ENVIRONMENTAL PROTECTION AGENCY

40 CFR Parts 136 and 503

[EPA–HQ–OW–2004–0014; FRL–8228–1]

RIN 2040–AE68

Guidelines Establishing Test Procedures for the Analysis of Pollutants; Analytical Methods for Biological Pollutants in Wastewater and Sewage Sludge: Final Rule

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This rule modifies the EPA's Guidelines that establish approved bacterial testing procedures for analysis and sampling under the Clean Water Act. EPA proposed these changes for public comment on August 16, 2005 and April 10, 2006. These changes include approval for new methods for monitoring microbial pollutants in wastewater and sewage sludge, including EPA methods, vendor-developed methods and methods developed by voluntary consensus bodies (VCSB) as well as updated versions of currently approved methods. The addition of new and updated methods to the wastewater regulations provides increased flexibility to the regulated community and laboratories in the selection of analytical methods. In addition, EPA has made a technical, non-substantive correction.

DATES: This regulation is effective April 25, 2007. The incorporation by reference of these methods is approved by the Director of the Federal Register on April 25, 2007. For judicial review purposes, this final rule is promulgated as of 1 p.m. (Eastern time) on April 9, 2007 as provided at 40 CFR 23.2 and 23.7.

ADDRESSES: EPA has established a docket for this action under Docket ID No. EPA–OW–2004–0014. All documents in the docket are listed on the *www.regulations.gov* Web site. Although listed in the index, some information is not publicly available, e.g., CBI or other information whose disclosure is restricted by statute. Certain other material, such as copyrighted material, is not placed on the Internet and will be publicly available only in hard copy form. Publicly available docket materials are available either electronically through *www.regulations.gov* or in hard copy at the HQ Water Docket Center, EPA/DC, EPA West, Room B102, 1301 Constitution Ave., NW., Washington, DC. The Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Public Reading Room is (202) 566–1744, and the telephone number is (202) 566–2426.

Note: The EPA Docket Center suffered damage due to flooding during the last week of June 2006. The Docket Center is continuing to operate. However, during the cleanup, there will be temporary changes to Docket Center telephone numbers, addresses, and hours of operation for people who wish to visit the Public Reading Room to view documents. Consult EPA's **Federal Register** notice at 71 FR 38147 (July 5, 2006) or the EPA website at *http://www.epa.gov/epahome/dockets.htm* for current information on docket status, locations and telephone numbers.

FOR FURTHER INFORMATION CONTACT: For information regarding the changes to wastewater regulations, contact Robin K. Oshiro, Engineering and Analysis Division (4303T), USEPA Office of Science and Technology, 1200 Pennsylvania Ave., NW., Washington, DC 20460, 202–566–1075 (e-mail: *oshiro.robin@epa.gov*).

SUPPLEMENTARY INFORMATION:

A. Potentially Regulated Entities

1. Clean Water Act

EPA Regions, as well as States, Territories and Tribes authorized to implement the National Pollutant Discharge Elimination System (NPDES) program, issue permits with conditions designed to ensure compliance with the technology-based and water quality-based requirements of the Clean Water Act (CWA). These permits may include restrictions on the quantity of pollutants that may be discharged as well as pollutant measurement and reporting requirements. If EPA has approved test procedures for analysis of a specific pollutant, an NPDES permittee (or applicant for an NPDES permit) must use an approved test procedure (or an approved alternate test procedure) for the specific pollutant when testing for the required waste constituent. Similarly, if EPA has established permit monitoring requirements, measurements taken and reported under an NPDES permit must comply with these requirements. Therefore, entities with NPDES permits will potentially be regulated by the actions in this rulemaking. Categories and entities that may potentially be subject to the requirements of today's rule include:

Category	Examples of potentially regulated entities
State, Territorial, and Indian Tribal Governments	States, Territories, and Tribes authorized to administer the NPDES permitting program; States, Territories, and Tribes providing certification under Clean Water Act section 401.
Industry	Facilities that must conduct monitoring to comply with NPDES permits.
Municipalities	POTWs that must conduct monitoring to comply with NPDES permits.

This table is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be regulated by this action. This table lists types of entities that EPA is now aware could potentially be regulated by this action. Other types of entities not listed in the table could also be regulated. To determine whether your facility is regulated by this action, you should carefully examine the applicability language at 40 CFR 122.1 (NPDES purpose and scope), 40 CFR 136.1 (NPDES permits and CWA), 40 CFR 403.1 (Pretreatment standards purpose and applicability). If you have questions regarding the applicability of this action to a particular entity, consult the appropriate person listed in the preceding **FOR FURTHER INFORMATION CONTACT** section.

What process governs judicial review of this rule?

Under Section 509(b)(1) of the Clean Water Act (CWA), judicial review of today's CWA rule may be obtained by filing a petition for review in the United States Circuit Court of Appeals within 120 days from the date of promulgation of this rule. For judicial review purposes, this final rule is promulgated as of 1 p.m. (Eastern time) on April 25, 2007 as provided at 40 CFR 23.2. The requirements of this regulation may also not be challenged later in civil or criminal proceedings brought by EPA.

Abbreviations and Acronyms Used in the Preamble and Final Rule

AOAC: Association of Official Analytical Chemists International

ASTM: American Society for Testing and Materials International

CWA: Clean Water Act
EPA: Environmental Protection Agency
VCSB: Voluntary Consensus Standard Body

Table of Contents

I. Statutory Authority

Clean Water Act

EPA is promulgating today's rule pursuant to the authority of sections 301(a), 304(h), and 501(a) of the Clean Water Act (''CWA'' or the ''Act''), 33 U.S.C. 1311(a), 1314(h), 1361(a). Section 301(a) of the Act prohibits the discharge of any pollutant into navigable waters unless the discharge complies with a National Pollutant Discharge Elimination System (NPDES) permit issued under section 402 of the Act. Section 304(h) of the Act requires the Administrator of the EPA to ''* * * promulgate guidelines establishing test procedures for the analysis of pollutants that shall include the factors which must be provided in any certification pursuant to [section 401 of this Act] or permit application pursuant to [section 402 of this Act].'' Section 501(a) of the Act authorizes the Administrator to ''* * * prescribe such regulations as are necessary to carry out this function under [the Act].'' EPA generally has codified its test procedure regulations (including analysis and sampling requirements) for CWA programs at 40 CFR Part 136, though some requirements are codified in other Parts (e.g., 40 CFR Chapter I, Subchapters N and O).

II. Summary of Final Rule

The following sections describe the changes EPA is making in today's final rule.

A. 40 CFR Part 136

This rule approves new and revised methods for inclusion in 40 CFR Part 136. These methods include EPA methods, vendor methods submitted by IDEXX and Hach, and voluntary consensus standards.

The following discussion briefly describes the changes to Part 136 methods approved today.

1. This rule amends the regulations at 40 CFR Part 136 to approve five *E. coli* and two enterococci methods for monitoring microbial pollutants in wastewaters. The *E. coli* methods include EPA Method 1603 (modified mTEC), and vendor methods Colilert® and Colilert-18®, and mColiBlue24®. The enterococci methods include EPA Method 1600 (mEI), and vendor method Enterolert™.

2. The rule approves two fecal coliform and one Salmonella method for monitoring microbial pollutants in sewage sludge (biosolids). The fecal coliform methods include EPA Methods 1680 (LT–EC) and 1681 (A–1) and the Salmonella Method 1682 (Modified MSRV). The methods approved today are alternative methods to those currently prescribed for measuring fecal coliform and salmonella in sewage sludge identified in 40 CFR § 503.8(b).

3. The rule amends the regulations by moving the microbial methods approved for use in ambient waters from Table IA to a new Table IH, and adding Table IH to section 136.3(a).

4. The rule extends the holding time for fecal coliforms using EPA Methods 1680 (LTB–EC) or 1681 (A–1) in sewage sludge for Class A composted, Class B aerobically or anaerobically digested sewage sludge.

5. The rule amends 40 CFR 136.1 to add a new provision that authorizes the use of the methods identified at 40 CFR 503.8(b) and the newly approved Part 136 methods for fecal coliform and Salmonella for permit applications and recordkeeping and reporting required under EPA's sewage sludge regulations at 40 CFR Part 503.

B. 40 CFR Part 503

This rule amends the regulations at 40 CFR Part 503 by adding a cross reference to the 40 CFR Part 136 methods in section 503.8(b).

III. Changes Between the Proposed Rule and the Final Rule

Except as noted below, the content of the final rule is the same as that of the proposed rule. In some instances, EPA revised for clarity the language of the final rule from that in the proposed rule.

A. Revision to 40 CFR Part 136, Applicability

Based on comment received on the Agency's proposal of methods for use in sewage sludge, EPA has amended the applicability provision to clarify that the applicable procedures of Part 136 and Part 503 must be used for measurements for sewage sludge permit applications and reporting and recordkeeping requirements under Part 503.

B. Revision to 40 CFR Part 136, Identification of Test Procedures

Section 553 of the Administrative Procedure Act, 5 U.S.C. 553(b)(B), provides that, when an agency for good cause finds that notice and public procedure are impracticable, unnecessary or contrary to the public interest, the agency may issue a rule without providing notice and an opportunity for public comment. EPA has determined that there is good cause for making today's changes to the rule final without prior proposal and opportunity for comment. Notice and opportunity for public comment is not necessary with respect to these changes because they are not substantive and merely correct errors in cross-referenced provisions as explained below.

Section 136.3(a) provides that discharge parameter values for which reports are required must be determined either by the standard analytical test procedures described in the tables in Part 136 or approved additional or alternate test procedures. EPA has modified the language of 40 CFR 136.3(a) to make three corrections. First, EPA has changed the citation in the last sentence before Table IA from ''paragraphs (b) or (c) of this section or 40 CFR 401.13'' to ''paragraphs (c) of this section, 40 CFR 136.5(a)–(d) and 40 CFR 401.13.'' Paragraph (b) does not describe circumstances in which alternate procedures may be approved while section 136.5 does.

Second, EPA has deleted the clause at the end of the last sentence which states that other test procedures may be used

''* * * when such other test procedures have been previously approved by the Regional Administrator of the Region in which the discharge will occur, and providing the Director of the State in which

the discharge will occur does not object to the use of such alternate test procedure * * *.''

Only two of the cited provisions require approval by the Regional Administrator or Director of a State. 40 CFR 401.13 does not because it pertains to variances of guidelines of national applicability.

The cross-referenced provisions authorize the use of additional or alternate test procedures in described circumstances. Thus, section 136.3(c) authorizes approval by the Regional Administrator (or Director of an approved State NPDES Program) for analysis of additional pollutants or parameters required to be reported for a particular discharge. Section 136.5(a)–(d) authorizes approval by the Regional Administrator of alternate procedures for use within a particular EPA Region. 40 CFR section 401.13 authorizes the use of analytical procedures that are specifically defined in 40 CFR Parts 402–699. This last category of analytical procedures that are promulgated for specific effluent limitations guidelines and pretreatment standards and not codified in Part 136 do not require the approval of the Director of a State as the current language erroneously implies.

Third, EPA removed an erroneous reference that was listed as a source for the methods listed in section 136.3.

EPA has modified the regulation to provide the correct citation and delete the inaccurate and misleading language. None of the changes EPA is promulgating today are themselves substantive but rather, as noted, only either correct an error in citing to the other applicable provisions of these regulations or correct inaccuracies. The substantive provisions in question were previously subject to notice and comment. Thus, notice and public procedures are unnecessary. EPA finds that this constitutes good cause under 5 U.S.C. 553(b)(B).

C. Revision to 40 CFR Part 136, Table IA Title

The rule revises the title to Table IA from ''List of Approved Biological Methods'' to ''List of Approved Biological Methods for Wastewater and Sewage Sludge.'' Today's action updating Table IA at § 136.3 more clearly defines the removal of approved microbiological methods for ambient waters from this table. Such methods have been moved to a new table, Table IH.

D. Revisions to 40 CFR Part 136, Table II and Footnotes

The rule revises Table II (Required Containers, Preservation Techniques, and Holding Times), and the footnotes to Table II at 40 CFR 136.3(e). Today's action updating Table II at § 136.3(e) more clearly defines the holding time for bacterial testing as 6 hours holding time with 2 hours to process samples.

E. Revision to 40 CFR Part 503, Sampling and Analysis

Based on comments received on the Agency's proposal of methods for use in sewage sludge, EPA is including a cross reference to 40 CFR Part 136 in 40 CFR 503.8(b) which prescribes the methods that must be used for sampling and analysis of sewage sludge.

IV. Response to Comments

EPA received 39 comments regarding methods included in this final rule from the August 16, 2005 proposal (70 FR 48256), and 9 comments on the April 10, 2006 Notice of Data Availability (NODA) (71 FR 18329). Commentors represented a number of different interests, including analytical laboratories, water utilities, instrument manufacturers, State and local governments, trade associations, scientists, and private citizens. The public docket for this rule includes the Agency's response to all comments. The majority of the comments were with regard to method inclusion, method use, and quality control requirements. The following is a summary of our response to comments about the lack of connecting language between 40 CFR Parts 136 and 503 for sewage sludge methods.

EPA proposed to approve methods in 40 CFR Part 136 for sewage sludge but did not include an appropriate cross reference in 40 CFR Part 503 to Part 136 so as to allow the use of appropriate 40 CFR 136.3 methods as alternative methods to those listed in 40 CFR 503.8. Based on comments to the proposal, EPA has amended the language in 40 CFR 503.8(b). In addition, as discussed above, EPA has also amended the language in 40 CFR 136.1 regarding the applicability of the methods in this section to 40 CFR Part 503.

V. Statutory and Executive Order Reviews

A. Executive Order 12866: Regulatory Planning and Review

This action is not a ''significant regulatory action'' under the terms of Executive Order (EO) 12866 (58 FR 51735, October 4, 1993) and is therefore not subject to review under the EO.

B. Paperwork Reduction Act

This action does not impose an information collection burden under the provisions of the Paperwork Reduction Act, 44 U.S.C. 3501 *et seq.* This rule does not impose any information collection, reporting, or recordkeeping requirements. This rule merely adds new and updated versions of testing procedures, withdraws some older testing procedures, and establishes new sample collection, preservation, and holding time requirements.

Burden means the total time, effort, or financial resources expended by persons to generate, maintain, retain, or disclose or provide information to or for a Federal agency. This includes the time needed to review instructions; develop, acquire, install, and utilize technology and systems for the purpose of collecting, validating, and verifying information, processing and maintaining information, and disclosing and providing information; adjust the existing ways to comply with any previously applicable instructions and requirements; train personnel to be able to respond to a collection of information; search data sources; complete and review the collection of information; and transmit or otherwise disclose the information.

An Agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. The OMB control numbers for EPA's regulations in 40 CFR are listed in 40 CFR Part 9.

C. Regulatory Flexibility Act

The RFA generally requires an agency to prepare a regulatory flexibility analysis of any rule subject to notice and comment rulemaking requirements under the Administrative Procedure Act or any other statute unless the agency certifies that the rule will not have a significant economic impact on a substantial number of small entities. Small entities include small businesses, small organizations, and small governmental jurisdictions.

For purposes of assessing the impacts of this rule on small entities for methods under the Clean Water Act, small entity is defined as: (1) A small business as defined by the Small Business Administration's (SBA) regulations at 13 CFR 121.201; (2) a small governmental jurisdiction that is a government of a city, county, town, school district or special district with a population less than 50,000; and (3) a small organization that is any not-for-profit enterprise which is independently owned and operated and is not dominant in its field.

After considering the economic impacts of today's final rule on small entities, I certify that this action will not have a significant economic impact on a substantial number of small entities.

This final rule will not impose any requirements on small entities. This action approves new and updated versions of testing procedures, withdraws some older testing procedures, and approves new sample collection, preservation, and holding time requirements. Generally, these changes will have a positive impact on small entities by increasing method flexibility, thereby allowing entities to reduce costs by choosing more cost-effective methods. In some cases, analytical costs may increase slightly due to the additional QC requirements included in the methods that are being approved. However, most laboratories that analyze samples for EPA compliance monitoring have already instituted QC requirements as part of their laboratory practices.

D. Unfunded Mandates Reform Act

Title II of the Unfunded Mandates Reform Act of 1995 (UMRA), Public Law 104–4, establishes requirements for Federal agencies to assess the effects of their regulatory actions on State, Tribal, and local governments and the private sector. Under section 202 of the UMRA, EPA generally must prepare a written statement, including a cost-benefit analysis, for proposed and final rules with ''Federal mandates'' that may result in expenditures to State, local, and Tribal governments, in the aggregate, or to the private sector, of $100 million or more in any one year. Before promulgating an EPA rule for which a written statement is needed, section 205 of the UMRA generally requires EPA to identify and consider a reasonable number of regulatory alternatives and adopt the least costly, most cost-effective or least burdensome alternative that achieves the objectives of the rule. The provisions of section 205 do not apply when they are inconsistent with applicable law. Moreover, section 205 allows EPA to adopt an alternative other than the least costly, most cost-effective or least burdensome alternative if the Administrator publishes with the final rule an explanation of why that alternative was not adopted.

Before EPA establishes any regulatory requirements that may significantly or uniquely affect small governments, including Tribal governments, it must have developed under section 203 of the UMRA a small government agency plan. The plan must provide for the notification of potentially affected small governments, enabling officials of affected small governments to have meaningful and timely input in the development of EPA regulatory proposals with significant Federal intergovernmental mandates, and informing, educating, and advising small governments on compliance with the regulatory requirements.

This rule contains no Federal mandates (under the regulatory provisions of Title II of UMRA) for State, local, or Tribal governments or the private sector. The rule imposes no enforceable duty on any State, local, or Tribal governments or the private sector. In fact, this rule should (on the whole) save money for governments and the private sector by increasing method flexibility, and allowing these entities to reduce monitoring costs by taking advantage of innovations. Thus, today's rule is not subject to the requirements of Sections 202 and 205 of the UMRA.

EPA has determined that this rule contains no regulatory requirements that might significantly or uniquely affect small governments. Generally, this action will have a positive impact by increasing method flexibility, thereby allowing method users to reduce costs by choosing more cost effective methods. In some cases, analytical costs may increase slightly due to changes in methods, but these increases are neither significant nor unique to small governments. This rule merely approves new and updated versions of testing procedures, withdraws some older testing procedures, and approves new sample collection, preservation, and holding time requirements. Thus, today's rule is not subject to the requirements of Section 203 of UMRA.

E. Executive Order 13132: Federalism

Executive Order 13132, entitled ''Federalism'' (64 FR 43255, August 10, 1999), requires EPA to develop an accountable process to ensure ''meaningful and timely input by State and local officials in the development of regulatory policies that have federalism implications.'' ''Policies that have federalism implications'' is defined in the Executive Order to include regulations that have ''substantial direct effects on the States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government.''

This final rule does not have federalism implications. It will not have substantial direct effects on the States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government, as specified in Executive Order 13132. This rule merely approves new and updated versions of testing procedures, withdraws some older testing procedures, and approves new sample collection, preservation, and holding time requirements. The costs to State and local governments will be minimal (in fact, governments may see a cost savings), and the rule does not preempt State law. Thus, Executive Order 13132 does not apply to this rule.

In the spirit of Executive Order 13132, and consistent with EPA policy to promote communications between EPA and State and local governments, EPA specifically solicited comment on the proposed rule from State and local officials.

F. Executive Order 13175: Consultation and Coordination With Indian Tribal Governments

Executive Order 13175, entitled ''Consultation and Coordination with Indian Tribal Governments'' (65 FR 67249, November 9, 2000), requires EPA to develop an accountable process to ensure ''meaningful and timely input by tribal officials in the development of regulatory policies that have tribal implications.''

This final rule does not have tribal implications, as specified in Executive Order 13175. It will not have substantial direct effects on Tribal governments, on the relationship between the Federal government and Indian tribes, or on the distribution of power and responsibilities between the Federal government and Indian tribes. This rule merely approves new and updated versions of testing procedures, withdraws some older testing procedures, and approves new sample collection, preservation, and holding time requirements. The costs to Tribal governments will be minimal (in fact, governments may see a cost savings), and the rule does not preempt State law. Thus, Executive Order 13175 does not apply to this rule.

G. Executive Order 13045: Protection of Children From Environmental Health Risks and Safety Risks

Executive Order 13045: ''Protection of Children from Environmental Health Risks and Safety Risks'' (62 FR 19885, April 23, 1997) applies to any rule that: (1) Is determined to be ''economically significant'' as defined under Executive Order 12866, and (2) concerns an environmental health or safety risk that EPA has reason to believe may have a disproportionate effect on children. If the regulatory action meets both criteria, the Agency must evaluate the environmental health or safety effects of the planned rule on children, and explain why the planned regulation is preferable to other potentially effective and reasonably feasible alternatives

considered by the Agency. This final rule is not subject to the Executive Order 13045 because it is not economically significant as defined in Executive Order 12866. Further it does not concern an environmental health or safety risk that EPA has reason to believe may have a disproportionate effect on children. This action approves new and updated versions of testing procedures, withdraws some older testing procedures, and approves new sample collection, preservation, and holding time requirements.

H. Executive Order 13211: Actions That Significantly Affect Energy Supply, Distribution, or Use

This rule is not subject to Executive Order 13211, ''Actions Concerning Regulations That Significantly Affect Energy Supply, Distribution, or Use'' (66 FR 28355 (May 22, 2001)) because it is not a significant regulatory action under Executive Order 12866.

I. National Technology Transfer and Advancement Act

As noted in the proposed rule, Section 12(d) of the National Technology Transfer and Advancement Act of 1995, (NTTAA), Public Law 104–113, section 12(d) (15 U.S.C. 272 note), directs EPA to use voluntary consensus standards in its regulatory activities unless to do so would be inconsistent with applicable law or otherwise impractical. Voluntary consensus standards are technical standards (e.g., material specifications, test methods, sampling procedures, and business practices) that are developed or adopted by voluntary consensus standard bodies. The NTTAA directs EPA to provide Congress, through the OMB, explanations when the Agency decides not to use available and applicable voluntary consensus standards. This rulemaking involves technical standards. EPA has decided to use *E. coli*, enterococci and fecal coliform methods published in Standard Methods and ASTM International.

The *E. coli* methods from Standard Methods are method 9223B (Standard Methods 18th, 19th and 20th Editions) and method 9223 B–97 (Standard Methods Online Edition), as well as AOAC method 991.15. The enterococci method from ASTM is method D6503–99. The fecal coliform methods from Standard Methods are methods 9221 C E (Standard Methods 18th, 19th and 20th Editions) and method 9221 C E–99 (Standard Methods Online Edition). Standard Methods can be obtained from American Public Health Association, 1015 15th Street, NW., Washington DC 20005, AOAC methods can be obtained from Association of Official Analytical Chemists International, 481 North Frederick Avenue, Suite 500, Gaithersburg, MD 20877–2417, and ASTM methods can be obtained from ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428. These newly approved procedures reflect improvements in science and technology. EPA believes that the addition of these methods offer a wider variety of options that may be more cost effective to conduct compliance monitoring of bacterial pollutants.

J. Congressional Review Act

The Congressional Review Act, 5 U.S.C. 801 *et seq.*, as added by the Small Business Regulatory Enforcement Fairness Act of 1996, generally provides that before a rule may take effect, the agency promulgating the rule must submit a rule report, which includes a copy of the rule, to each House of the Congress and to the Comptroller General of the United States. EPA will submit a report containing this rule and other required information to the U.S. Senate, the U.S. House of Representatives, and the Comptroller General of the United States prior to publication of the rule in the **Federal Register**. A major rule cannot take effect until 60 days after it is published in the **Federal Register**. This action is not a ''major rule'' as defined by 5 U.S.C. 804(2). This rule will be effective April 25, 2007.

List of Subjects

40 CFR Part 136

Environmental protection, Incorporation by reference, Reporting and recordkeeping requirements, Water pollution control.

40 CFR Part 503

Environmental protection, Reporting and recordkeeping requirements, Waste treatment and disposal, Water pollution control.

Dated: September 28, 2006.

Stephen L. Johnson,

Administrator.

Editorial Note: The Office of the Federal Register received this document on March 8, 2007.

■ For the reasons set out in the preamble, title 40, chapter I of the Code of Federal Regulations, is amended as follows:

PART 136—GUIDELINES ESTABLISHING TEST PROCEDURES FOR THE ANALYSIS OF POLLUTANTS

■ 1. The authority citation for Part 136 continues to read as follows:

Authority: Secs. 301, 304(h), 307, and 501(a) Pub. L. 95–217, 91 Stat. 1566, *et seq.* (33 U.S.C. 1251, *et seq.*) (The Federal Water Pollution Control Act Amendments of 1972 as amended by the Clean Water Act of 1977.)

■ 2. Section 136.1 is revised to read as follows:

§ 136.1 Applicability.

(a) The procedures prescribed herein shall, except as noted in § 136.5, be used to perform the measurements indicated whenever the waste constituent specified is required to be measured for:

(1) An application submitted to the Administrator, or to a State having an approved NPDES program for a permit under section 402 of the Clean Water Act of 1977, as amended (CWA), and/or to reports required to be submitted under NPDES permits or other requests for quantitative or qualitative effluent data under parts 122 to 125 of title 40, and,

(2) Reports required to be submitted by dischargers under the NPDES established by parts 124 and 125 of this chapter, and,

(3) Certifications issued by States pursuant to section 401 of the CWA, as amended.

(b) The procedure prescribed herein and in part 503 of title 40 shall be used to perform the measurements required for an application submitted to the Administrator or to a State for a sewage sludge permit under section 405(f) of the Clean Water Act and for recordkeeping and reporting requirements under part 503 of title 40.

■ 3. Section 136.3 is amended as follows:

■ a. By revising paragraph (a) introductory text and Table IA.

■ b. In paragraph (a) by adding Table IH after the notes of Table IG.

■ c. In paragraph (b) by revising the introductory text and by revising references 2, 6, 10, 11, 34, 38, 39, and 52 through 62; and by adding references 70 through 72.

■ d. By revising paragraph (e).

§ 136.3 Identification of test procedures.

(a) Parameters or pollutants, for which methods are approved, are listed together with test procedure descriptions and references in Tables IA, IB, IC, ID, IE, IF, IG, and IH. In the event of a conflict between the reporting requirements of 40 CFR Parts 122 and 125 and any reporting requirements associated with the methods listed in these tables, the provisions of 40 CFR Parts 122 and 125 are controlling and will determine a permittee's reporting requirements. The full text of the referenced test procedures are incorporated by reference into Tables

IA, IB, IC, ID, IE, IF, IG, and IH. The incorporation by reference of these documents, as specified in paragraph (b) of this section, was approved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR Part 51. Copies of the documents may be obtained from the sources listed in paragraph (b) of this section. Documents may be inspected at EPA's Water Docket, EPA West, 1301 Constitution Avenue, NW., Room B102, Washington, DC (Telephone: 202–566–2426); or at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html*. These test procedures are incorporated as they exist on the day of approval and a notice of any change in these test procedures will be published in the **Federal Register**. The discharge parameter values for which reports are required must be determined by one of the standard analytical test procedures incorporated by reference and described in Tables IA, IB, IC, ID, IE, IF, IG, and IH or by any alternate test procedure which has been approved by the Administrator under the provisions of paragraph (d) of this section and §§ 136.4 and 136.5. Under certain circumstances paragraph (c) of this section, § 136.5(a) through (d) or 40 CFR 401.13, other additional or alternate test procedures may be used.

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS FOR WASTEWATER AND SEWAGE SLUDGE

Parameter and units	Method [1]	EPA	Standard methods 18th, 19th, 20th ed.	Standard methods online	AOAC, ASTM, USGS	Other
Bacteria:						
1. Coliform (fecal), number per 100 mL or number per gram dry weight.	Most Probable Number (MPN),[5] tube 3 dilution, or	p. 132 [3] ..... 1680 [12,14] ..... 1681 [12,19]	9221 C E	9221 C E–99.		
	Membrane filter (MF) [2], single step.	p. 124 [3]	9222 D	9222 D–97	B–0050–85 [5].	
2. Coliform (fecal) in presence of chlorine, number per 100 mL.	MPN, 5 tube, 3 dilution, or	p. 132 [3]	9221 C E	9221 C E–99.		
	MF [2], single step	p. 124 [3]	9222 D	9222 D–97.		
3. Coliform (total), number per 100 mL.	MPN, 5 tube, 3 dilution, or	p. 114 [3]	9221 B	9221 B–99.		
	MF [2], single step or two step.	p. 108 [3]	9222 B	9222 B–97	B–0025–8 [5].	
4. Coliform (total), in presence of chlorine, number per 100 mL.	MPN, 5 tube, 3 dilution, or	p. 114 [3]	9221 B	9221 B–99.		
	MF [2] with enrichment ...	p. 111 [3]	9222 (B+B.5c)	9222 (B+B.5c) – 97.		
5. *E. coli*, number per 100 mL [20].	MPN [7,9,15] multiple tube/multiple well.	.....	9223 B [13]	9223 B–97 [13]	991.15 [11]	Colilert®[13,17] Colilert-18®[13,16,17]
	MF [2,6,7,8,9] single step ..	1603 [21]	.....	.....	.....	mColiBlue-24®[18]
6. Fecal streptococci, number per 100 mL.	MPN, 5 tube 3 dilution,	p. 139 [3]	9230 B	9230 B–93.		
	MF [2], or	p. 136 [3]	9230 C	9230 C–93	B–0055–85 [5].	
	Plate count	p. 143 [3].				
7. Enterococci, number per 100 mL [20].	MPN [7,9], multiple tube/multiple well.	.....	.....	.....	D6503–99 [10]	Enterolert® [13,23]
	MF [2,6,7,8,9] single step ..	1600 [24].				
8. Salmonella, number per gram dry weight [12].	MPN multiple tube	1682 [22].				
Aquatic Toxicity:						
9. Toxicity, acute, fresh water organisms, LC_{50}, percent effluent.	*Ceriodaphnia dubia* acute.	2002.0 [25].				
	Daphnia puplex and *Daphnia magna* acute.	2021.0 [25].				
	Fathead Minnow, *Pimephales promelas*, and Bannerfin *shiner*, *Cyprinella leedsi*, acute.	2000.0 [25].				

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS FOR WASTEWATER AND SEWAGE SLUDGE—Continued

Parameter and units	Method [1]	EPA	Standard methods 18th, 19th, 20th ed.	Standard methods online	AOAC, ASTM, USGS	Other
	Rainbow Trout, *Oncorhynchus mykiss*, and brook trout, *Salvelinus fontinalis*, acute.	2019.0 [25].				
10. Toxicity, acute, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, LC_{50}, percent effluent.	Mysid, *Mysidopsis bahia*, acute.	2007.0 [25].				
	Sheepshead Minnow, *Cyprinodon* variegatus, acute.	2004.0 [25].				
	Silverside, *Menidia beryllina*, *Menidia menidia*, and *Menidia peninsulae*, acute.	2006.0 [25].				
11. Toxicity, chronic, fresh water organisms, NOEC or IC_{25}, percent effluent.	Fathead minnow, *Pimephales promelas*, larval survival and growth.	1000.0 [26].				
	Fathead minnow, *Pimephales promelas*, embryo-larval survival and teratogenicity.	1001.0 [26].				
	Daphnia, *Ceriodaphnia dubia*, survival and reproduction.	1002.0 [26].				
	Green alga, *Selenastrum capricornutum*, growth.	1003.0 [26].				
12. Toxicity, chronic, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, NOEC or IC_{25}, percent effluent.	Sheepshead minnow, *Cyprinodon variegatus*, larval survival and growth.	1004.0 [27].				
	Sheepshed minnow, *Cyprinodon variegatus*, embryo-larval survival and teratogenicity.	1005.0 [27].				
	Inland silverside, *Menidia beryllina*, larval survival and growth.	1006.0 [27].				
	Mysid, *Mysidopsis bahia*, survival, growth, and fecundity.	1007.0 [27].				
	Sea urchin, *Arbacia punctulata*, fertilization.	1008.0 [27].				

[1] The method must be specified when results are reported.

[2] A 0.45 μm membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth.

[3] USEPA. 1978. Microbiological Methods for Monitoring the Environment, Water, and Wastes. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH, EPA/600/8–78/017.

[4] [Reserved].

[5] USGS. 1989. U.S. Geological Survey Techniques of Water-Resource Investigations, Book 5, Laboratory Analysis, Chapter A4, Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples, U.S. Geological Survey, U.S. Department of the Interior, Reston, VA.

[6] Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies.

[7] Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample.

[8] When the MF method has been used previously to test waters with high turbidity, large numbers of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results.

[9] To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines.

[10] ASTM. 2000, 1999, 1996. Annual Book of ASTM Standards—Water and Environmental Technology. Section 11.02. ASTM International. 100 Barr Harbor Drive, West Conshohocken, PA 19428.

[11] AOAC. 1995. Official Methods of Analysis of AOAC International, 16th Edition, Volume I, Chapter 17. Association of Official Analytical Chemists International. 481 North Frederick Avenue, Suite 500, Gaithersburg, MD 20877–2417.

[12] Recommended for enumeration of target organism in sewage sludge.

[13] These tests are collectively known as defined enzyme substrate tests, where, for example, a substrate is used to detect the enzyme β-glucuronidase produced by *E. coli*.

[14] USEPA. July 2006. Method 1680: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation Using Lauryl-Tryptose Broth (LTB) and EC Medium. US Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–06–012.

[15] Samples shall be enumerated by the multiple-tube or multiple-well procedure. Using multiple-tube procedures, employ an appropriate tube and dilution configuration of the sample as needed and report the Most Probable Number (MPN). Samples tested with Colilert® may be enumerated with the multiple-well procedures, Quanti-Tray® Quanti-Tray® 2000, and the MPN calculated from the table provided by the manufacturer.

[16] Colilert-18® is an optimized formulation of the Colilert® for the determination of total coliforms and *E. coli* that provides results within 18 h of incubation at 35 °C rather than the 24 h required for the Colilert® test and is recommended for marine water samples.

[17] Descriptions of the Colilert®, Colilert-18®, Quanti-Tray®, and Quanti-Tray®/2000 may be obtained from IDEXX Laboratories, Inc., 1 IDEXX Drive, Westbrook, ME 04092.

[18] A description of the mColiBlue24® test, Total Coliforms and *E. coli*, is available from Hach Company, 100 Dayton Ave., Ames, IA 50010.

[19] USEPA. July 2006. Method 1681: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using A–1 Medium. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–06–013.

[20] Recommended for enumeration of target organism in wastewater effluent.

[21] USEPA. July 2006. Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (modified mTEC). U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–06–011.

[22] USEPA. July 2006. Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–06–014.

[23] A description of the Enterolert® test may be obtained from IDEXX Laboratories, Inc., 1 IDEXX Drive, Westbrook, ME 04092.

[24] USEPA. July 2006. Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI). U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–06–009.

[25] USEPA. October 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA/821/R–02/012.

[26] USEPA. October 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition, U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA/821/R–02/013.

[27] USEPA. October 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Third Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA/821/R–02/014.

* * * * *

TABLE IH.—LIST OF APPROVED MICROBIOLOGICAL METHODS FOR AMBIENT WATER

Parameter and units	Method [1]	EPA	Standard methods 18th, 19th, 20th Ed.	Standard methods online	AOAC, ASTM, USGS	Other
Bacteria:						
1. E. coli, number per 100 mL.	MPN [6,8,14] multiple tube,	.....	9221 B.1/9221 F [11,13].	9221 B.1–99/ 9221 F [11,13].		
	Multiple tube/multiple well,	.....	9223 B [12]	9223 B–97 [12] ...	991.15 [10]	Colilert® [12,16] Colilert-18® [12,15,16].
	MF [2,5,6,7,8] two step, or	1103.1 [19]	9222 B/9222 G [18], 9213 D.	9222 B–97/ 9222 G [18].	D5392–93 [9].	
	Single step	1603 [20], 1604 [21]	.....	.....	.....	mColiBlue-24® [17].
2. Enterococci, number per 100 mL.	MPN [6,8] multiple tube,	.....	9230 B	9230 B–93.		
	Multiple tube/multiple well.	.....	.....	.....	D6503–99 [9]	Enterolert® [12,22].
	MF [2,5,6,7,8] two step	1106.1 [23]	9230 C	9230 C–93	D5259–92 [9].	
	Single step, or	1600 [24].				
	Plate count	p. 143 [3].				
Protozoa:						
3. *Cryptosporidium* ..	Filtration/IMS/FA	1622 [25], 1623 [26].				
4. *Giardia*	Filtration/IMS/FA	1623 [26].				

[1] The method must be specified when results are reported.

[2] A 0.45 μm membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth.

[3] USEPA. 1978. Microbiological Methods for Monitoring the Environment, Water, and Wastes. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH. EPA/600/8–78/017.

[4] [Reserved]

[5] Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies.

[6] Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample.

[7] When the MF method has not been used previously to test waters with high turbidity, large number of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results.

[8] To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines.

[9] ASTM. 2000, 1999, 1996. Annual Book of ASTM Standards—Water and Environmental Technology. Section 11.02. ASTM International. 100 Barr Harbor Drive, West Conshohocken, PA 19428.

[10] AOAC. 1995. Official Methods of Analysis of AOAC International, 16th Edition, Volume I, Chapter 17. Association of Official Analytical Chemists International. 481 North Frederick Avenue, Suite 500, Gaithersburg, MD 20877–2417.

[11] The multiple-tube fermentation test is used in 9221B.1. Lactose broth may be used in lieu of lauryl tryptose broth (LTB), if at least 25 parallel tests are conducted between this broth and LTB using the water samples normally tested, and this comparison demonstrates that the false-positive rate and false-negative rate for total coliform using lactose broth is less than 10 percent. No requirement exists to run the completed phase on 10 percent of all total coliform-positive tubes on a seasonal basis.

[12] These tests are collectively known as defined enzyme substrate tests, where, for example, a substrate is used to detect the enzyme β-glucuronidase produced by *E. coli*.

[13] After prior enrichment in a presumptive medium for total coliform using 9221B.1, all presumptive tubes or bottles showing any amount of gas, growth or acidity within 48 h ± 3 h of incubation shall be submitted to 9221F. Commercially available EC–MUG media or EC media supplemented in the laboratory with 50 μg/mL of MUG may be used.

[14] Samples shall be enumerated by the multiple-tube or multiple-well procedure. Using multiple-tube procedures, employ an appropriate tube and dilution configuration of the sample as needed and report the Most Probable Number (MPN). Samples tested with Colilert® may be enumerated with the multiple-well procedures, Quanti-Tray® or Quanti-Tray® 2000, and the MPN calculated from the table provided by the manufacturer.

[15] Colilert-18® is an optimized formulation of the Colilert® for the determination of total coliforms and E. coli that provides results within 18 h of incubation at 35 °C rather than the 24 h required for the Colilert® test and is recommended for marine water samples.

[16] Descriptions of the Colilert®, Colilert-18®, Quanti-Tray®, and Quanti-Tray®/2000 may be obtained from IDEXX Laboratories, Inc., 1 IDEXX Drive, Westbrook, ME 04092.

[17] A description of the mColiBlue24® test, Total Coliforms and *E. coli*, is available from Hach Company, 100 Dayton Ave., Ames, IA 50010.

[18] Subject total coliform positive samples determined by 9222B or other membrane filter procedure to 9222G using NA–MUG media.

[19] USEPA. July 2006. Method 1103.1: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC). U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–06–010.

[20] USEPA. July 2006. Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC). U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–06–011.

[21] Preparation and use of MI agar with a standard membrane filter procedure is set forth in the article, Brenner et al. 1993. ''New Medium for the Simultaneous Detection of Total Coliform and *Escherichia coli* in Water.'' Appl. Environ. Microbiol. 59:3534–3544 and in USEPA. September 2002.: Method 1604: Total Coliforms and *Escherichia coli* (*E. coli*) in Water by Membrane Filtration by Using a Simultaneous Detection Technique (MI Medium). U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA 821–R–02–024.

[22] A description of the Enterolert® test may be obtained from IDEXX Laboratories, Inc., 1 IDEXX Drive, Westbrook, ME 04092.

[23] USEPA. July 2006. Method 1106.1: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus-Esculin Iron Agar (mE–EIA). U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–06–008.

[24] USEPA. July 2006. Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI). U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–06–009.

[25] Method 1622 uses filtration, concentration, immunomagnetic separation of oocysts from captured material, immunofluorescence assay to determine concentrations, and confirmation through vital dye staining and differential interference contrast microscopy for the detection of *Cryptosporidium*. USEPA. 2001. Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–01–026.

[26] Method 1623 uses filtration, concentration, immunomagnetic separation of oocysts and cysts from captured material, immunofluorescence assay to determine concentrations, and confirmation through vital dye staining and differential interference contrast microscopy for the simultaneous detection of *Cryptosporidium* and *Giardia* oocysts and cysts. USEPA. 2001. Method 1623. *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA–821–R–01–025.

(b) The full texts of the methods from the following references which are cited in Tables IA, IB, IC, ID, IE, IF, IG and IH are incorporated by reference into this regulation and may be obtained from the source identified. All costs cited are subject to change and must be verified from the indicated source. The full texts of all the test procedures cited are available for inspection at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html*.

References, Sources, Costs, and Table Citations

* * * * *

(2) USEPA. 1978. Microbiological Methods for Monitoring the Environment, Water, and Wastes. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA/600/8–78/017. Available at *http://www.epa.gov/clariton/srch.htm* or from: National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Pub. No. PB–290329/A.S. Table IA, Note 3; Table IH, Note 3.

* * * * *

(6) American Public Health Association. 1992, 1995, and 1998. Standard Methods for the Examination of Water and Wastewater. 18th, 19th, and 20th Edition (respectively). Available from: American Public Health Association, 1015 15th Street, NW., Washington, DC 20005. Standard Methods Online is available through the Standard Methods Web site (*http://www.standardmethods.org*). Tables IA, IB, IC, ID, IE, and IH.

* * * * *

(10) ASTM International. Annual Book of ASTM Standards, Water, and Environmental Technology, Section 11, Volumes 11.01 and 11.02, 1994, 1996, 1999, Volume 11.02, 2000, and individual standards published after 2000. Available from: ASTM International, 100 Barr Harbor Drive, P.O. Box C700, West Conshohocken, PA 19428–2959, or *http://www.astm.org*. Tables IA, IB, IC, ID, IE, and IH.

* * * * *

(11) USGS. 1989. U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Laboratory Analysis, Chapter A4, Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples, U.S. Geological Survey, U.S. Department of the Interior, Reston, Virginia. Available from USGS Books and Open-File Reports Section, Federal Center, Box 25425, Denver, Colorado 80225. Table IA, Note 5; Table IH.

* * * * *

(34) USEPA. October 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA 821–R–02–012. Available at *http://www.epa.gov/epahome/index/*

sources.htm or from National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Pub. No. PB2002–108488. Table IA, Note 25.

* * * * *

(38) USEPA. October 2002. Short-Term Methods for Measuring the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA 821–R–02–013. Available at *http://www.epa.gov/epahome/index/sources.htm* or from National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Pub. No. PB2002–108489. Table IA, Note 26.

(39) USEPA. October 2002. Short-Term Methods for Measuring the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Third Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC EPA 821–R–02–014. Available at *http://www.epa.gov/epahome/index/sources.htm* or from National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Pub. No. PB2002–108490. Table IA, Note 27.

* * * * *

(52) IDEXX Laboratories, Inc. 2002. Description of Colilert®, Colilert-18®, Quanti-Tray®, Quanti-Tray®/2000, Enterolert® methods are available from IDEXX Laboratories, Inc., One Idexx Drive, Westbrook, Maine 04092. Table IA, Notes 17 and 23; Table IH, Notes 16 and 22.

(53) Hach Company, Inc. Revision 2, 1999. Description of m-ColiBlue24® Method, Total Coliforms and *E. coli*, is available from Hach Company, 100 Dayton Ave, Ames IA 50010. Table IA, Note 18; Table IH, Note 17.

(54) USEPA. July 2006. Method 1103.1: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC). U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–621–R–06–010. Available at *http://www.epa.gov/waterscience/methods/*. Table IH, Note 19.

(55) USEPA. July 2006. Method 1106.1: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus-Esculin Iron Agar (mE–EIA). U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–621–R–06–008. Available at *http://www.epa.gov/waterscience/methods/*. Table IH, Note 23

(56) USEPA. July 2006. Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC). U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–R–06–011. Available at *http://www.epa.gov/waterscience/methods/*. Table IH, Note 19; Table IH, Note 20.

(57) Brenner *et al.* 1993. New Medium for the Simultaneous Detection of Total Coliforms and *Escherichia coli* in Water. Appl. Environ. Microbiol. 59:3534–3544. Available from the American Society for Microbiology, 1752 N Street NW., Washington DC 20036. Table IH, Note 21.

(58) USEPA. September 2002. Method 1604: Total Coliforms and *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium). U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–R–02–024. Available at *http://www.epa.gov/waterscience/methods/*. Table IH, Note 20.

(59) USEPA. July 2006. Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI). U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–R–06–009. Available at *http://www.epa.gov/waterscience/methods/*. Table IA, Note 24; Table IH, Note 24.

(60) USEPA. April 2001. Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water, Washington DC EPA–821–R–01–026. Available at *http://www.epa.gov/waterscience/methods/*. Table IH, Note 25.

(61) USEPA. April 2001. Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA–821–R–01–025. Available at *http://www.epa.gov/waterscience/methods/*. Table IH, Note 26.

(62) AOAC. 1995. Official Methods of Analysis of AOAC International, 16th Edition, Volume I, Chapter 17. AOAC International, 481 North Frederick Avenue, Suite 500, Gaithersburg, Maryland 20877–2417. Table IA, Note 11; Table IH.

* * * * *

(70) USEPA. July 2006. Method 1680: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using Lauryl Tryptose Broth (LTB) and EC Medium. U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA 821–R–06–012. Available at *http://www.epa.gov/waterscience/methods/*.

(71) USEPA. July 2006. Method 1681: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using A–1 Medium. U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA 821–R–06–013. Available at *http://www.epa.gov/waterscience/methods/*.

(72) USEPA. July 2006. Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium. U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA 821–R–06–014. Available at *http://www.epa.gov/waterscience/methods/*.

* * * * *

(e) Sample preservation procedures, container materials, and maximum allowable holding times for parameters are cited in Tables IA, IB, IC, ID, IE, IF, IG and IH are prescribed in Table II. Information in the table takes precedence over information in specific methods or elsewhere. Any person may apply for a variance from the prescribed preservation techniques, container materials, and maximum holding times applicable to samples taken from a specific discharge. Applications for variances may be made by letters to the Regional Administrator in the Region in which the discharge will occur. Sufficient data should be provided to assure such variance does not adversely affect the integrity of the sample. Such data will be forwarded by the Regional Administrator, to the Alternate Test Procedure Program Coordinator, Washington, DC, for technical review and recommendations for action on the variance application. Upon receipt of the recommendations from the Alternate Test Procedure Program Coordinator, the Regional Administrator may grant a variance applicable to the specific discharge to the applicant. A decision to approve or deny a variance will be made within 90 days of receipt of the application by the Regional Administrator.

TABLE II.—REQUIRED CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES

Parameter No./name	Container [1]	Preservation [2,3]	Maximum holding time [4]
Table IA—Bacterial Tests:			
1–5. Coliform, total, fecal, and *E. coli*	PA, G	Cool, <10 °C, 0.0008% $Na_2S_2O_3$ [5]	6 hours.[22,23]
6. Fecal streptococci	PA, G	Cool, <10 °C, 0.0008% $Na_2S_2O_3$ [5]	6 hours.[22]
7. Enterococci	PA, G	Cool, <10 °C, 0.0008% $Na_2S_2O_3$ [5]	6 hours.[22]
8. Salmonella	PA, G	Cool, <10 °C, 0.0008% $Na_2S_2O_3$ [5]	6 hours.[22]
Table IA—Aquatic Toxicity Tests:			
9–11. Toxicity, acute and chronic	P, FP, G	Cool, ≤6 °C [16]	36 hours.
Table IB—Inorganic Tests:			
1. Acidity	P, FP, G	Cool, ≤6 °C [18]	14 days.
2. Alkalinity	P, FP, G	Cool, ≤6 °C [18]	14 days.
4. Ammonia	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH<2	28 days.
9. Biochemical oxygen demand	P, FP, G	Cool, ≤6 °C [18]	48 hours.
10. Boron	P, FP, or Quartz	HNO_3 to pH<2	6 months.
11. Bromide	P, FP, G	None required	28 days.
14. Biochemical oxygen demand, carbonaceous.	P, FP G	Cool, ≤6 °C [18]	48 hours.
15. Chemical oxygen demand	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH<2	28 days.
16. Chloride	P, FP, G	None required	28 days.
17. Chlorine, total residual	P, G	None required	Analyze within 15 minutes.
21. Color	P, FP, G	Cool, ≤6 °C [18]	48 hours.
23–24. Cyanide, total or available (or CATC)	P, FP, G	Cool, ≤6 °C [18], NaOH to pH>12 [6], reducing agent [5].	14 days.
25. Fluoride	P	None required	28 days.
27. Hardness	P, FP, G	HNO_3 or H_2SO_4 to pH<2	6 months.
28. Hydrogen ion (pH)	P, FP, G	None required	Analyze within 15 minutes.
31, 43. Kjeldahl and organic N	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH<2	28 days.
Table IB—Metals: [7]			
18. Chromium VI	P, FP, G	Cool, ≤6 °C [18], pH = 9.3–9.7 [20]	28 days.
35. Mercury (CVAA)	P, FP, G	HNO_3 to pH<2	28 days.
35. Mercury (CVAFS)	FP, G; and FP-lined cap [17].	5 mL/L 12N HCl or 5 mL/L BrCl [17]	90 days.[17]
3, 5–8, 12, 13, 19, 20, 22, 26, 29, 30, 32–34, 36, 37, 45, 47, 51, 52, 58–60, 62, 63, 70–72, 74, 75. Metals, except boron, chromium VI, and mercury.	P, FP, G	HNO_3 to pH<2, or at least 24 hours prior to analysis [19].	6 months.
38. Nitrate	P, FP, G	Cool, ≤6 °C [18]	48 hours.
39. Nitrate-nitrite	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH<2	28 days.
40. Nitrite	P, FP, G	Cool, ≤6 °C [18]	48 hours.
41. Oil and grease	G	Cool to ≤6 °C [18], HCl or H_2SO_4 to pH<2.	28 days.
42. Organic Carbon	P, FP, G	Cool to ≤6 °C [18], HCl, H_2SO_4, or H_3PO_4 to pH<2.	28 days.
44. Orthophosphate	P, FP, G	Cool, ≤6 °C [18]	Filter within 15 minutes; Analyze within 48 hours.
46. Oxygen, Dissolved Probe	G, Bottle and top	None required	Analyze within 15 minutes.
47. Winkler	G, Bottle and top	Fix on site and store in dark	8 hours.
48. Phenols	G	Cool, ≤6 °C [18], H_2SO_4 to pH<2	28 days.
49. Phosphorous (elemental)	G	Cool, ≤6 °C [18]	48 hours.
50. Phosphorous, total	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH<2	28 days.
53. Residue, total	P, FP, G	Cool, ≤6 °C [18]	7 days.
54. Residue, Filterable	P, FP, G	Cool, ≤6 °C [18]	7 days.
55. Residue, Nonfilterable (TSS)	P, FP, G	Cool, ≤6 °C [18]	7 days.
56. Residue, Settleable	P, FP, G	Cool, ≤6 °C [18]	48 hours.
57. Residue, Volatile	P, FP, G	Cool, ≤6 °C [18]	7 days.
61. Silica	P or Quartz	Cool, ≤6 °C [18]	28 days.
64. Specific conductance	P, FP, G	Cool, ≤6 °C [18]	28 days.
65. Sulfate	P, FP, G	Cool, ≤6 °C [18]	28 days.
66. Sulfide	P, FP, G	Cool, ≤6 °C [18], add zinc acetate plus sodium hydroxide to pH>9.	7 days.
67. Sulfite	P, FP, G	None required	Analyze within 15 minutes.
68. Surfactants	P, FP, G	Cool, ≤6 °C [18]	48 hours.
69. Temperature	P, FP, G	None required	Analyze.
73. Turbidity	P, FP, G	Cool, ≤6 °C [18]	48 hours.
Table IC—Organic Tests [8]			

TABLE II.—REQUIRED CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES—Continued

Parameter No./name	Container [1]	Preservation [2,3]	Maximum holding time [4]
13, 18–20, 22, 24–28, 34–37, 39–43, 45–47, 56, 76, 104, 105, 108–111, 113. Purgeable Halocarbons.	G, FP-lined septum	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$ [5]	14 days.
6, 57, 106. Purgeable aromatic hydrocarbons	G, FP-lined septum	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$ [5], HCl to pH 2 [9].	14 days.[9]
3, 4. Acrolein and acrylonitrile	G, FP-lined septum	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$ [5], pH to 4–5 [10].	14 days.[10]
23, 30, 44, 49, 53, 77, 80, 81, 98, 100, 112. Phenols [11].	G, FP-lined cap	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$ [5]	7 days until extraction, 40 days after extraction.
7, 38. Benzidines [11, 12]	G, FP-lined cap	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$ [5]	7 days until extraction.[13]
14, 17, 48, 50–52. Phthalate esters [11]	G, FP-lined cap	Cool, ≤6 °C [18]	7 days until extraction, 40 days after extraction.
82–84. Nitrosamines [11, 14]	G, FP-lined cap	Cool, ≤6 °C [18], store in dark, 0.008% $Na_2S_2O_3$ [5].	7 days until extraction, 40 days after extraction.
88–94. PCBs [11]	G, FP-lined cap	Cool, ≤6 °C [18]	1 year until extraction, 1 year after extraction.
54, 55, 75, 79. Nitroaromatics and isophorone [11].	G, FP-lined cap	Cool, ≤6 °C [18], store in dark, 0.008% $Na_2S_2O_3$ [5].	7 days until extraction, 40 days after extraction.
1, 2, 5, 8–12, 32, 33, 58, 59, 74, 78, 99, 101. Polynuclear aromatic hydrocarbons [11].	G, FP-lined cap	Cool, ≤6 °C [18], store in dark, 0.008% $Na_2S_2O_3$ [5].	7 days until extraction, 40 days after extraction.
15, 16, 21, 31, 87. Haloethers [11]	G, FP-lined cap	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$ [5]	7 days until extraction, 40 days after extraction.
29, 35–37, 63–65, 107. Chlorinated hydrocarbons [11].	G, FP-lined cap	Cool, ≤6 °C [18]	7 days until extraction, 40 days after extraction.
60–62, 66–72, 85, 86, 95–97, 102, 103. CDDs/CDFs [11].			
Aqueous Samples: Field and Lab Preservation	G	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$ [5], pH<9.	1 year.
Solids and Mixed-Phase Samples: Field Preservation.	G	Cool, ≤6 °C [18]	7 days.
Tissue Samples: Field Preservation	G	Cool, ≤6 °C [18]	24 hours.
Solids, Mixed-Phase, and Tissue Samples: Lab Preservation.	G	Freeze, ≤ −10 °C	1 year.
Table ID—Pesticides Tests:			
1–70. Pesticides [11]	G, FP-lined cap	Cool, ≤6 °C [18], pH 5–9 [15]	7 days until extraction, 40 days after extraction.
Table IE—Radiological Tests:			
1–5. Alpha, beta, and radium	P, FP, G	HNO_3 to pH<2	6 months.
Table IH—Bacterial Tests:			
1. *E. coli*	PA, G	Cool, <10 °C, 0.0008% $Na_2S_2O_3$ [5]	6 hours.[22]
2. Enterococci	PA, G	Cool, <10 °C, 0.0008% $Na_2S_2O_3$ [5]	6 hours.[22]
Table IH—Protozoan Tests:			
8. Cryptosporidium	LDPE; field filtration	0–8 °C	96 hours.[21]
9. Giardia	LDPE; field filtration	0–8 °C	96 hours.[21]

[1] "P" is polyethylene; "FP" is fluoropolymer (polytetrafluoroethylene (PTFE; Teflon®), or other fluoropolymer, unless stated otherwise in this Table II; "G" is glass; "PA" is any plastic that is made of a sterlizable material (polypropylene or other autoclavable plastic); "LDPE" is low density polyethylene.

[2] Except where noted in this Table II and the method for the parameter, preserve each grab sample within 15 minutes of collection. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sampler; see 40 CFR 122.21(g)(7)(i) or 40 CFR Part 403, Appendix E), refrigerate the sample at ≤6 °C during collection unless specified otherwise in this Table II or in the method(s). For a composite sample to be split into separate aliquots for preservation and/or analysis, maintain the sample at ≤6 °C, unless specified otherwise in this Table II or in the method(s), until collection, splitting, and preservation is completed. Add the preservative to the sample container prior to sample collection when the preservative will not compromise the integrity of a grab sample, a composite sample, or an aliquot split from a composite sample; otherwise, preserve the grab sample, composite sample, or aliquot split from a composite sample within 15 minutes of collection. If a composite measurement is required but a composite sample would compromise sample integrity, individual grab samples must be collected at prescribed time intervals (e.g., 4 samples over the course of a day, at 6-hour intervals). Grab samples must be analyzed separately and the concentrations averaged. Alternatively, grab samples may be collected in the field and composited in the laboratory if the compositing procedure produces results equivalent to results produced by arithmetic averaging of the results of analysis of individual grab samples. For examples of laboratory compositing procedures, see EPA Method 1664A (oil and grease) and the procedures at 40 CFR 141.34(f)(14)(iv) and (v) (volatile organics).

[3] When any sample is to be shipped by common carrier or sent via the U.S. Postal Service, it must comply with the Department of Transportation Hazardous Materials Regulations (49 CFR Part 172). The person offering such material for transportation is responsible for ensuring such compliance. For the preservation requirements of Table II, the Office of Hazardous Materials, Materials Transportation Bureau, Department of Transportation has determined that the Hazardous Materials Regulations do not apply to the following materials: Hydrochloric acid (HCl) in water solutions at concentrations of 0.04% by weight or less (pH about 1.96 or greater); Nitric acid (HNO_3) in water solutions at concentrations of 0.15% by weight or less (pH about 1.62 or greater); Sulfuric acid (H_2SO_4) in water solutions at concentrations of 0.35% by weight or less (pH about 1.15 or greater); and Sodium hydroxide (NaOH) in water solutions at concentrations of 0.080% by weight or less (pH about 12.30 or less).

[4] Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before the start of analysis and still be considered valid (e.g., samples analyzed for fecal coliforms may be held up to 6 hours prior to commencing analysis). Samples may be held for longer periods only if the permittee or monitoring laboratory has data on file to show that, for the specific types of samples under study, the analytes are stable for the longer time, and has received a variance from the Regional Administrator under § 136.3(e). For a grab sample, the holding time begins at the time of collection. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sampler; see 40 CFR 122.21(g)(7)(i) or 40 CFR Part 403, Appendix E), the holding time begins at the time of the end of collection of the composite sample. For a set of grab samples composited in the field or laboratory, the holding time begins at the time of collection of the last grab sample in the set. Some samples may not be stable for the maximum time period given in the table. A permittee or monitoring laboratory is obligated to hold the sample for a shorter time if it knows that a shorter time is necessary to maintain sample stability. See § 136.3(e) for details. The date and time of collection of an individual grab sample is the date and time at which the sample is collected. For a set of grab samples to be composited, and that are all collected on the same calendar date, the date of collection is the date on which the samples are collected. For a set of grab samples to be composited, and that are collected across two calendar dates, the date of collection is the dates of the two days; e.g., November 14–15. For a composite sample collected automatically on a given date, the date of collection is the date on which the sample is collected. For a composite sample collected automatically, and that is collected across two calendar dates, the date of collection is the dates of the two days; e.g., November 14–15.

[5] Add a reducing agent only if an oxidant (e.g., chlorine) is present. Reducing agents shown to be effective are sodium thiosulfate ($Na_2S_2O_3$), ascorbic acid, sodium arsenite ($NaAsO_2$), or sodium borohydride ($NaBH_4$). However, some of these agents have been shown to produce a positive or negative cyanide bias, depending on other substances in the sample and the analytical method used. Therefore, do not add an excess of reducing agent. Methods recommending ascorbic acid (e.g., EPA Method 335.4) specify adding ascorbic acid crystals, 0.1–0.6 g, until a drop of sample produces no color on potassium iodide (KI) starch paper, then adding 0.06 g (60 mg) for each liter of sample volume. If $NaBH_4$ or $NaAsO_2$ is used, 25 mg/L $NaBH_4$ or 100 mg/L $NaAsO_2$ will reduce more than 50 mg/L of chlorine (see method "Kelada-01" and/or Standard Method 4500–CN^- for more information). After adding reducing agent, test the sample using KI paper, a test strip (e.g. for chlorine, SenSafe™ Total Chlorine Water Check 480010) moistened with acetate buffer solution (see Standard Method 4500–Cl.C.3e), or a chlorine/oxidant test method (e.g., EPA Method 330.4 or 330.5), to make sure all oxidant is removed. If oxidant remains, add more reducing agent. Whatever agent is used, it should be tested to assure that cyanide results are not affected adversely.

[6] Sample collection and preservation: Collect a volume of sample appropriate to the analytical method in a bottle of the material specified. If the sample can be analyzed within 48 hours and sulfide is not present, adjust the pH to > 12 with sodium hydroxide solution (e.g., 5% w/v), refrigerate as specified, and analyze within 48 hours. Otherwise, to extend the holding time to 14 days and mitigate interferences, treat the sample immediately using any or all of the following techniques, as necessary, followed by adjustment of the sample pH to > 12 and refrigeration as specified. There may be interferences that are not mitigated by approved procedures. Any procedure for removal or suppression of an interference may be employed, provided the laboratory demonstrates that it more accurately measures cyanide. Particulate cyanide (e.g., ferric ferrocyanide) or a strong cyanide complex (e.g., cobalt cyanide) are more accurately measured if the laboratory holds the sample at room temperature and pH > 12 for a minimum of 4 hours prior to analysis, and performs UV digestion or dissolution under alkaline (pH=12) conditions, if necessary.

(1) Sulfur: To remove elemental sulfur (S_8), filter the sample immediately. If the filtration time will exceed 15 minutes, use a larger filter or a method that requires a smaller sample volume (e.g., EPA Method 335.4 or Lachat Method 01). Adjust the pH of the filtrate to > 12 with NaOH, refrigerate the filter and filtrate, and ship or transport to the laboratory. In the laboratory, extract the filter with 100 mL of 5% NaOH solution for a minimum of 2 hours. Filter the extract and discard the solids. Combine the 5% NaOH-extracted filtrate with the initial filtrate, lower the pH to approximately 12 with concentrated hydrochloric or sulfuric acid, and analyze the combined filtrate. Because the detection limit for cyanide will be increased by dilution by the filtrate from the solids, test the sample with and without the solids procedure if a low detection limit for cyanide is necessary. Do not use the solids procedure if a higher cyanide concentration is obtained without it. Alternatively, analyze the filtrates from the sample and the solids separately, add the amounts determined (in μg or mg), and divide by the original sample volume to obtain the cyanide concentration.

(2) Sulfide: If the sample contains sulfide as determined by lead acetate paper, or if sulfide is known or suspected to be present, immediately conduct one of the volatilization treatments or the precipitation treatment as follows: Volatilization—Headspace expelling. In a fume hood or well-ventilated area, transfer 0.75 liter of sample to a 4.4 L collapsible container (e.g., Cubitainer™). Acidify with concentrated hydrochloric acid to pH < 2. Cap the container and shake vigorously for 30 seconds. Remove the cap and expel the headspace into the fume hood or open area by collapsing the container without expelling the sample. Refill the headspace by expanding the container. Repeat expelling a total of five headspace volumes. Adjust the pH to > 12, refrigerate, and ship or transport to the laboratory. Scaling to a smaller or larger sample volume must maintain the air to sample volume ratio. A larger volume of air will result in too great a loss of cyanide (> 10%). Dynamic stripping: In a fume hood or well-ventilated area, transfer 0.75 liter of sample to a container of the material specified and acidify with concentrated hydrochloric acid to pH < 2. Using a calibrated air sampling pump or flowmeter, purge the acidified sample into the fume hood or open area through a fritted glass aerator at a flow rate of 2.25 L/min for 4 minutes. Adjust the pH to > 12, refrigerate, and ship or transport to the laboratory. Scaling to a smaller or larger sample volume must maintain the air to sample volume ratio. A larger volume of air will result in too great a loss of cyanide (> 10%). Precipitation: If the sample contains particulate matter that would be removed by filtration, filter the sample prior to treatment to assure that cyanide associated with the particulate matter is included in the measurement. Ship or transport the filter to the laboratory. In the laboratory, extract the filter with 100 mL of 5% NaOH solution for a minimum of 2 hours. Filter the extract and discard the solids. Combine the 5% NaOH-extracted filtrate with the initial filtrate, lower the pH to approximately 12 with concentrated hydrochloric or sulfuric acid, and analyze the combined filtrate. Because the detection limit for cyanide will be increased by dilution by the filtrate from the solids, test the sample with and without the solids procedure if a low detection limit for cyanide is necessary. Do not use the solids procedure if a higher cyanide concentration is obtained without it. Alternatively, analyze the filtrates from the sample and the solids separately, add the amounts determined (in μg or mg), and divide by the original sample volume to obtain the cyanide concentration. For removal of sulfide by precipitation, raise the pH of the sample to > 12 with NaOH solution, then add approximately 1 mg of powdered cadmium chloride for each mL of sample. For example, add approximately 500 mg to a 500-mL sample. Cap and shake the container to mix. Allow the precipitate to settle and test the sample with lead acetate paper. If necessary, add cadmium chloride but avoid adding an excess. Finally, filter through 0.45 micron filter. Cool the sample as specified and ship or transport the filtrate and filter to the laboratory. In the laboratory, extract the filter with 100 mL of 5% NaOH solution for a minimum of 2 hours. Filter the extract and discard the solids. Combine the 5% NaOH-extracted filtrate with the initial filtrate, lower the pH to approximately 12 with concentrated hydrochloric or sulfuric acid, and analyze the combined filtrate. Because the detection limit for cyanide will be increased by dilution by the filtrate from the solids, test the sample with and without the solids procedure if a low detection limit for cyanide is necessary. Do not use the solids procedure if a higher cyanide concentration is obtained without it. Alternatively, analyze the filtrates from the sample and the solids separately, add the amounts determined (in μg or mg), and divide by the original sample volume to obtain the cyanide concentration. If a ligand-exchange method is used (e.g., ASTM D6888), it may be necessary to increase the ligand-exchange reagent to offset any excess of cadmium chloride.

(3) Sulfite, thiosulfate, or thiocyanate: If sulfite, thiosulfate, or thiocyanate is known or suspected to be present, use UV digestion with a glass coil (Method Kelada-01) or ligand exchange (Method OIA–1677) to preclude cyanide loss or positive interference.

(4) Aldehyde: If formaldehyde, acetaldehyde, or another water-soluble aldehyde is known or suspected to be present, treat the sample with 20 mL of 3.5% ethylenediamine solution per liter of sample.

(5) Carbonate: Carbonate interference is evidenced by noticeable effervescence upon acidification in the distillation flask, a reduction in the pH of the absorber solution, and incomplete cyanide spike recovery. When significant carbonate is present, adjust the pH to ≥12 using calcium hydroxide instead of sodium hydroxide. Allow the precipitate to settle and decant or filter the sample prior to analysis (also see Standard Method 4500–CN.B.3.d).

(6) Chlorine, hypochlorite, or other oxidant: Treat a sample known or suspected to contain chlorine, hypochlorite, or other oxidant as directed in footnote 5.

[7] For dissolved metals, filter grab samples within 15 minutes of collection and before adding preservatives. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sampler; see 40 CFR 122.21(g)(7)(i) or 40 CFR Part 403, Appendix E), filter the sample within 15 minutes after completion of collection and before adding preservatives. If it is known or suspected that dissolved sample integrity will be compromised during collection of a composite sample collected automatically over time (e.g., by interchange of a metal between dissolved and suspended forms), collect and filter grab samples to be composited (footnote 2) in place of a composite sample collected automatically.

[8] Guidance applies to samples to be analyzed by GC, LC, or GC/MS for specific compounds.

[9] If the sample is not adjusted to pH 2, then the sample must be analyzed within seven days of sampling.

[10] The pH adjustment is not required if acrolein will not be measured. Samples for acrolein receiving no pH adjustment must be analyzed within 3 days of sampling.

[11] When the extractable analytes of concern fall within a single chemical category, the specified preservative and maximum holding times should be observed for optimum safeguard of sample integrity (i.e., use all necessary preservatives and hold for the shortest time listed). When the analytes of concern fall within two or more chemical categories, the sample may be preserved by cooling to ≤6 °C, reducing residual chlorine with 0.008% sodium thiosulfate, storing in the dark, and adjusting the pH to 6–9; samples preserved in this manner may be held for seven days before extraction and for forty days after extraction. Exceptions to this optional preservation and holding time procedure are noted in footnote 5 (regarding the requirement for thiosulfate reduction), and footnotes 12, 13 (regarding the analysis of benzidine).

[12] If 1,2-diphenylhydrazine is likely to be present, adjust the pH of the sample to 4.0 ± 0.2 to prevent rearrangement to benzidine.

[13] Extracts may be stored up to 30 days at < 0 °C.

[14] For the analysis of diphenylnitrosamine, add 0.008% $Na_2S_2O_3$ and adjust pH to 7–10 with NaOH within 24 hours of sampling.

[15] The pH adjustment may be performed upon receipt at the laboratory and may be omitted if the samples are extracted within 72 hours of collection. For the analysis of aldrin, add 0.008% $Na_2S_2O_3$.

[16] Sufficient ice should be placed with the samples in the shipping container to ensure that ice is still present when the samples arrive at the laboratory. However, even if ice is present when the samples arrive, it is necessary to immediately measure the temperature of the samples and confirm that the preservation temperature maximum has not been exceeded. In the isolated cases where it can be documented that this holding temperature cannot be met, the permittee can be given the option of on-site testing or can request a variance. The request for a variance should include supportive data which show that the toxicity of the effluent samples is not reduced because of the increased holding temperature.

[17] Samples collected for the determination of trace level mercury (<100 ng/L) using EPA Method 1631 must be collected in tightly-capped fluoropolymer or glass bottles and preserved with BrCl or HCl solution within 48 hours of sample collection. The time to preservation may be extended to 28 days if a sample is oxidized in the sample bottle. A sample collected for dissolved trace level mercury should be filtered in the laboratory within 24 hours of the time of collection. However, if circumstances preclude overnight shipment, the sample should be filtered in a designated clean area in the field in accordance with procedures given in Method 1669. If sample integrity will not be maintained by shipment to and filtration in the laboratory, the sample must be filtered in a designated clean area in the field within the time period necessary to maintain sample integrity. A sample that has been collected for determination of total or dissolved trace level mercury must be analyzed within 90 days of sample collection.

[18] Aqueous samples must be preserved at ≤6 °C, and should not be frozen unless data demonstrating that sample freezing does not adversely impact sample integrity is maintained on file and accepted as valid by the regulatory authority. Also, for purposes of NPDES monitoring, the specification of "≤°C" is used in place of the "4 °C" and "< 4 °C" sample temperature requirements listed in some methods. It is not necessary to measure the sample temperature to three significant figures (1/100th of 1 degree); rather, three significant figures are specified so that rounding down to 6 °C may not be used to meet the ≤6 °C requirement. The preservation temperature does not apply to samples that are analyzed immediately (less than 15 minutes).

[19] An aqueous sample may be collected and shipped without acid preservation. However, acid must be added at least 24 hours before analysis to dissolve any metals that adsorb to the container walls. If the sample must be analyzed within 24 hours of collection, add the acid immediately (see footnote 2). Soil and sediment samples do not need to be preserved with acid. The allowances in this footnote supersede the preservation and holding time requirements in the approved metals methods.

[20] To achieve the 28-day holding time, use the ammonium sulfate buffer solution specified in EPA Method 218.6. The allowance in this footnote supersedes preservation and holding time requirements in the approved hexavalent chromium methods, unless this supersession would compromise the measurement, in which case requirements in the method must be followed.

[21] Holding time is calculated from time of sample collection to elution for samples shipped to the laboratory in bulk and calculated from the time of sample filtration to elution for samples filtered in the field.

[22] Samples analysis should begin immediately, preferably within 2 hours of collection. The maximum transport time to the laboratory is 6 hours, and samples should be processed within 2 hours of receipt at the laboratory.

[23] For fecal coliform samples for sewage sludge (biosolids) only, the holding time is extended to 24 hours for the following sample types using either EPA Method 1680 (LTB–EC) or 1681 (A–1): Class A composted, Class B aerobically digested, and Class B anaerobically digested.

PART 503—STANDARDS FOR THE USE OR DISPOSAL OF SEWAGE SLUDGE

■ 3. The authority citation for Part 503 continues to read as follows:

Authority: Secs. 405(d) and (e) of the Clean Water Act, as amended by Pub. L. 95–217, sec. 54(d), 91 Stat. 1591 (33 U.S.C. 1345(d) and (e)); and Pub. L. 100–4, title IV, sec. 406(a), (b), 101 Stat., 71, 72 (33 U.S.C. 1251 et seq.).

■ 4. Section 503.8 is amended by revising paragraph (b) introductory text to read as follows:

§ 503.8 Sampling and analysis.

* * * * *

(b) *Methods.* The materials listed below are incorporated by reference in this part. These incorporations by reference were approved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. The materials are incorporated as they exist on the date of approval, and notice of any change in these materials will be published in the **Federal Register**. They are available for inspection at the HQ Water Docket Center, EPA/DC, EPA West, Room B102, 1301 Constitution Ave., NW., Washington, DC, and at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html.*

Copies may be obtained from the standard producer or publisher listed in the regulation. The methods in the materials listed below (or in 40 CFR Part 136) shall be used to analyze samples of sewage sludge.

* * * * *

[FR Doc. 07–1455 Filed 3–23–07; 8:45 am]

BILLING CODE 6560–50–P