3</sup> The last approved version of this historical standard is referenced on www.astm.org.

### 3. Terminology

3.1 *Definitions—Definitions:* For definitions of terms used in this test method, refer to Terminology [D1129](#), Terminology [E131](#), and Practice [D3415](#).

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### 4. Summary of Test Method

4.1 This test method consists of fluorescence analysis of dilute solutions of PAHs or petroleum oils in appropriate solvents (spectroquality solvents such as cyclohexane or other appropriate solvents, for example, ethanol, depending on polarity considerations of the sample). The test method requires an initial qualitative characterization step involving both fluorescence emission and synchronous spectroscopy in order to select appropriate calibration standards with similar fluorescence spectra as compared to the samples (see [Annex A1](#) for the definition of spectral similarity). Intensities of peak maxima of suitable emission spectra are then used to develop calibration curves for quantification.

NOTE 1—Although some sections of the characterization part of this test method are similar to Test Method [D3650](#), there are also significant differences (see [Annex A1](#)). Since the purpose and intent of the two test methods are different, one should not be substituted for the other.

### 5. Significance and Use

5.1 This test method is useful for characterization and rapid quantification of PAH mixtures including petroleum oils, fuels, creosotes, and industrial organic mixtures, either waterborne or obtained from tanks.

5.2 The unknown PAH mixture is first characterized by its fluorescence emission and synchronous scanning spectra. Then a suitable site-specific calibration standard with similar spectral characteristics is selected as described in [Annex A1](#). This calibration standard may also be well-characterized by other independent methods such as gas chromatography (GC), GC-mass spectrometry (GC-MS), or high performance liquid chromatography (HPLC). Some suggested independent analytical methods are included in References [\(1-7\)](#)<sup>4</sup> and Test Method [D4657](#). Other analytical methods can be substituted by an experienced analyst depending on the intended data quality objectives. Peak maxima intensities of appropriate fluorescence emission spectra are then used to set up suitable calibration curves as a function of concentration. Further discussion of fluorescence techniques as applied to the characterization and quantification of PAHs and petroleum oils can be found in References [\(8-18\)](#).

5.3 For the purpose of the present test method polynuclear aromatic hydrocarbons are defined to include substituted polycyclic aromatic hydrocarbons with functional groups such as carboxyl acid, hydroxy, carbonyl and amino groups, and heterocycles giving similar fluorescence responses to PAHs of similar molecular weight ranges. If PAHs in the more classic definition, that is, unsubstituted PAHs, are desired, chemical reactions, extractions, or chromatographic procedures may be required to eliminate these other components. Fortunately, for the most commonly expected PAH mixtures, such substituted PAHs and heterocycles are not major components of the mixtures and do not cause serious errors.

### 6. Interferences

6.1 The fluorescence spectra may be distorted or quantification may be affected if the sample is contaminated with an appreciable amount of other fluorescent chemicals that are excited and which fluoresce in the same spectral regions with relatively high fluorescence yields. Usually the fluorescence spectra would be distorted at levels greater than 1 to 2 % of such impurities before the quantification would be seriously affected. (**Warning**—Storage of samples in improper containers (for example, plastics other than TFE-fluorocarbon) may result in contamination.)

NOTE 2—**Caution:** Storage of samples in improper containers (for example, plastics other than TFE-fluorocarbon) may result in contamination.

NOTE 2—*Spectroquality* solvents may not have low enough fluorescence background to be used as solvent blanks. Solvent lots vary in the content of fluorescent impurities that may increase with storage time even for unopened bottles.

NOTE 3—This test method is normally used without a matrix spike due to possible fluorescence interference by the spike. If a spike is to be used, it must fluoresce in a spectral region where it will not interfere with the quantification process. Compounds that could be used are dyes that fluoresce at longer wavelengths than the emission of the PAH mixture.

6.2 If the PAH mixture to be analyzed is a complex mixture such as an oil or creosote, it is assumed that a well-characterized sample of the same or similar material is available as a calibration standard so the fluorescent fraction of the mixture can be ratioed against the total mixture. Otherwise, since the samples and standards are weighed, the nonfluorescent portion of the mixture would bias the quantification although the characterization portion of the test method for PAHs given in [Annex A1](#) would be unaffected.

### 7. Apparatus

7.1 *Fluorescence Spectrometer*—An instrument recording in the spectral range of 250 nm to at least 600 nm for both excitation and emission responses and capable of scanning both monochromators simultaneously at a constant speed with a constant

<sup>4</sup> The boldface numbers in parentheses refer to a list of references at the end of this standard.

wavelength offset between them for synchronous scanning. The instrument should meet the specifications in **Table 1**. (Also known as spectrofluorometer or fluorescence spectrophotometer.) Consult manufacturer's instrument manuals for specific operating instructions.

**NOTE 4**—Although the characterization section of this test method (given in **Annex A1**) is similar to Test Method **D3650** in many respects, there are differences in the purpose and intents of the two test methods. The purpose of the characterization step of this test method is to find an oil with similar fluorescence properties as the sample in order to serve as an appropriate calibration standard for quantification. Other differences between the test methods are instrumentation requirements and the use of synchronous spectra as well as emission spectra for this test method.

**7.2 Excitation Source**—A high-pressure xenon lamp (a 150-W continuous xenon lamp or a 10-W pulsed xenon lamp has been proven acceptable). Other continuum sources (either continuous or pulsed) having sufficient intensity throughout the ultraviolet and visible regions may also be used.

**7.3 Fluorescence Cells**—Standard cells made from fluorescence-free fused silica with a path length of 10 mm and a height of at least 45 mm. Stopped cells may be preferred to prevent sample evaporation and contamination.

**7.4 Data Recording System**—Preferably the instrument should be interfaced to a suitable computer system compatible with the instrument and with suitable software for spectral data manipulation. Use of a strip chart or X-Y recorder with a response time of less than 1 s for full-scale deflection is acceptable.

**7.5 Micropipet**, glass, 10 to 50- $\mu$ L capacity.

**7.6 Weighing Pans**, 5 to 7-mm diameter, 18-mm thick, made of aluminum or equivalent. Check pans for contamination.

## 8. Reagents and Materials

**8.1 Purity of Reagents**—Use spectroquality grade reagents in all instances unless otherwise stated. Since the goal is to have as low a fluorescence blank as possible, and since different brands and lots of spectroquality solvent may vary, check reagents frequently.

**8.2 Purity of Water**—References to water mean Type IV water conforming to Specification **D1193**. Since fluorescent organic impurities in the water may introduce an interference, check the purity of the water by analyzing a water blank using the same instrumental conditions as for the solvent blank.

**8.3 Acetone**, spectroquality, ( $\text{CH}_3\text{COCH}_3$ ).

**8.4 Cyclohexane**, spectroquality or HPLC grade. The fluorescence solvent blank must be as low as possible and less than 5 % of the intensity of the maximum emission peak for the lowest concentration of PAHs analyzed. Dispense cyclohexane during the procedure from either a TFE-fluorocarbon or glass wash bottle, but, for prolonged storage, store cyclohexane only in glass.

**8.5 Nitric Acid (1 + 1)**—Carefully add one volume of concentrated  $\text{HNO}_3$  (sp gr 1.42) to one volume of water.

**8.6 TFE-Fluorocarbon Strips**, 25 mm by 75 mm, 0.25-mm thickness. Use TFE strips when sampling neat PAH films on water as described in Practices **D4489**.

## 9. Sampling and Sample Preparation

**9.1** Collect a representative sample (see Practices **D4489** for water samples).

**9.2** Preserve samples in containers as specified in Practice **D3325**. Do not cool samples below 5°C to avoid dewaxing of oil or creosote samples.

**9.3** Neat PAH samples (including surface films or layers on water) require only dilution in spectroquality cyclohexane. Prepare initial concentration for the unknown at 100  $\mu\text{g}/\text{mL}$  for a check of the fluorescence signal. Further dilutions down to 1  $\mu\text{g}/\text{mL}$  may

**TABLE 1 Specifications for Fluorescence Spectrometers**

Wavelength Reproducibility	
Excitation monochromator	$\pm 2$ nm or better
Emission monochromator	$\pm 2$ nm or better
Gratings (Typical Values)	
Excitation monochromator	minimum of 600 lines/mm blazed at 300 nm
Emission monochromator	minimum of 600 lines/mm blazed at 300 nm or 500 nm
Photomultiplier Tube	
S-20 or S-5 response or equivalent	
Spectral Resolutions	
Excitation monochromator	spectral bandpass of 2.5 nm or less
Emission monochromator	spectral bandpass 2.5 nm or less
Maximum bandpasses for both monochromators at least 10 nm	

be needed to bring the fluorescence signal into the linear range and to avoid self-absorption effects in the solution. Most PAH mixtures and oils have been found to be soluble in cyclohexane at the concentrations listed. Alternative solvents can be substituted with appropriate tests.

9.4 If any unknown PAH mixture is dissolved in water, test the mixture with appropriate dilutions or preconcentrations as required. The assumption is that no naturally-occurring fluorescent materials such as humic or fulvic acids are present at levels interfering with the determination (refer to [Fig. A2.5](#) and [Fig. A2.6](#) to show that humic acid does not interfere with the test method even at high ( $\mu\text{g/L}$ ) levels). This usually becomes a problem only at PAH levels in the low  $\mu\text{g/L}$  range. Extraction methods (or separation by column chromatography) are listed in Practice [D3326](#).

9.4.1 An extraction method that proved satisfactory for the collaborative test is as follows:

9.4.1.1 Pour 50.0 mL of the sample into a separatory funnel, add 5.0 mL of cyclohexane and shake for 2 min. Vent the separatory funnel occasionally. Withdraw the aqueous layer (keep this for a second extraction). Collect the cyclohexane extract in a 10-mL volumetric flask. Add 5.0 mL of cyclohexane to the aqueous layer and perform a second extraction. Combine the two extracts and dilute to 10.0 mL with cyclohexane.

9.4.1.2 For field use, it has proven satisfactory to use a reagent bottle instead of a separatory funnel. Pour 50.0 mL of the sample in the bottle and add 5.0 mL of cyclohexane, shake for 2 min and collect most of the top layer with a Pasteur pipet. It is important to collect most of the top layer to maximize percent recovery (tilt the flask to see the separation between the two layers more easily). Add 5.0 mL of cyclohexane to the aqueous layer and perform a second extraction. Combine the two cyclohexane extracts and dilute to 10.0 mL with cyclohexane.

9.4.1.3 See [12.6](#) to check extraction recoveries. Other extraction methods can be used at the discretion of the analyst, by adding an appropriate solvent exchange step to cyclohexane and by checking for recoveries and interferences. As is always the case, the analyst shall demonstrate method performance when changing the method. At the  $\text{mg/L}$  level or above, the PAH mixture might not be totally in solution. If the PAH mixture is emulsified in water, is sparingly soluble in water, or if the concentration of the unknown must be known more accurately, it may be necessary to evaporate the solution to dryness or to extract the PAH mixture into a suitable solvent, followed by evaporation, weighing, and redissolving in cyclohexane.

9.4.1.4 At the  $\text{mg/L}$  level or above, the PAH mixture in water might not be totally in solution.

9.5 Sample bottles must be made of glass, precleaned with dilute nitric acid (1 + 1) and sealed with plastic screw caps having TFE-fluorocarbon liners. Solutions must be prepared in precleaned volumetric flasks. Because many aromatics are subject to photodegradation, flasks must be low-actinic (amber) or covered with aluminum foil. Volumetric flasks and fluorescence cells must be cleaned with dilute nitric acid followed by rinsing with water and then air-drying them. To remove the water more quickly, use a triple rinse with spectroquality acetone. As a final step, triple rinse glassware and cells with the solvent used for analysis, usually cyclohexane.

## 10. Preparation of Apparatus

10.1 Set up and calibrate the fluorescence spectrometer according to the manufacturer's instructions and Practices [E169](#) and [E275](#) and Test Methods [E388](#), [E578](#), and [E579](#). Include in the calibration procedures a check of wavelength accuracy using a low pressure mercury lamp (or similar line source). Allow an appropriate period of time (usually 15 min) for the instrument electronics to stabilize. The instrument specifications must meet the specifications of [Table 1](#), with fixed or variable slits capable of covering the range of spectral resolution specified in the test method (2.5 nm to 10 nm) and capable of scanning both monochromators synchronously as well as individually.

## 11. Procedure

11.1 Select an appropriate standard based on the characterization procedure described in [Annex A1](#) that entails examination of fluorescence emission and synchronous spectra of unknown sample(s). Do not use this quantification procedure until the sample is characterized and a suitable calibration standard is selected based on the procedure in [Annex A1](#). This PAH standard must be site-specific and should consist of a sample of unweathered or weathered oil that might be the same oil or an oil of the same type with similar fluorescence spectral properties. Preferably select a PAH mixture that has been well characterized by other methods (GC, GC-MS, HPLC, see test methods listed in Test Method [D4657](#) and References [\(1-7\)](#)). If this is not possible, one must rely on the known composition of similar oils. If a neat sample of the unknown PAH mixture is available, compare the fluorescence intensity of this material at known weight/volume ratio in the spectroquality solvent to the selected standard under the same instrumental and experimental conditions. For best quantification results, the intensities must agree to within 10 % of the fluorescence intensity at peak maxima. Empirically, PAH mixtures with very similar spectral characteristics have been usually found to have similar fluorescence intensities. In some cases, for example, an aromatic solvent spill, use an appropriate single aromatic compound or simple PAH mixture as the standard.

11.2 Once an appropriate calibration standard is selected, prepare standard solutions, starting at 100  $\mu\text{g/mL}$  in spectroquality cyclohexane and diluting down. These standard solutions, depending on instrumental conditions, can span a range from 5  $\mu\text{g/mL}$  to 5  $\text{ng/mL}$  or lower. Use these data to generate a calibration plot, which should be linear over this range. Higher concentrations would require dilution to avoid self-absorption (inner-filter effect) and to stay in the linear range. It is preferable to prepare solutions fresh each day, but they may be held up to 3 days if stored in a refrigerator. In all cases, treat sample and calibration

solutions in the same manner. For each concentration, scan the emission spectra and take the maximum intensity value for a data point. Once the wavelength corresponding to the maximum emission is known, record the emission intensity at the wavelength corresponding to the peak maximum for a fixed period of time (usually 1 s) for subsequent samples rather than scanning the whole spectrum. If the whole spectrum is recorded, use either the emission intensity of the peak maximum or the area under the fluorescence spectral envelope for quantification. For some PAH mixtures, spectral areas may yield better quantitative results than peak maxima. In each case, use these peak maxima or spectral area values to create the calibration curve. Preliminary data indicates that the peak maxima usually are satisfactory for quantification. The time scan at the emission peak maximum allows for faster sample analysis. Multichannel detectors may also be used with an appropriate intensity value recorded. If it is necessary to change instrumental conditions, check instrument conditions and determine the correction factor. Suggested instrumental conditions are as follows: excitation monochromator bandpass 10 nm or less, emission monochromator bandpass 2.5 nm or less, and an excitation wavelength of 254 nm (for oil), other PAH mixtures may require different excitation wavelengths. Measure and subtract the solvent blank, preferably in the same cell, if necessary, with each measurement. Make all measurements with the same instrumental conditions.

11.3 Create a calibration curve by plotting the intensity measurements against the concentration of standards.

11.4 Once the calibration plot for quantification has been generated, prepare and measure unknown samples in the same fashion, provided that their characterization spectra show good agreement with the spectra of the calibration standard (see Annex A1). If an extraction step is necessary, weigh the original sample (before and after drying). The extracted sample may also need to be evaporated down and weighed, or measure in a known volume. Compare the spectral intensity of the unknown sample with the calibration curve. Since the lamp intensity of the fluorescence spectrometer may fluctuate with time, repeat at least one standard at frequent intervals to check the stability of the source and instrumentation as needed. Analyze at least 3 different concentrations of the standard with each set of samples.

11.5 Determine the concentration of the diluted unknown sample solution by referring the intensity to the calibration curve.

11.5.1 Calculate concentration of the original extracted sample as follows:

$$\text{concentration, } \mu\text{g/mL} = C_c (V_s/V_T) \tag{1}$$

where:

- $C_c$  = concentration from calibration curve,  $\mu\text{g/mL}$ ,
- $V_s$  = volume of diluted extract, mL, and
- $V_T$  = volume of water that sample was extracted from, mL.

Since the original concentration and  $C_c$  are related to a site-specific standard, express concentration either as total oil or as total PAH (if the percentage of PAH in the original standard is known or if the standard is 100 % PAH).

11.6 The reliability of this fluorescence method will depend critically on the proper choice of standards for each site or project.

## 12. Quality Control Measures

12.1 Calibrate the fluorescence spectrometer frequently to check the wavelength accuracy with an appropriate mercury or other line source and check relative peak ratios for appropriate PAHs (as a check on any spectral correction factor). Check its sensitivity periodically (weekly) using appropriate PAH standards (plastic standards, commercially available, or PAH mixtures in cyclohexane). Naphthalene and anthracene are recommended as instrumental standards. Pyrene, chrysene, or ovalene emit at longer wavelengths and are appropriate for heavier PAH mixtures that also have emission maxima at longer wavelengths.

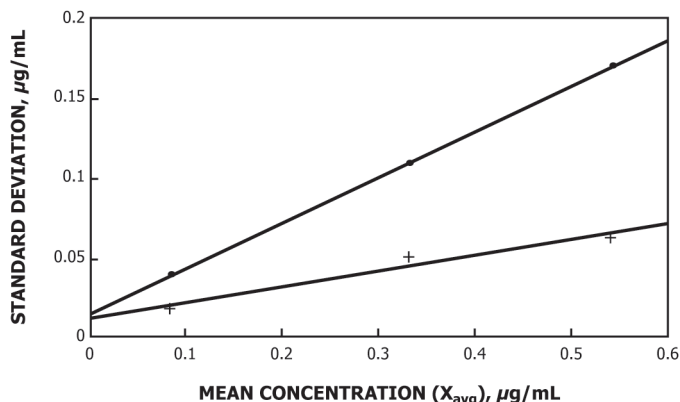


FIG. 1 Total and Single-Operator Standard Deviation

**TABLE 2 Recoveries of Known Amount of Oil from Reagent Water**

Amount Added, $\mu\text{g/mL}$	Amount Found, $\mu\text{g/mL}$	% Bias	Statistically Significant (95 % Conf. Level)
0.091	0.085	-6	no
0.440	0.333	-24	yes
0.782	0.541	-31	yes

12.2 Measure solvent blanks with each sample measurement to check the purity of the solvent and the cleanliness of the fluorescence cells. At low concentrations it may be necessary to subtract out solvent blanks for accurate quantification. Treat sample and standard spectra in the same manner.

12.3 For each set of samples, measure one sample in triplicate using separate aliquots of the same sample extract. For each set of samples, carry one sample through the entire sample extraction, preparation and analysis procedure in triplicate.

12.4 For test method validation (or when a new type of matrix is being extracted) make at least three separate determinations (taking each sample through the entire sample extraction and analysis procedure) for at least five concentrations.

12.5 Measure standards (PAH mixtures or site-specific, well-characterized oils) with each set of samples. Standard solutions can be kept up to 3 days, if stored in the refrigerator and away from light. Generate a new calibration curve when the standard changes or when deviations are noted from the standard curve for fresh standard solutions. Set control limits depending on the desired accuracy for the experiment.

12.6 Check recoveries, where extraction steps are involved for a few selected samples, by extracting the same material with a second aliquot of solvent. Where the amount of PAH material extracted in the second aliquot exceeds a certain amount (15 to 30 %) depending on desired accuracy, combine the two aliquots and perform a third extraction. (This might indicate the need for a different extraction solvent or procedure.)

12.7 For a complex PAH mixture, spikes of a specific PAH are not appropriate, but for a single aromatic compound or simple PAH mixture, a PAH spike can be added that does not interfere spectrally with the determination. Such a spike should be carried throughout the whole procedure including sample extraction. Also, such a PAH spike can be introduced into a clean matrix as an alternate check on extraction efficiency.

12.8 For situations requiring an additional degree of reliability it is desirable that an independent method be used to define the calibration curve.

### 13. Precision and Bias

13.1 An interlaboratory study was conducted using an unknown oil and four standard oils: Prudhoe Bay Crude, Arabian Light Crude, South Louisiana Crude and #2 Fuel Oil. The laboratories participating were asked to characterize the unknown oil by comparing it with the emission and synchronous fluorescence spectra of the standard oils and then to select an appropriate standard (with similar spectral shape and intensity). After the characterization was reported, they proceeded to quantify the three different concentrations of unknown oil. The precision and bias statements were based on Practice **D2777**.

13.2 *Precision*—Based on the results of seven laboratories, conducting triplicate test on three levels of concentrations, the precision of the test method within its designed range is linear with concentration in accordance with **Fig. 1** and may be expressed as:

$$\text{Reagent water: } S_t = 0.285x + 0.0145 \quad (2)$$

$$S_o = 0.0975x + 0.0122 \quad (3)$$

where:

$S_t$  = overall precision,  $\mu\text{g/mL}$ ,

$S_o$  = pooled single-operator precision,  $\mu\text{g/mL}$ , and

$x$  = concentration of oil in water,  $\mu\text{g/mL}$ .

13.3 *Bias*—Recoveries of known amount of oil from reagent water were as shown in **Table 2**. These collaborative test data were obtained on reagent-grade water. Single operator data obtained on tap water were also consistent with the results of the collaborative study. These data may not apply to untested matrices, which should be tested by the analyst.

13.4 The data from the seven participating laboratories show that a negative bias is expected when performing this test method. A negative bias would be expected of any test method having an extraction step; the magnitude of the bias in this test method would depend on the efficiency of the extraction and the volatility of the light components of the oil. In this test method cyclohexane, a not very efficient solvent, is used in the extraction step because of its ease of use under field conditions, its low fluorescence interference and background. Other extraction techniques using a more efficient solvent have to be tested by the chemist before