Method Comparison Table

SM 4500 CN^- E	**14561CN – June 1999**
1.0 Scope and Application The cyanide ions in the alkaline distillate from preliminary treated sample are converted to cyanogen chloride (CNCl) by reaction with chloramine-T. After the reaction is completed, the (CNCl) forms a red-blue complex color with the addition of a pyridine-barbituric acid reagent. Using a spectrophotometer or filter photometer the absorbance of this red-violet complex is measured at a wavelength of 570 to 580 nm. This method is capable of measuring cyanide in the range of 0.02-0.2mg/L. Additional sample manipulation could cause increased opportunity for error. The minimum level (ML) for this method is implied (0.02 mg/L) based on the test range cited. This method is applicable for use in the Environmental Protection Agency's (EPA's) survey and monitoring programs under the Clean Water Act.	**1.0 Scope and Application** The cyanide ions in the alkaline distillate from preliminary treated sample are converted to cyanogen chloride by reaction with dichloroisocyanuric acid. After the reaction is complete cyanogen chloride forms a red-blue complex color on the addition of a 1,3 dimethyl barbituric acid-Pyridine carboxylic acid mixture (Appendix V). Using a spectrophotometer or filter photometer the absorbance of this red-violet complex is measured at 605 nm This method is capable of measuring cyanide ions in the range of 0.010-0.50 mg/L. The ML for this method is 0.010 mg/L, which is 2 times more sensitive than the ML for the standard reference method, SM 4500 CN^-E. The color complex formed using 1,3-barbituric acid has a maximum absorbance reading at 605 nm (Appendix II and IV). This method is applicable for use in the Environmental Protection Agency's (EPA's) survey and monitoring programs under the Clean Water Act.
2.0 Summary of Method An aliquot of alkaline distillate from preliminary treatment (SM 4500CN^- B) is treated with a chlorinating agent (Chloramine-T) which converts the available CN into Cyanogen Chloride (CNCl). The Chloramine-T reaction time is two minutes. Through the addition of a pyridine-barbituric acid solution, the CNCl forms a red-violet color complex, which is read photometrically (exactly eight minutes after reagent addition) at a wavelength between 570 – 580 nm (maximum is 575 to 582 nm). Specifications for spectrophotometers are that the wavelength be set at 578 nm	**2.0 Summary of Method** The sample to be tested is preliminary treated according to SM 4500- CN^- B The Spectroquant® CN Cell Test contains a chlorinating agent 1,3 dichloro cyanuric acid, an equivalent to chloramine-T in the reference method. A 5 ml volume pre-treated (and pH adjusted) sample is added to the Spectroquant® CN Cell Test. After mixing, a premixed dose (100 mg) of 1,3, dimethyl barbituric acid-pyridine carboxylic acid (Spectroquant® Reagent CN-3K) reagent is added to the cell and mixed. The reaction producing the red-violet dye complex is allowed to complete over a period of 10 minutes. This overall time for color reaction is the same as that stated in the reference method. The intensity of the red-violet pyridine-barbituric acid complex which has been formed is measured photometrically near at 605-nm wavelength (605 nm is the maximum for absorbance of the color complex). The data presented in Appendix II and IV support this choice of maximum wavelength for measurement.

3.0 Definitions See section 18.0 – There are no terms, acronyms, or symbols which have been defined in this method.	**3.0 Definitions** See section 18.0 – This method defines, in great detail, the terms, acronyms, and symbols, which appear in the body of the method.
4.0 Interferences All known interferences are eliminated or reduced to a minimum by distillation. pH level of the sample must be <8 upon addition of the Chloramine-T reagent. Sulfides adversely affect the colorimetric procedures. Samples with high nitrate and or nitrite concentrations may yield high CN results. The nitrate and nitrite may form nitrous acid, which will react with some organic compounds to form oximes. These compounds will decompose under the conditions of the test, generating HCN. The addition of sulfamic acid eliminates this interference.	**4.0 Interferences** All oxidizing and reducing reagent can interfere, but most interferences are eliminated or reduced to a minimum by the distillation procedure. Bromium interferes in a concentration $\geq$ 0.1 mg Bromium/L. Preliminary treatment by distillation (SM 4500 CN B) does not decompose cobalt-cyano complexes; hence cobalt interferes at concentrations >1 mg Co^{2+}/L. Thiocyanate (SCN) interferes, as it reacts similarly to cyanide ions, when present at concentrations >0.05 mg SCN^-/L. Hg^{2+} interferes if concentration in sample is >0.1mg Hg^{2+} /L. pH level of the sample prior to addition into the test cell reagents is critical. The level must be between 2 – 10. Sulfides adversely affect the colorimetric procedures. Samples with high nitrate and or nitrite concentrations may yield high CN results. The nitrate and nitrite may form nitrous acid, which will react with some organic compounds to form oximes. These compounds will decompose under the conditions of the test, generating HCN. The addition of sulfamic acid eliminates this interference caused by nitrite. See Appendix III.
5.0 Safety The reference method defines potential health risks associated with the use of the chemicals in this method, and the formation of toxic CNCl gas. The reference method cautions the user to avoid the inhalation of cyanogen chloride, which is a toxic gas. KCN employed in the preparation of the standard solutions is highly toxic and contact or inhalation must be avoided.	**5.0 Safety** This method employs the pre-measured reagents and a calibrated dosing system for the addition of the color forming reagent (Spectroquant® Reagent CN-3K), hence the handling of hazardous chemicals is significantly reduced. CNCl is a toxic gas, and as in the reference method, the user should avoid inhalation. This method, employing the small reagent volumes, and closed system reduces this risk. As with any analytical procedure, the analyst is cautioned to become familiar with the potential health hazards described in the reference Material Safety Data Sheet (MSDS) records.

	Each Spectroquant® CN Cell Test is also clearly labeled as to the contents, and any potential health hazards. This helps to insure the safety of the analysts involved.
6.0 Equipment and Supplies This method employs standard laboratory glassware for sampling (1L glass or plastic bottle). Standard glassware for sample handling include pipettes, culture tubes (16 x 100-mm, 20 x 150-mm, and 25 x 150-mm). A spectrophotometer or filter photometer is used to measure the red-violet pyridine-barbituric complex. The wavelength is set at 570-580 nm for the determination.	**6.0 Equipment and Supplies** This method employs all supplies, which are specified in method 4500 CN D. The photometric determination is accomplished using a filter photometer or other photometric devices. **Spectroquant®CN Cell Test** The chlorinating reagent is pre-measured in 16 x 100 mm tubes (the Spectroquant® CN Cell Test). This approach eliminates the possibility of contamination due to residues remaining on laboratory washed glassware. A Merck Spectroquant® system photometer or other photometer can be used to measure the red-violet pyridine-barbituric complex. The wavelength is set at or near 605 nm for the determination. **Merck Spectroquant® system photometer** CN cell identification – The Merck Spectroquant® system photometers are equipped with a Sample ID system. Each cell test is bar coded, and when placed correctly in the cell compartment, the instrument recognizes the cell, and sets the instrument to the proper measuring parameters that is, item no., test range, cell format, wavelength, calibration data (Appendix I). Merck Spectroquant® system photometer can also store the sample information within its data files, for printing, downloading to alternate data storage location, or for easy retrieval. **Calibration** The use of other photometric equipment, which may be substituted for use with this method, is consistent with the reference method specifications. Merck Spectroquant® system photometer's are factory calibrated (Appendix I).
7.0 Reagents and Standard This method incorporates the following chemicals which are prepared by the laboratory analyst: Chlorinating agent – Chloramine T dissolved 1.0g in	**7.0 Reagents and Standards** This method incorporates chemicals equivalent to the Standard Method SM 4500 CN E. They include Dichloro isocyanuric acid-chlorinating agent

100ml water-chlorinating agent. . Pyridine-barbituric acid reagent– 15g barbituric acid and 75 ml pyridine mixed in water to which 15mL conc, hydrochloric acid is added and mixed-color complexing reagents. Stock, intermediate, and working cyanide solutions – Highly toxic KCN dissolved with sodium hydroxide in water	1,3, dimethyl barbituric acid and pyridine carboxylic acid-color complexing reagents. CN standard solutions-equivalent to reference method. The chlorinating agent (dichloro isocyanuric acid) used in this method has identical to chloramine-T, which is specified in the reference method. The chlorinating capacity of this reagent is equivalent to that of chloramine-T. Furthermore, this method uses 1,3, dimethyl barbituric acid, which is more water soluble and more stable than barbituric acid. Unlike pyridine employed in the reference method, the pyridine carboxylic acid is non-toxic and does not produce the foul odor associated with pyridine. The mixture 1,3 dimethyl barbituric acid-pyridine carboxylic acid also has a greater buffering capacity than that of the reference chemicals. Hence, fluctuation from the optimal pH during the reaction time for forming of violet red dye complex are less likely to occur. The pre-measured reagents reduce the risk of error in preparation of the chemical reagents. Each Spectroquant® CN Cell Test is clearly labeled with the product number and hazard symbol to eliminate erroneous results. The CN-standard solutions have been incorporated into the quality control program, as initial precision and recovery (IPR), ongoing precision and recovery (OPR), and matrix spiking (MS) solutions. The use of these standard solutions increases the laboratory's potential to produce accurate data.
8.0 Sample Collection, Preservation, and Storage Samples for Cyanide are typically collected as grab samples. The samples should be preserved with NaOH to a pH of up to 12. The samples should be analyzed within 14 days after collection. Some interferences call for special sampling treatments. Test the sample for chlorine at the time of sampling, and remove excess chlorine by adding ascorbic acid. Sulfide interferes with the final results, therefore the sample should be checked for the presence of sulfide prior to preservation.	**8.0 Sample Collection, Preservation, and Storage** The sampling is performed in accordance with Standard Methods. There are no differences in the way the samples are collected, preserved or stored in this method.

9.0 Quality Control The method suggests that at least one of the calibration standards be carried through the entire distillation procedure to check the efficiency of the system.	**9.0 Quality Control** This method includes guidelines for initial demonstration of laboratory capability, quality control and quality assurance measurements. Initial demonstration of performance of the method is required. After initial performance has been established, the analyst is required to provide proof of continued performance through the analysis of ongoing precision and recovery standards. These are tested in conjunction with the entire analytical quality control batch (for up to 20 samples), which include: (1) laboratory blank, (1) laboratory control sample (OPR), (1) Spiked sample (MS), and (1) spiked sample duplicate (MSD).
10.0 Calibration and Standardization The spectrophotometer is calibrated using a blank and at least five (5) potassium hydrogen phthalate standards covering the concentration range of samples to be tested. The absorbances of the standards is plotted against the concentration. Absorbance readings from samples are plotted against this curve to obtain a concentration value. The calibration curve is checked "periodically", and each time a new reagent is prepared.	**10.0 Calibration and Standardization** Calibrate the analytical balance with class S certified weights. Merck Spectroquant® system photometers are shipped factory calibrated. The calibration data is stored within the unit, and cannot be altered by the user. The manufacturer updates the information, when they supply a new MemoChip (transponder). The curve may be verified by running a calibration standard, and determining the concentration, as stated in Section 10. Calibration curves for absorbance mode measurements on any instrument are prepared and performed as stated in the reference method. The calibration curve may be checked with a mid-range standard, and should be prepared when the linearity varies by more than 10%.
11.0 Procedure Distillation removes interferences, therefore should be performed on samples prior to analysis. A 500 ml of sample is treated by distilling as stated in Section B of 4500-CN-. The CN is swept from the acidified solution into a basic scrubber solution (NaOH). This distillate is then diluted to 250 ml in a volumetric flask. To a portion of the sample, up to 50 ml, chloramine-T is added to convert the CN to CNCl. After two minutes reaction with the Chloramine-T, the color reagent (pyridine-barbituric acid) is added. After exactly 8 minutes reaction time, the absorbance of the color solution is read photometrically at a wavelength between 570 – 580 nm.	**11.0 Procedure** As in the reference method, samples are treated for interferences, and distillated using the SM 4500-CN B method. An aliquot of alkaline distillate from preliminary treatment (SM 4500CN^- B) is pH adjusted to pH 2-10. The Spectroquant® CN Cell Test contains the chlorinating agent 1,3 dichloro cyanuric acid. A volume of 5 ml pretreated sample is added to the Spectroquant® CN Cell Test. After mixing to dissolve the reagents, a dose (100 mg) of 1,3, dimethyl barbituric acid-pyridine carboxylic acid mixture is added and mixed. The reaction is allowed complete over a period of 10 minutes. The intensity of the red-violet pyridine-barbituric acid complex , which has

	been formed, is measured photometrically at or near a 605-nm wavelength. The reagent formulations equivalent to those cited in the reference method yet are expanded to encompass a broad 0.010 – 0.500 mg/L concentration range.
12.0 Data Analysis and Calculations The reference method defines the calculation for distilled samples from the concentration obtained on the calibration curve. The method does not address significant digits. The Minimum Level (ML) is implied from the concentration range as 0.02 mg/L.	**12.0 Data Analysis and Calculations** This method defines the calculations involved for computing the QC acceptance criteria. The method also defines calculations for dilution and distillation correction factors. This method lists a ML of 0.010 mg/L, an MDL of 0.003 mg/L, and three significant figures are to be used when reporting data.
13.0 Method Performance Referenced in the method is precision and bias data obtained from up to 27 measurements of CN levels in Type II reagent water and selected water matrices.	**13.0 Method Performance** As in the SM 4500 CN E reference method, this method incorporates the same chemical components, in the same proportions, and is determined similarly via photometric measurement. The precision and bias stated in the reference method are achievable by this method.
14.0 Pollution Prevention The reference method does not discuss pollution prevention.	**14.0 Pollution Prevention** The label on each Spectroquant® CN Cell Test informs the user of the contents and potential health risks. Furthermore, the Material Safety Data Sheets (MSDS) provide guidance for waste management. The packaging and use of pre-measured Spectroquant® CN Cell Test s is designed to minimize risks of spillage, and to reduce the amounts of the chemicals used. The laboratory is reminded to properly manage these reagents in the laboratory to reduce any threat to the environment. General practices, such as ordering of supplies, can seriously impact the amount of materials, which require disposal in the laboratory. It is suggested that the laboratory only order supplies as demand dictates, to minimize expired materials requiring disposal.
15.0 Waste Management The reference method does not discuss waste management.	**15.0 Waste Management** Referenced in section 15.3 are two waste management documents for further information on this subject. In using this method, the laboratory must comply with all federal, state, and local regulations governing waste management.

16.0 References The reference method lists two journal publications in its bibliography. There are references regarding overcoming chloride interferences in the introduction section of this method.	**16.0 References** This proposed method includes in the reference section, existing method documents for CN, supplemental and related documents for safety and general laboratory practices.
17.0 Tables, Diagrams, Flowcharts, and Validation Data The reference method has a table in section 6, which summarizes the Precision and Bias achieved by this test procedure.	**17.0 Tables, Diagrams, Flowcharts, and Validation Data** Three tables are included in this method. **Table 1** This table summarizes for Spectroquant®CN Cell Test concentration range, sample volume required, and cell type used for photometric measurement. **Table 2** This table outlines the procedure for preparing calibration curve solutions for calibration of photometric devices using the absorbance measurement mode. **Table 3** This table outlines the performance acceptance criteria, cited for methods listed in 40 CFR Part 136, Table IB. This data must be achieved when the analyst initiates the method, attempts to prove initial demonstration of performance, and also ongoing performance of the method.
18.0 Glossary The reference method does not have a glossary.	**18.0 Glossary** See Section 3.0 The glossary defines terminology used in the body of method. Much of the terms defined are specific to the quality control section of the method. These terms are not used, or defined, in the reference method. This section clearly defines the nomenclature of the products being proposed for use.