WEST VIRGINIA
SECRETARY OF STATE
KEN HECHLER
ADMINISTRATIVE LAW DIVISION

Form #1

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SECRETARY OF STATE

NOTICE OF PUBLIC HEARING ON A PROPOSED RULE

AGENCY: State Water Resources Board TITLE NUMBER: 46

RULE TYPE: Legislative ; CITE AUTHORITY §§20-5A,20-5E,20-5F and 20-5G

AMENDMENT TO AN EXISTING RULE: YES____ NO X

IF YES, SERIES NUMBER OF RULE BEING AMENDED: _____

TITLE OF RULE BEING AMENDED: _____

IF NO, SERIES NUMBER OF NEW RULE BEING PROPOSED: 11

TITLE OF RULE BEING PROPOSED: Proposed Rules Governing Laboratory Certification and Standards of Performance

DATE OF PUBLIC HEARING: September 12, 1988 TIME: 7:00 P.M.

LOCATION OF PUBLIC HEARING: State Capitol Complex Conference Room B

COMMENTS LIMITED TO: ORAL____, WRITTEN____, BOTH X

COMMENTS MAY ALSO BE MAILED TO THE FOLLOWING ADDRESS: State Water Resources Board
1260 Greenbrier Street
Charleston, WV 25311

The Department requests that persons wishing to make comments at the hearing make an effort to submit written comments in order to facilitate the review of these comments.

The issues to be heard shall be limited to the proposed rule.

ATTACH A **BRIEF** SUMMARY OF YOUR PROPOSAL

Frances E Hunter

STATE OF WEST VIRGINIA
STATE WATER RESOURCES BOARD
1206 Greenbrier Street
Charleston, West Virginia 25311
(304) 348-4002

August 3, 1988

The Honorable Ken Hechler
Secretary of state
State Capitol
Charleston, West Virginia 25305

Re: Notice of Hearing on Proposed Rules

Attn: Rich Hartman

Dear Mr. Secretary:

Enclosed is a copy of proposed amendments to the State Water Resources Board's Series I, Water Quality Standards along with a new Series 11, proposed Legislative Rules Governing Environmental Laboratory Certification and Standards of Performance, for filing in the State Register pursuant to the Administrative Procedures Act.

If you have any questions do not hesitate to contact this office.

Very truly yours,

STATE WATER RESOURCES BOARD

Frances E. Hunter

Frances E. Hunter
Executive Secretary

enclosures

NOTICE OF
PUBLIC HEARING
ON PROPOSED
AMENDMENTS
AND REVISIONS
TO THE WATER
QUALITY STANDARDS
SERIES I
AND PROPOSED
RULES GOVERNING
ENVIRONMENTAL
CERTIFICATION
AND STANDARDS
OF PERFORMANCE
SERIES II

Public Notice Date: August 3, 1988

The West Virginia State Water Resources Board will, in accordance with applicable State and federal requirements, hold public hearings on proposed amendments and revisions to Series I of the Legislative Rules "Requirements Governing Water Quality Standards" and proposed Legislative Rules "Governing Environmental Laboratory Certification and Standards of Performance".

The hearing will be held in the State Capitol Complex Conference Room B on Monday, September 12, 1988 at 7:00 P.M.

People wishing to make comments on the proposed rules are invited to be present or represented at the hearings. Although oral statements will be accepted, written statements are encouraged for the accuracy of the record.

Copies of the proposed rules may be obtained by contacting the Board office at 1260 Greenbrier Street, Charleston, WV 25311 (304) 348-4002.

(500447)

TITLE 46

LEGISLATIVE RULES

WATER RESOURCES BOARD

SERIES 11

PROPOSED RULES GOVERNING

LABORATORY CERTIFICATION AND STANDARDS

OF PERFORMANCE

NOTE: This is an entirely new series; therefore underscoring has been omitted.

Mailing Address: State Water Resources Board
1260 Greenbrier Street
Charleston, WV 25311

Phone (304)348-4002

Regulations Governing Environmental Laboratory Certification and Standards Of Performance

REGULATIONS GOVERNING ENVIRONMENTAL LABORATORIES

CERTIFICATION AND STANDARDS OF PERFORMANCE

Chapter 20, Article Series

Section 1 - General Provisions

1.1 Scope and Authority

This chapter, adopted in conjunction with the Water Pollution Control Act (Chapter 20-5A-1 et seq.), Hazardous Waste Management Act (Chapter 20-5E-1 et seq.), Solid Waste Management Act (Chapter 20-5F-1 et seq.) and the Hazardous Waste Emergency Response Fund (Chapter 20-5G-1 et seq.), constitutes the Department of Natural Resources regulations governing certification of laboratories conducting environmental analysis performed as referenced by regulations or orders issued pursuant to those acts. This chapter establishes the procedures for obtaining and maintaining certifications and the criteria and procedures laboratories shall follow in analyzing samples.

1.2 Construction

These regulations shall be liberally construed to permit the Department to discharge its statutory functions and to effectuate the purposes of the laboratory certification program.

1.3 Purpose of the Regulations

(a) This Chapter is promulgated for the following purposes:

(1) To establish the administrative procedures to be followed by certified laboratories and laboratories seeking certification.

(2) To establish the categories and parameters in which laboratories may be certified.

(3) To establish the standards, criteria and procedures for laboratory equipment and supplies, practices, methodology, quality control, personnel, facilities, data reporting, and maintenance which a certified laboratory or laboratory seeking certification shall continually meet.

(4) To establish the enforcement procedures the Department shall follow to ensure that all certified laboratories or laboratories seeking certification

are in compliance with this chapter.

1.4 Certification Program Requirements

(a) Any laboratory wishing to analyze samples for compliance with adopted regulations, permits, or orders issued pursuant to an applicable act shall follow the procedures set forth herein in order to obtain and maintain certification.

(b) Certified laboratories and laboratories seeking certification shall analyze all samples in accordance with the procedures and methods required by this chapter.

1.5 Incorporation by Reference

The Department hereby adopts and incorporates into these regulations the "Guidelines Establishing Test Procedures for the Analysis of Pollutants" as amended, 40 CFR Part 136, and future supplements and amendments to those regulations; the "Test Methods for Evaluating Solid Waste", U.S. EPA Manual SW-846 as amended, and future supplements and amendments.

1.6 Program Information

Unless otherwise specified, any questions concerning the requirements of this chapter should be directed to the Department of Natural Resources, Division of Water Resources, Quality Assurance Office, 1201 Greenbrier Street, Charleston, West Virginia 25311. Telephone number 304-348-0478.

1.7 Definitions

The following words and terms, when used in the chapter, shall have the following meanings unless the context clearly indicates otherwise.

"Accredited" means having the approval conferred upon institutions or programs where appropriate by a nationally recognized accrediting agency or association as determined by the Department.

"Analytical reagent (AR) grade, ACS reagent grade, and reagent grade" are synonymous terms for reagents which conform to the current specifications of the Committee on Analytical Reagents of

the American Chemical Society.

"Category" means a group of parameters for which certification is offered.

"Certification parameter" is a parameter which is identified in a performance evaluation sample test and is used to evaluate the overall analytical performance of a laboratory on the specific method.

"Certification year" means that period of time following the date upon which the laboratory first receives certification for any parameter or category and lasting for 365 consecutive days.

"Certified thermometer" is a thermometer that has documentation from the manufacturer showing that it has been compared against a National Bureau of Standards thermometer for the temperature ranges employed by the laboratory and the correction factors from the comparison.

"Chief" means the Chief of the Department's Division of Water Resources.

"Compliance analysis" means the analysis of a sample that is required to be analyzed by a department regulation, permit, or order.

"Confluent growth" means a bacterial growth (coliform or non-coliform) which covers the entire filtration area of the filter with no discrete colonies.

"Department" means the West Virginia Department of Natural Resources.

"EPA" means the United States Environmental Protection Agency.

"Laboratory pure water" means distilled or deionized water which is free of contaminants that may interfere with the analytical test in question.

"Laboratory seeking certification" means an uncertified laboratory which has submitted an acceptable application and the appropriate fee for the category in which it desires certification, and a laboratory holding a valid interim approval.

"Performance evaluation sample" is a sample containing a known amount of a specific or combination of parameters used in part to evaluate the performance of a laboratory.

"Personal and direct supervision" means that a qualified supervisor is available at all times when laboratory procedures are being performed.

"Primary standard" is a highly pure reagent used as a reference for standardizing other reagent solutions.

"Quality Assurance Office" is a subdivision of the Department of Natural Resources.

"Replicate sample" is a sample prepared by dividing a homogeneous sample into separate parts so that each part is also homogenous and representative of the original sample.

"Standard curve" is a curve plotting concentrations of a known parameter standard minus a blank, versus the standard's absorbance or percent transmittance.

"Standard Methods" means the latest EPA and State approved edition of Standard Methods for the Examination of Water and Wastewater, American Public Health Association.

"Subsequent to graduation" means laboratory training and experience acquired after receipt of the degree specified.

"USEPA" means United States Environmental Protection Agency.

1.8 Severability

If any section, subsection, provision, clause, or portion of this chapter is adjudged unconstitutional or invalid by a court of competent jurisdiction, the remainder of this chapter shall not be

affected thereby.

Section 2 - Program, Procedures, and Requirements

2.1 Requirement of Certification

(a) All sample analyses performed for the purpose of determining compliance with chemical, microbiological, bioassay and radiological requirements of the States natural resources and environmental programs or when required by order issued by the Department shall be performed in laboratories certified or, during the program phase-in, an interim status laboratory certified for this purpose pursuant to this chapter. Analyses performed in laboratories not so certified shall not be accepted by the Department as being in compliance with the requirements, regulations or orders of the Department.

(b) Laboratories in other states where the certifying agency grants reciprocal certifications to laboratories located in West Virginia, certified under conditions no less strigent than those required by this chapter by the agency having primary enforcement responsiblility under Federal programs delegated to such other state, shall be considered to be certified for the purpose of this chapter once they have complied with the provisions of section 2.4 (a) thru (d).

(c) Only laboratories certified pursuant to these regulations or maintained by the USEPA may be called State Certified Environmental Laboratories and no laboratory may adopt any name or make any oral or written statement intended or likely to mislead the public with respect to its certification status.

2.2 Categories for Certification

(a) A laboratory may apply for certification in any one or more of the following categories and shall be certified in those parameters within the category for which it demonstrates acceptable performance on performance evaluation samples, where available, and meets all other requirements of this chapter. The laboratory certificate shall specify the categories and the parameters within each category for which the laboratory is certified and it shall be displayed in a location visible to the public. The certification categories are as follows:

1. Atomic absorption (AA), which comprises tests or analyses for which the atomic absorption method is applicable or required. Tests for the AA category must be conducted in accordance with the method and procedures specified in 40 CFR Part 136 or EPA SW-846 manual as appropriate.

2. Limited chemistry, which comprises chemical tests or analyses required to determine compliance with the States' Acts and Regulations covered by this chapter except those analyses for which the atomic absorption, gas chromatography and/or mass spectrometry methods are specifically required. Tests for the limited chemistry category must be conducted in accordance with the methods and procedures specified in 40 CFR Part 136 or EPA manual SW-846 as appropriate for compliance with the States Acts and Regulations covered by this chapter.

3. Gas Chromatography (GC) and/or mass spectrometry (MS), which comprises tests or analyses required to determine compliance with the State Acts and Regulations covered by this chapter for which the gas chromatograph and/or mass spectrometry method is applicable or required. Tests for the GC or MS categories must be conducted in accordance with the methods and procedures specified in 40 CFR Part 136 or EPA manual SW-846 as appropriate.

4. Microbiological Testing, which comprises tests for coliform bacteria must be conducted in accordance with the methods and procedures specified in 40 CFR Part 136 for compliance with the Water Pollution Control Act and the State Pollutant Discharge Elimination System Regulations.

5. Bioassay Testing, which shall include any bioassay analyses required to determine compliance with the Acts and Regulations covered by this chapter. Bioassay analyses must be conducted in accordance with the methods and procedures specified in Section 6 of these regulations.

6. Radiological Testing, which comprises those tests or analyses for radioactivity required to determine compliance with the State's Acts and Regulations covered by this chapter. Tests for the Radiological category must be conducted in accordance with the methods and procedures specified in 40 CFR Part 136.3, Table 1E. 7.

7. Solid and Hazardous Waste Testing, which comprises those tests or analyses required to determine compliance with the States' Acts and Regulations covered by this chapter. Tests for the hazardous waste characteristics, composition of the waste, and groundwater associated with hazardous waste must be conducted in accordance with the methods and procedures in EPA manual SW-846.

2.3 Application Procedures and Requirements for Laboratories

Located in West Virginia Including Special Provisions for the Phase-in of the West Virginia Environmental Laboratory Certification Program.

(a) The owner of a laboratory in West Virginia who wishes to be certified in any or all of the categories and parameters thereof (described in section 2.2) or, if already certified, who wishes to add a category or a parameter within a category, shall apply for certification to the West Virginia Department of Natural Resources, Water Resources Division, Quality Assurance Office, Charleston, WV 25311 (304-348-0478). The applicant shall submit the appropriate fee.

(b) If the applicant fails to submit all the information requested or fails to submit the appropriate fee, the Department shall reject the application without prejudice.

(c) If the applicant submits a complete application, the appropriate fee, performance evaluation data, if required, and the information submitted meets the minimum requirements of this chapter for the category or categories for which certification is requested, the application shall be accepted. Acceptance of the application does not authorize the laboratory to perform analyses regulated by this chapter. The applicant shall be notified of the acceptance and shall participate in the following laboratory evaluation:

1. Microbiological and Bioassay Testing

   i. The Department shall contact the laboratory after the application is accepted to arrange a mutually acceptable date for an on-site inspection;

   ii. The laboratory shall be evaluated and inspected to determine if it is in compliance with the requirements of this chapter.

   iii. If the laboratory demonstrates to the Quality Assurance Office that it is in compliance with the requirements of this chapter it will be certified for the category for which it has requested certification.

2. Atomic Absorption, Limited Chemistry, Solid and Hazardous Waste, Gas Chromatography and/or Mass Spectrometry:

   i. The Quality Assurance Office shall contact the laboratory to arrange a mutually acceptable date for an on-site laboratory inspection;

   ii. The Quality Assurance Officer shall send to the laboratory a set of performance evaluation samples, if

available, for the parameters for which certification is requested after acceptance of the laboratory's application by the Department;

iii. The laboratory shall analyze the performance evaluation samples and return the data generated from the analyses within 45 days of its receipt of the samples to the Department of Natural Resources, Water Resources Division, Quality Assurance Office, 1201 Greenbrier Street, Charleston, WV 25311;

iv. The laboratory shall have satisfied the requirements for testing for a parameter when it acceptably analyzes both high and low values for that parameter within a given set of performance evaluation samples;

v. Acceptable analysis for a value in all water pollution parameters occurs when the reported value falls within the 99 percent confidence interval calculated for that sample from available performance evaluation data;

vi. Acceptable analysis for a value in all solid and hazardous waste parameters occurs when the reported value falls within the stated confidence limits or standard deviations for each parameter as given in USEPA Manual SW-846.

vii. The laboratory shall have three separate opportunities within 90 days to acceptably analyze one of three different sets of performance evaluation samples for each parameter. Labs who fail to successfully analyze the samples in this time period will not be reevaluated for a period of one year;

vii. If the laboratory's analytical values for the performance evaluation samples are acceptable, the Quality Assurance Office shall contact the laboratory to arrange a mutually acceptable date for an on-site inspection;

viii. If the laboratory demonstrates to the Quality Assurance Office that it is in compliance with the requirements of this chapter, and that it can acceptably analyze performance evaluation samples then it shall be certified in the category and the parameters within the category for which it has acceptably analyzed performance evaluation samples.

ix. If performance evaluation samples are not available, then the evaluation of the laboratory will be based on the application and the on-site laboratory inspection.

3. Radiological Testing

i. Laboratories seeking certification in the Radiological category shall have participated in the USEPA's radiological proficiency testing program during the immediately preceding twelve months and shall submit copies of the USEPA's performance evaluation reports demonstrating that for each parameter in which the laboratory is seeking certification at least four (4) performance test average values have been within the control limits established for that parameter.

ii. Laboratories which intend to seek certification in the Radiological category, but which have not participated in the USEPA's radiological proficiency testing program may obtain information concerning that program from the department.

iii. The Quality Assurance Office shall contact the laboratory after the application is accepted to arrange a mutually acceptable date for an on-site laboratory inspection, and the inspection will be conducted by representatives of the Quality Assurance Office and the USEPA; and

iv. If the laboratory demonstrates to the Quality Assurance Office during the inspection that it is in compliance with the requirements of this chapter it shall be certified for the category and the parameters within the category for which it has requested certification.

(d) Certifications may contain conditions requiring recertification of minor deficiencies identified by the Quality Assurance Office by a date or dates specified therein, but only if such minor deficiencies do not affect the accuracy of the analytical results.

(e) An applicant for certification who either does not perform acceptably on the performance evaluation samples or does not meet the requirements of this chapter shall be notified that certification has been denied. Laboratories notified of denial of certification must immediately cease performing analyses required to be performed in a certified laboratory for compliance with the Acts and Regulations covered by this chapter.

Applicants receiving such a notification may not reapply for certification until the laboratory assures the Quality Assurance Officer in writing that all reasons for denial of certification have been rectified.

Owners, principal officers, directors and supervisors may not reapply for certification of this same facility by the simple expedient of changing the company or laboratory name.

The laboratory facility may reapply by preparing a new application if the facility is sold and has a change of principal officer, directors and supervisors.

(f) The following special provisions are applicable to the phase-in of the West Virginia Environmental Laboratory Certification Program:

1. During the phase-in period all laboratories that desire to continue performing analyses that the Department will find acceptable and to receive interim approval must contact the Quality Assurance Office for an Interim Status Environmental Laboratories Certification and License form, complete, and submit the form with the appropriate fee to the Quality Assurance Office within 60 days after these regulations become final.

2. The laboratory that has been granted an interim approval is authorized to perform analysis for the programs covered by this chapter while the laboratory is being evaluated for certification providing:

   i. Such laboratories follow the procedures and meet the requirements of all previous subsections of this section;

   ii. Interim approvals shall be valid until the laboratory is audited and an approval or denial decision is made or until three (3) years after promulgation of these regulations, whichever is earlier;

   iii. A laboratory that fails to acceptably analyze the performance evaluation samples or otherwise fails to meet the requirements of this chapter for certification shall be allowed to remain in an interim approved status for 30 days after being notified of deficiencies if the laboratory submits an acceptable plan to correct the deficiencies within 10 days of receiving notification of its deficiencies, to the Quality Assurance Office.

   iv. Laboratories notified that their interim approval has been revoked shall immediately cease performing analyses required to be performed in a certified laboratory for compliance with the Acts and Regulations covered by this chapter and shall

comply with subsection (e) above before reapplying for certification.

2.4 Procedures for laboratories not located in West Virginia

(a) The owner of a laboratory, located in a state other than West Virginia, which has been certified (by the state where it is located) under conditions no less stringent than those required by this chapter by the agency having primary enforcement responsibility for the programs covered by this chapter and who wishes to perform analyses covered by this chapter shall:

1. Annually complete the application form provided by the West Virginia Department of Natural Resources, Water Resources Division, Quality Assurance Office, 1201 Greenbrier Street, Charleston, WV 25311, 304-348-0478;

2. Have the form certified to by the agency having primary enforcement responsibility or delegated administrative responsibility; and

3. Return the form with the proper fee to the Quality Assurance Office of West Virginia.

(b) The Quality Assurance Office shall review the application and if it finds it complete and the appropriate fee has been paid, shall assign or reassign the laboratory a certification number to be used in all correspondence with the Department.

(c) The receipt of the certification number authorizes the laboratory to perform analyses for the category and the parameters within the category for which it has requested certification for the Acts and Regulations covered by this chapter.

(d) If the laboratory's certification is revoked by the agency having primary enforcement responsibility or delegated administrative responsibility, the West Virginia authorization is thereby automatically cancelled. The laboratory manager shall notify the Quality Assurance Office and all clients in West Virginia that utilize the laboratory of the revocation within 48 hours of receipt of notice of revocation.

(e) The owner of a laboratory in a state other than West Virginia which is not certified by the state or is certified under conditions less stringent than those required by this chapter and who wishes to perform analyses in any or all of the

categories described in section 2.2 for the Acts and Regulations covered by this chapter shall apply for interim approval or certification in accordance with the procedure set forth in section 2.3. In addition, prior to conducting the on-site laboratory inspection, the laboratory shall submit to the Quality Assurance Office as an additional fee the sum the Department determines to be sufficient to cover the travel, room and board expenses of the certification inspections.

2.5 Renewal of Certification

Applications for renewals of certification shall be submitted no later than 120 days before the expiration date of either interim approval or certification and shall be on the forms provided therefor and shall be accompanied by the appropriate fee.

2.6 Fees

(a) Owner of laboratories applying for certification or renewal of certification, for the certification year shall submit the appropriate fee obtained from the annual schedule below along with the required application materials. Fees are nonrefundable. Laboratories owned or operated by the State of West Virginia or an agency of the Federal Government are exempt from this fee requirement, but shall make appropriate application for certification in accordance with the other provisions of these regulations.

Environmental Laboratory Certification Annual Fee Schedule:

	FEES
Any one of the following chemistry categories: Limited chemistry, atomic absorption, Gas Chromatography and/or Mass Spectrometry	$ 500.00
Any two of the above mentioned chemistry categories	600.00
All three of the above mentioned chemistry categories	700.00
Any one or two radiological parameters	250.00 and 50 per any additional parameter
Solid and Hazardous Waste Testing	500.00
Bioassay Testing	500.00
Microbiological Testing	500.00

(b) This section is also applicable to interim approved laboratories.

(c) All application fees collected under these regulations shall be paid into the state treasury into a special fund

designated "The Water Resources Management Fund" for defraying the cost of administering these regulations.

2.7 Required Laboratory Personnel Qualifications

(a) Every certified laboratory and laboratories seeking certification shall have sufficient properly qualified personnel commensurate with the workload and types of tests or analyses required to be performed for the parameters for which the laboratory is certified, or is seeking certification, pursuant to this chapter.

1. One individual shall be designated as the person in responsible charge and irrespective of any local title or designation, is herein referred to as the laboratory director.

2. The laboratory shall have one or more supervisors who shall be qualified in accordance with the provisions of 2.7 (d) below to perform the tests or analyses required to be performed within the category or categories for which the laboratory is certified, or seeks certifications. The laboratory director may also serve as laboratory supervisor, depending upon the size and functions of the laboratory, provided that the laboratory director meets the qualifications for laboratory supervisor.

3. The laboratory shall have a sufficient number of laboratory technical personnel commensurate with the volume and diversity of the tests performed.

(b) Current employee records shall be maintained, which shall include a resume' documenting each employee's training, degrees held, experience, duties, and date or dates of relevant employment.

(c) Work assignments shall be consistent with qualifications.

1. The following tests require the analyst to have at least a bachelor's degree in chemistry or biological science as appropriate: Arsenic (colorimetric), Total Kjeldahl Nitrogen, Ammonia, Phosphorus, Phenol, Oil and Grease, Fluoride, Cyanide, Coliform, Group Bacteria, Total Organic Carbon, Metals by AA, Organics by GC and Hazardous Waste characteristics.

2. If a laboratory performs tests or analyses utilizing gas chromatography/mass spectrometry (GC/MS), the GC/MS operator shall:

1. Meet the requirements of 2.7 (d) 5; and

2. Have completed a formal training course in GC/MS; and

3. Have six months experience in the operation of GC/MS equipment.

(d) The laboratory supervisor shall possess the qualifications for the category which he or she supervises.

1. If the laboratory performs tests in the category of Microbiological testing, the supervisor shall hold at least a bachelor's degree in a biological science or chemistry from an accredited institution with at least three credits in bacteriology and, subsequent to graduation, shall have had at least one year of laboratory training or experience in bacteriology.

2. If a laboratory performs tests or analysis in the category of limited chemistry for chlorine residual, pH, temperature, turbidity, or settleable solids, the supervisor shall have had at least one year of laboratory training or experience in chemistry.

3. If the laboratory performs tests or analyses in the categories of solid and hazardous waste or limited chemistry for the parameters other than chlorine residual, pH, temperature, turbidity or settleable solids, the supervisor shall hold at least a bachelor's degree in chemistry or in a biological science from an accredited institution and, subsequent to graduation, shall have had at least one year of laboratory training or experience in chemistry.

4. If the laboratory performs tests or analyses in the category of Atomic Absorption the supervisor shall:

   i. Hold at least a bachelor's degree from an accredited institution either in chemistry or in a biological science; and

   ii. Have, subsequent to graduation, at least one year of laboratory training or experience in chemistry; and

   iii. Have either six months experience in the operation of atomic absorption equipment or have completed a formal training course in the operation of atomic absorption equipment; and

iv. Demonstrate competence in the operation of atomic absorption equipment and analytical procedures during an inspection by a representative of the Department.

5. If they perform tests or analyses in the category of Gas Chromatography and/or Mass Spectrometry, supervisor shall:

i. Hold at least a bachelor's degree from an accredited institution either in chemistry or in a biological science; and

ii. Have, subsequent to graduation, at least one year of laboratory training or experience in chemistry; and

iii. Have either six months experience in the operation of gas chromatography equipment and/or mass spectrometry equipment as appropriate or have completed a formal training course in the operation of gas chromatography or mass spectrometry equipment; and

iv. Demonstrate competency in the operation of gas chromatography equipment and/or mass spectrometry equipment, as appropriate and analytical procedures during an inspection by a representative of the Department.

6. If the laboratory performs tests or analyses in the category of Radiological Testing, the Supervisor shall:

i. Hold at least a bachelor's degree from an accredited institution in chemistry, radiochemistry, radioisotope technology, biology, physics, or any of the applied sciences; and

ii. Have, subsequent to graduation, at least five years laboratory training or experience in any of the above, two years of which shall be in low-level radiation measurements and radiochemical procedures being considered for certification; and

iii. Demonstrate competency in the operation of radiological equipment and radiological procedures during an inspection by a representative of the Department.

7. If the laboratory performs tests in the category of Bioassay, the supervisor shall:

i. Hold at least a bachelor's degree from an accredited institution in a biological science or chemistry which shall include two courses in any of the following subjects:

(1) General Zoology

(2) Biological Methods and Experimental Design

(3) Icthyology

(4) Comparative Physiology

(5) Environmental Science; and

ii. Have, subsequent to graduation, at least one year of laboratory training or experience in the bioassay procedure being considered for certification; or

(1) A masters degree from an accredited institution in an environmental science may be substituted for the one year of laboratory training or experience requirement, as specified in 7.ii above, provided the applicant can demonstrate competency in the operation of bioassay equipment and methodologies by having successfully completed at least six definitive bioassays prior to applying for supervisor. Competency in test organism handling, sample handling/preservation, test and date methodologies must be documented. This documentation shall be available during inspection by a representative of the Department; and

iii. Demonstrate competency in the operation of bioassay equipment and methodologies during an inspection by a representative of the Department.

8. If a laboratory performs tests or analyses in the category of Solid and Hazardous Waste Testing, the supervisor shall:

i. Hold at least a bachelor's degree from an accredited institution in chemistry; and

ii. Have either six months experience in the operation of equipment used for determining characteristics or have completed a course in the use and operation of such equipment; and

iii. Meet the requirements of the other categories in this section when the equipment in other categories is used in performing solid and hazardous waste determinations; and

iv. Demonstrate competence in the operation of all equipment and analytical procedures applicable to solid and hazardous waste testing during an inspection by a representative of the Department.

9. During the phase-in period and for laboratories that receive interim status approval the supervisor shall;

i. Have had one year of pertinent laboratory experience working in a laboratory performing compliance analyses in a category or categories covered by this chapter; and

ii. Demonstrate the ability of complying with the testing, analytical, and quality control requirements contained in the chapter.

(e) For those municipal laboratories who employ personnel that do not possess the minimum education and experience requirements established by these regulations, the laboratory may become certified if: (1) the laboratory director and supervisor have been certified as having received instruction and education pertaining to laboratory operations and (2) individual analysts have been certified as competent to run the specific discharge parameters commonly required of municipal treatment facilities and that the individuals certification training included standard principles, techniques, and instruction on uses of chemicals and equipment as well as on the possible irregularities one might encounter in both pre and post analysis. Such non- State individual certification programs must be formally recognized, by the Chief of the Water Resources Division of the DNR. The analyst certification along with meeting all other program certification requirements will be grounds for awarding certification to the laboratory. If certified personnel are employed then the laboratory director must make and have written arrangements on file for obtaining the advice of a chemist.

(f) Experience in a certified laboratory which was gained prior to acquiring a bachelors degree may be substituted on an equivalency basis of one year of such experience for every one year of post-degree training and experience required.

2.8 Duties and Responsibilities of Laboratory Personnel

(a) Laboratory directors shall have the following responsibilities:

1. The laboratory director shall serve the laboratory on either a full time or a regular part time basis, and shall administer the operations of the laboratory including the reporting of tests and analyses. The director shall be readily available for personal or telephone consultation, and, if the director is to be absent, the director shall arrange for a substitute. Where the director is acting as laboratory supervisor the substitute shall meet the requirements of section 2.7 (d).

2. The laboratory director shall be responsible for the employment of an adequate number of qualified personnel, commensurate with the workload of the laboratory and the diversity of tests or analyses performed, and for the inservice training of such personnel.

3. The laboratory director shall report the discovery of an analytical error to the Quality Assurance Office and the person requesting an analysis within 72 hours of discovering the error if the error may effect the validity of a reported analytical result.

(b) Laboratory supervisors shall have the following responsibilities:

1. Each laboratory supervisor shall provide personal and direct supervision of technical personnel and the reporting of tests and analyses within the category or categories for which the supervisor is qualified.

2. Each laboratory supervisor shall be able to perform tests or analysis within the category or categories for which the lab is qualified.

3. Each laboratory supervisor shall be held responsible by the Department for the proper performance of all laboratory procedures, test and analyses, within the category or categories for which he is certified.

2.9 Management of Laboratories

(a) A certified laboratory may offer as a service those laboratory tests, analyses, or procedures that are within the category or categories for which it is certified provided it has a qualified supervisor in accordance with the provisions of section 2.7 (d) and for which adequate personnel,

equipment and facilities are available.

(b) A laboratory that is certified shall accept only samples which are properly labeled, and for which there is assurance that the samples have been collected, preserved, processed, stored, and transported in such a manner as to assure identity and the stability of the sample with respect to the requested tests or analyses; or if the stability of the sample has not been assured the laboratory shall refuse the sample.

(c) This section is also applicable to laboratories holding interim approvals.

2.10 Proficiency Testing

(a) Except when determined by the Quality Assurance Office that an appropriate performance evaluation test is not readily available, all certified laboratories or laboratories seeking certification shall participate in a performance evaluation testing program covering all tests, analyses and analytical methods as made available within the category and categories in which the laboratory is certified or seeks certification.

1. In the Gas Chromatograph and/or Mass Spectrometry categories, a laboratory's performance on a specific analytical method may be determined by the ability of the laboratory to acceptably analyze at least four certification parameters during a performance evaluation test.

(b) Appropriate samples shall be distributed by the Quality Assurance Office or its designee to such laboratories at such times and frequencies as designated by the Quality Assurance Office.

(c) Laboratories certified, or seeking certification shall:

1. Receive, examine and analyze such samples; and

2. Maintain records of such performance evaluation testing results; and

3. For all categories except radiological testing, submit the results for such testing within 30 days from the date of receipt of the performance evaluation samples, to the Quality Assurance Office for evaluation; or

4. For radiological performance evaluation testing, submit results in accordance with the directions of the USEPA.

(d) The laboratory shall be informed of the results of such evaluation and if the laboratory has not analyzed the performance evaluation samples acceptably, the Quality Assurance Office may require the laboratory to analyze additional performance evaluation samples.

(e) The results shall be considered by the Quality Assurance Office when making recommendations for improvement in laboratory procedures, and in evaluating whether the certification of the laboratory should be granted, denied, revoked, or suspended.

(f) Results of performance evaluation testing during the preceding twelve months shall be made available by the laboratory, upon request, to any person utilizing or requesting the services of the laboratory.

(g) Certified laboratories that desire to extend the range of tests or analyses offered shall submit a written request, comply with the requirements of section 2.3 or 2.4, as appropriate, and shall demonstrate satisfactory results in at least one round of performance evaluation sample testing prior to the inclusion of this test or analysis in the list of tests or analyses for which proficiency has been established.

2.11 Laboratory Inspections

(a) As a condition of obtaining and maintaining certification, a laboratory shall permit and facilitate inspections by personnel of the Department.

(b) The Department shall conduct at least one on-site inspection of a laboratory seeking certification in any parameter to determine whether or not the laboratory meets the Quality Assurance Office standards, as set forth in this chapter, for performing analyses for that parameter. The on-site inspection shall be performed prior to making a decision concerning the requested certification.

(c) Regular inspections of laboratories certified in accordance with this chapter shall be conducted during reasonable hours at intervals of not more than two years. Such inspections shall be conducted by representatives of the Department upon presentation of credentials. Laboratories that have moved to a new location shall comply with section 2.14 and shall be inspected by the Department after notification has been received by the Quality Assurance Office of such changes of location.

(d) Authorized representatives of the Department may make an announced or unannounced inspection or examination of a certified or an interim approved laboratory whenever the Department in its discretion considers such an inspection or examination necessary to determine the extent of the laboratories compliance with the condition of its certification and these regulations. Any refusal to allow entry to the Department's representative shall constitute a violation of a condition of certification and grounds for revocation of certification.

(e) During inspections, consideration will be given to competence and attitude of staff; working conditions, including adequacy of space; lighting, equipment and supplies, efficient organization of the laboratory; testing or analytical methods used; quality control procedures and practices; maintenance of all required records; and compliance with the requirements of this chapter.

(f) Following inspections, laboratories shall be furnished with inspection reports which shall list deficiencies found, and a listing of the parameters for which the laboratories have demonstrated proficiency during inspections, such inspection reports and listings shall be deemed public information or records, and shall be made available to any person utilizing or requesting the services of the laboratory.

(g) Whenever deviations from the requirements of this chapter are found, the laboratory shall be afforded not less than fifteen days, nor more than thirty days from the date the inspection report is mailed to the laboratory in which to correct such deficiencies. If deficiencies affecting the accuracy of results are found, the certification shall be immediately suspended or revoked, in accordance with the provisions of section 2.12.

2.12 Cancellation, Suspension, and Revocation of Certification

(a) Any certified laboratory may cancel its certification in any category or parameter by notifying the Quality Assurance Office in writing of the laboratory's decision to cancel its certification. The laboratory shall enclose its Environmental Laboratory Certification and License with the letter of notification. This cancellation notification shall not entitle the laboratory to any refund of its certification fee.

(b) The Quality Assurance Office may temporarily suspend a laboratories certification in any or all categories or in any

parameter when the laboratory fails to fully meet the standards of this chapter and the failure does not merit immediate decertification action. The Quality Assurance Office shall notify the laboratory by letter of its suspension and the reason therefor. Suspensions may be invoked for, but are not limited to, the following reasons:

1. Failure to submit in a timely manner a complete renewal application or the appropriate fee;

2. Failure to submit a timely or acceptable response to the laboratory evaluation report;

3. For all categories except Radiological Testing, failure to submit results of performance evaluation samples within 45 days of receipt of such proficiency samples;

4. For the Radiological category, failure to submit results of performance evaluation samples to the USEPA in a timely manner;

5. For all categories except Radiological, Gas Chromatography and/or Mass Spectrometry Testing, failure to acceptably analyze both the high and low values for any one parameter during a performance evaluation test shall be grounds for suspension in the parameter;

6. For the Radiological category, failing to acceptably analyze two performance test average values for any one parameter during any consecutive twelve month period shall be grounds for suspension in the parameter;

7. For the Gas Chromatography and/or Mass Spectrometry categories, failing to acceptably analyze both the high and low values for any one parameter during a performance evaluation test shall be grounds for suspension in all parameters covered by those chromatography and/or mass spectrometry methods.

(c) Certification may be revoked for due cause, including, but not limited to:

1. Violation of a condition of the Certification;

2. Violation of a statute, regulation, or order of the Department;

3. Misrepresentations made to the Department;

4. Demonstrable nonconformance with the requirements of this chapter;

5. Substantial changes in personnel, facilities or techniques without disclosure thereof to the Quality Assurance Office;

6. Nonpayment of applicable fee;

7. For all categories except Radiological Testing, failure to analyze a set of performance evaluation samples within 45 days of the receipt of such proficiency samples;

8. For the Radiological category, failure to submit results of performance evaluation samples to the USEPA in a timely manner;

9. For all categories except Radiological, Gas Chromatography and/or Mass Spectrometry Testing, failing to acceptably analyze both the high and low values for any one parameter during a performance evaluation test shall be grounds for decertification in the parameter;

10. For the Radiological Category, failing to acceptably analyze two performance test average values for any one parameter during any consecutive twelve month period shall be grounds for decertification in the parameter; or

11. For the Gas Chromatography and/or Mass Spectrometry category, failing to acceptably analyze both the high and low values for any one parameter during a performance evaluation test shall be grounds for decertification in all parameters covered by those gas chromatography and/or mass spectrometry methods; or

12. Performing and charging for additional tests or analyses that have not been requested by the customer, falsifying analyses, or engaging in other unethical or fraudulent practices.

(d) Interim approval may be revoked for due cause with right to a hearing thereon.

2.13 Effect and Duration of Suspension Notification and Revocation

(a) The results of any tests or analyses performed after issuance of a suspension notification or revocation for any category or parameter suspended or revoked shall not be accepted by the Department as an indication of the compliance status with the requirements of the Acts and Regulations covered by this chapter.

(b) Suspension shall not be withdrawn until all basis for the suspension have been eliminated or rectified.

(c) Revocations shall provide that reapplication for certification shall not be considered until all basis for revocation have been eliminated or rectified.

2.14 Notice of Changes

In the event there are any changes in the name, location, ownership, post office address, telephone number or personnel of a laboratory to which the provisions of this chapter apply, then the laboratory shall immediately submit written notice thereof to the Department of Natural Resources, Division of Water Resources, Quality Assurance Office, 1201 Greenbrier Street, Charleston, West Virginia 25311. In the case of change in supervisor(s) the qualifications of the new supervisor(s) showing compliance with the requirements of section 2.7 (d) shall be furnished. In the case of change in laboratory analysts both the laboratory and the analyst must notify the Quality Assurance Office of the change (ie: performing different analysis or leaving employment of the laboratory).

Section 3 Criteria and Procedures for Microbiological Testing and Analysis

3.1 Scope

This section establishes the Department's requirements which a certified laboratory or a laboratory seeking certification shall continually meet and follow when performing microbiological analyses.

3.2 Laboratory Facilities and Safety

(a) Laboratory space and facilities shall be adequate to properly carry out the services performed in, or offered by, the laboratory.

(b) Laboratory work areas shall be arranged so as to minimize problems in transportation and communication.

(c) Workbench space within the laboratory shall be ample for the tests or analyses to be performed, and shall be well-lighted and convenient to a sink, and such water, gas, suction and electrical outlets as are necessary to properly carry out the specific tests or analyses performed in the laboratory.

(d) The temperature and humidity within the laboratory shall be maintained within the limits required for the proper performance of each test or analysis and for the proper operation of instruments which may be affected by temperature variations.

(e) Each laboratory shall have available to it facilities, equipment, and instruments, including but not limited to water baths, incubators, sterilizers, and refrigerators, which shall be adequate to properly perform the tests and analyses for the parameters within this category for which the laboratory is certified or is seeking certification.

(f) Adequate fire precautions shall be taken, including but not limited to having readily available a fire extinguisher rated for the types of fires that reasonably may be foreseen given the types of tests and analyses performed by the laboratory.

(g) Appropriate occupational safety and health laws shall be posted and observed.

3.3 Laboratory Equipment, Supplies and Materials

(a) Laboratories performing microbiological tests and analyses shall have on the premises and under the control of the laboratory supervisor the equipment and instruments listed in this section necessary for the preparation of the specific media and analysis of the samples for which the laboratory is seeking certification or is certified. Such instruments, when required, shall meet the following specifications:

1. The pH meters shall have an accuracy of and scale graduations within ± 0.1 unit.

2. Top-loader or pan balances shall meet the following requirements:

   i. Balances shall be clean, not corroded, and shall be provided with appropriate weights of good quality; and

   ii. Balances shall tare out and detect a weight of 100 mg when used for general media preparation.

3. Temperature-monitoring devices shall meet the following requirements:

   i. Glass or metal thermometers shall be graduated in 0.5 degrees centigrade increments for all analyses except fecal coliform analysis; in which case glass or metal thermometers shall be graduated in 0.2 degrees centigrade increments;

   ii. Continuous temperature recording devices shall be sensitive and accurate to within 1.0 degrees centigrade;

   iii. The column of liquid in glass thermometers shall have no separation; and

   iv. A certified thermometer shall be available for use by the laboratory.

4. Air or water-jacketed incubators, aluminum block incubators, incubator rooms, and water baths shall meet the following requirements:

   i. Incubators, incubator rooms, and water baths shall be of sufficient size to accomodate periods of peak work load;

ii. Incubators and water baths must maintain internal temperatures of 35.0 ± 0.5 degrees centigrade for total coliform and fecal streptococci analysis, and 44.5 ± 0.2 degrees centigrade for fecal coliform analysis, in the area of use at maximum loading;

5. The autoclave shall meet the following requirements:

i. It shall be in good operating condition when observed during its operational cycle or when time-temperature charts are read, and, for most efficient operation, use of a double-walled autoclave constructed of stainless steel is suggested;

ii. It shall have pressure and temperature gauges on the exhaust side, and shall have a safety-valve that is in good operating condition;

iii. The requirement for a separate pressure gauge shall be waived provided the laboratory has documentation from the manufacturer of the autoclave certifying that the equipment will operate safely without a pressure gauge.

iv. It shall reach the sterilization temperature of 121 degrees centigrade and pressure of 1.1 lb/cm (15 psi) and shall maintain that temperature and pressure throughout the sterilization cycle. The sterilization cycle shall be for 15 minutes or 15 minutes per liter of sample, whichever is longer, but no more than 45 minutes in any case;

v. During depressurization the autoclave shall not produce air bubbles in the fermentation media.

6. The hot air oven shall meet the following requirements:

i. The hot air oven shall be constructed in a manner which shall ensure a stable sterilization temperature;

ii. Use of the hot air oven is recommended for sterilization of glass pipets, bottles, flasks, culture dishes, and other laboratory glassware and utensils; and

iii. A calibrated thermometer in at least 10 degrees

centigrade increments with its bulb placed in sand shall be placed on one of the shelves in use within the hot air oven.

7. The refrigerator shall maintain an internal temperature of 1 degrees to 4.4 degree centigrade (34 degrees to 40 degrees Farenheit).

8. Laboratories shall have available the following optical, counting, and lighting equipment:

   i. At least one low power magnification device, preferably a binocular microscope with 10 to 15X magnification, for use in counting fecal coliform and fecal streptococci MF colonies;

   ii. At least one low power magnification device, preferably a binocular microscope with 10 to 15X magnification, with a fluorescent light source for use in counting total coliform MF colonies; and

   iii. A mechanical hand tally for use in counting bacteria colonies.

9. Inoculation equipment shall meet the following requirements:

   i. The diameter of inoculation loops shall be at least 3 mm and the loops shall be constructed of 24 to 26 gauge Nichrome, chromel, or platinum-iridium wire;

   ii. Either single-service metal inoculation loops, pre-sterilized plastic inoculation loops, or reusable metal inoculation loops shall be used; and

   iii. Disposable dry-heat-sterilized hardwood applicator sticks may be used.

10. Membrane filtration (MF) equipment shall meet the following requirements:

    i. Units used in MF procedures shall be made of stainless steel, glass, or autoclavable plastic;

    ii. MF equipment shall not leak and shall not be corroded; and

iii. Field equipment may be used for coliform detection; however, standard laboratory MF procedures must be followed when using field equipment.

11. Membrane filters and pads shall meet the following requirements:

i. Membrane filters shall be manufactured from cellulose ester materials, and shall be white, grid-marked, and have a 47-mm diameter and 0.45 um pore size; however, another pore size may be used when the performance data provided by the manufacturer show the performance of that pore size to be equal to or better than the performance of the 0.45 um membrane filter; and

ii. Membrane filters and pads shall be either autoclavable or presterilized.

12. Laboratory glassware, plastic ware, and metal utensils shall meet the following requirements:

i. Glassware and metal utensils shall be resistant to the effects of corrosion, high temperatures, and vigorous cleaning operations;

ii. Flasks, beakers, dilution bottles, culture dishes, culture tubes, and other glassware shall be of borosilicate glass and free of chips, cracks, and excessive etching;

iii. Volumetric glassware should be Class A and need not be calibrated before use;

iv. Plastic items shall be of clear, inert, nontoxic materials and shall retain accurate calibration marks after repeated autoclaving; and

v. It is recommended that metal utensils made of stainless steel be used.

13. Sample bottles shall meet the following requirements:

i. Either wide-mouthed hard glass and stoppered sample bottles, or plastic sample bottles with screw caps, shall be used, and all sample bottles shall have a capacity of at least 120 mL.;

ii. Glass-stoppered bottles shall be stored so that they are protected from contamination by dust and the caps shall be covered with either aluminum foil or kraft paper;

iii. Screw caps shall have leakproof nontoxic liners which are capable of withstanding repeated sterilizations, at temperatures of 121 degrees centigrade sustained for 30 minutes per sterilization;

iv. Sterile sample bottles shall contain 10 mg. of dechlorinating agent per 100 mL. of sample; and

v. Presterilized plastic bags containing 10 mg. of sodium thiosulfate may be used for collecting samples for coliform analyses.

14. Pipets shall meet the following requirements:

i. Sterile, glass or plastic pipets shall be used for measuring quantities of 10 mL or less;

ii. Glass pipets shall be made of borosilicate glass;

iii. Pipets shall deliver the required volume quickly and accurately within a 2.5 percent tolerance;

iv. Pipets shall not be excessively etched, mouthpiece or delivery tips shall not be chipped, and graduation marks shall be legible.

15. Pipet containers shall meet the following requirements:

i. Open packets of disposable sterile pipets shall be resealed after each use; and

ii. Pipet containers shall be made of aluminum or stainless steel.

16. Culture dishes shall meet the following requirements:

i. Sterile plastic culture dishes with tight or loose lids, or glass culture dishes with loose lids shall be used; and

ii. When culture dishes with loose lids are used,

the relative humidity in the incubator shall not be less than 90 percent.

17. Culture dish containers shall meet the following requirements:

    i. Culture dish containers shall be made of either aluminum or stainless steel, or the culture dishes may be wrapped in heavy aluminum foil or char-resistant paper; and

    ii. Open packs of disposable sterile culture dishes shall be resealed after each use.

18. Culture tubes and closures shall meet the following requirements:

    i. Culture tubes shall be made of borosilicate glass or other corrosion resistant glass and shall be of a sufficient size to contain both the culture medium and the sample portions to be tested, without being more than three-quarters full; furthermore, it is recommended that the fermentation vial be 10 mm x 75 mm and extend above the medium; and

    ii. Caps should be made of snug-fitting stainless steel or plastic; however, loose-fitting aluminum caps or screw caps are also acceptable.

3.4 Sample Collection, Handling, and Preservation

(a) The sample volume shall be at least 100 mL. The sample bottle shall be filled only to the shoulder.

(b) Sample bottles shall have a capacity of at least 120 mL and shall be made of either sterile plastic or hard glass, and shall be wide mouthed with either a plastic screw cap or glass stopper. Sample bottles shall be capable of withstanding repeated sterilization. Ten milligrams of sodium thiosulfate per one hundred mL of sample shall be added to all sample bottles during preparation of the bottles.

(c) The sample report form shall be completed immediately after collection and shall state at a minimum the sampling location, date and time of collection, chlorine residual, collectors name, and any remarks.

(d) The following chain of custody procedures shall be employed in collecting and handling samples and the information shall be reported on the sample report form or a chain of custody form.

1. Sterilized and decontaminated containers shall be used for sampling.

2. Tie-on or affixed labels with an identification number shall be used for labeling all samples.

3. After the sample has been collected, the appropriate information as to the identity of the sample shall be written on the label. If the label has been removed it shall be reaffixed before removing the label from any other container.

4. After collecting the sample, the label shall remain affixed to the sample container and shall not be removed until the required analyses have been completed and the surplus sample has been discarded.

5. Immediately upon delivery of the sample to the laboratory, the sample collector shall complete the appropriate chain-of-custody section of the sample report form or chain of custody form.

6. The chain-of-custody information reported on the sample report form or the chain of custody form shall list at a minimum the following information:

   i. Sample number;

   ii. Description of samples;

   iii. Specific location of sample collection;

   iv. Identity of person collecting the sample;

   v. Date and time of sample collection;

   vi. Date and time of custody transfer to laboratory;

   vii. Identity of person accepting custody;

   viii. Date and time of initiation of analyses;

   ix. Identity of person performing analyses;

   x. Name of laboratory performing the analyses;

7. Prior to accepting custody of the sample, the laboratory personnel who will accept the sample shall be reasonably assured that the sample has met the collection, handling and preservation requirements. If the sample fails to meet those requirements, the chain of custody section of the sample report form or the chain of custody form shall so indicate and the sample shall be rejected.

8. The laboratory personnel accepting responsibility for the sample shall sign the form containing the chain of custody information.

9. When it is necessary to send samples by mail, bus, courier service, or private shipping, the chain of custody form shall be completed by the sampler prior to the shipping of the sample and shall accompany the sample during shipping. Upon receipt of the sample in the laboratory steps (d)6 thru 8 above shall be followed.

(e) The holding time between sample collection and analysis shall not exceed the specified parameter time. Samples that fail to meet this holding time shall be rejected and a new sample requested.

(f) Fecal coliform samples collected for WVPDES compliance analysis shall be analyzed within the holding time recommended in 40 CFR 136. Samples that fail to meet this holding time shall be rejected and a new sample requested.

(g) WVPDES samples that cannot be analyzed within one hour following collection shall be stored in iced coolers during transit to the laboratory and refrigerated upon delivery until such analyses can be performed.

(h) A laboratory that has received either certification or interim approval shall accept only samples that are properly labeled and for which assurance is given that the samples have been collected, preserved, processed, stored and transported in a manner that will assure both the identity of the sample and that the sample is sufficiently stable to be used in the requested tests or analyses.

3.5 Methodology

(a) Test procedures required by 40 CFR 136 shall be utilized for the analysis of WVPDES parameters.

(b) All procedures other than those set forth in subsection (a) above are considered alternative analytical techniques as described in 40 CFR 136.4. Laboratories shall make special application to the Department for the use of alternative

analytical methods and such application shall include a showing of acceptable comparability data.

(c) All laboratories which have previously been granted approval to use an alternate analytical method by the USEPA shall be allowed to continue using such method after it submits written proof of the approval to the Department.

(d) The membrane filter (MF) procedure used for Water Analysis should show good colony development over the entire surface. The golden green metallic sheen colonies shall be counted and recorded as the total coliform density per 100 mL of water sample. The following rules for reporting any problem with MF results shall be followed:

1. In the case of total coliform analysis, if there is confluent growth, with or without discrete sheet colonies, covering the entire filtration area of the membrane, the results shall be reported as "confluent growth per 100 mL, with (or without) total coliforms", and a new sample shall be requested.

2. In the case of fecal coliform and fecal streptococci analysis, if there is confluent growth, with or without typical discrete colonies, covering the entire filtration area of the membrance, the results shall be reported as "confluent growth per 100 mL, with (or without) fecal coliforms (or fecal streptococci)", and a new sample shall be requested.

3. When the total number of bacterial colonies on the membrane is greater than 200 total colonies, or is not sufficiently distinct, or both, the results shall be reported as "Too numerous to count (TNTC) per 100 mL, with (or without) total coliforms, (or fecal coliform, or fecal streptococci)" and a new sample shall be requested.;

4. When both confluent growth and TNTC are present, a new sample shall be requested and, if the MF procedure is used, the sample volumes filtered shall be adjusted by increasing dilution of the sample; otherwise the most probable number (MPN) procedure shall be used.

5. If the laboratory has elected to use the MPN test on discharges that have a history of confluent growth or TNTC with the MF procedure, all presumptive tubes from the MPN test to check for the suppression of coliforms; the count shall be adjusted based upon confirmation and a new sample shall be requested. In addition, this procedure should be carried out on samples collected from

water supplies or discharges known to have such a history, at a frequency of at least once every three months.

3.6 General Laboratory Practices

(a) Laboratory sterilization procedures shall meet the following requirements:

1. The following times and temperatures shall be used for sterilization of materials by auto-claving:

Material	Temperature/Minimum Time
Membrane filter and pads	121 degrees centigrade/10 min.
Carboyhdrate-containing media (lauryl tryptose, brilliant green lactose bile broth, etc.)	121 degrees centigrade/12-15 min.
Contaminated materials and discarded tests	121 degrees centigrade/30 min.
Membrane filter assemblies (wrapped), sample collection bottles (empty), individual glassware items	121 degrees centigrade/15 min.
Rinse water volumes of 500 ml to 1,000 ml	121 degrees centigrade/15 min.
Rinse water in excess of 1,000 ml	121 degrees centigrade/time adjusted for volume; check for sterility
Dilution water blanks	121 degrees centigrade/15 min.

2. Membrane filter assemblies made of metal shall be autoclaved after each sample filtration series, the end of which is marked by the lapse of 30 minutes or more between sample filtrations;

3. Membrane filters assemblies made of glass or plastic may be sterilized by 2 minutes of exposure in an ultraviolet sterilizer unit, provided its use does not affect the validity of the results and the ultraviolet lamps are tested with a light meter and a spread plate irradiation test is performed quarterly.

4. At least two minutes of ultraviolet light or boiling

water may be used on a membrane filter assembly to prevent bacterial carry-over between filtrations; and

5. Dried glassware shall be sterilized in a hot air oven at 170 degrees centigrade for a minimum of two hours.

(b) Laboratory pure water, including distilled, deionized, or other processed waters, shall meet the following requirements:

1. An analyst shall either test the quality of the laboratory pure water or have the laboratory pure water tested by another State certified laboratory; and

2. Only laboratory pure water meeting the requirements set forth in the 16th Edition of Standard Methods shall be used in performing bacteriological analyses.

(c) Rinse water and dilution water used by the laboratory shall meet the following requirements:

1. Stock buffer solution shall be prepared in accordance with Standard Methods, 16th Edition or Microbiological Methods - EPA, using laboratory pure water adjusted to pH 7.2;

2. Stock buffer shall be either autoclaved or filter-sterilized, and must be labeled, dated, and stored at 1 degree to 4.4 degrees centigrade;

3. The stored buffer solution shall be free of turbidity; and

4. Rinse and dilution water shall be prepared by adding 1.25 mL of stock buffer solution and 5 ml of magnesium chloride solution per liter of laboratory pure water, and the final pH shall be 7.2 + 0.1.

(d) Media shall be prepared and stored in accordance with the following requirements:

1. Laboratories shall use commercial dehydrated media for routine bacteriological procedures;

2. All media shall be prepared according to the procedures for media preparation set out in Standard Methods, 16th Edition, or Microbiological Methods - EPA, however, lactose broth shall not be used.

3. Dehydrated media containers shall be kept tightly closed and stored in a cool, dry location, to prevent

discoloration and caking; laboratories shall not use discolored or caked dehydrated media;

4. Dissolution of the media using laboratory pure water shall be completed before dispensing to culture tubes or bottles;

5. The membrane filter broth and agar media shall be heated in a boiling water bath until completely dissolved;

6. MF broths shall be stored and refrigerated no longer than 96 hours and MF agar media shall be stored, refrigerated and used within two weeks;

7. MPN media prepared in tubes with loose-fitting caps shall be used within one week, but if MPN media are refrigerated after sterilization, they shall be incubated overnight at 35 degrees centigrade to confirm usability, and tubes showing growth or gas bubbles shall be discarded;

8. Media in screw cap containers may be held up to three months, provided that the media are stored in an enclosed area so that no light may enter and provided that evaporation does not exceed 0.5 mL per 10 mL total volume; in addition, commercially prepared liquid and agar media supplies may be used; and

9. Ampouled media shall be stored at 1 degree to 4.4 degrees centigrade (34 degrees to 40 degrees Farenheit), and storage time shall be limited to the manufacturer's expiration date.

(e) When measuring sample volumes of more than 10 mL, graduated cylinders or graduated membrane filter funnels having an accuracy within 2.5 percent tolerance shall be used.

3.7 Quality Control Program

(a) Each laboratory shall develop and have on file and available for inspection a written description of the current laboratory quality control program. Such written description shall outline the procedures which the laboratory will use in meeting the quality control requirements set forth in this section and section 3.4 and section 3.6. Management, supervisors, and analysts should participate in developing the quality control program. Each participant within the laboratory should have a copy of the quality control program and detailed guidelines for implementation of the participant's responsibility. A record of analytical control

tests and quality control checks on media, materials, and equipment shall be prepared by the laboratory and retained for at least five years.

1. Laboratories shall perform the following analytical quality control tests to ensure that general laboratory practices and methodology are in compliance with the requirements of this subchapter:

   i. When analyzing water samples, the laboratory shall verify at least five sheen or borderline sheen total coliform bacterial colonies from each membrane containing five or more such colonies. Bacteria counts shall be adjusted based on this verification. The verification procedure shall be conducted by transferring growth from the total coliform bacterial colonies into lauryl tryptose broth (hereinafter referred to as LTB) tubes and then transferring growth from gas-positive LTB cultures to brilliant green lactose bile (hereinafter referred to as BGLB) tubes. Colonies shall not be transferred exclusively to BGLB. However, colonies may be transferred to LTB and BGLB simultaneously. Negative LTB tubes shall be reincubated on the day following the verification procedure and shall be confirmed if gas is produced. It is recommended that laboratories verify all sheen colonies and borderline sheen colonies.

   ii. A start and finish MF sterile control test of rinse water, media and supplies shall be conducted for each sample filtration series. If the MF sterile control tests indicate contamination of rinse water, media, or supplies, then all data which has been generated through tests involving the use of the contaminated rinse water, media, or supplies shall be rejected and the laboratory shall request immediate resampling of those waters involved in the laboratory error.

   iii. The MPN test for water samples shall be carried to completion on 10 percent of positive confirmed samples except that gram staining shall not be performed; but, if no positive tubes result from the tested water samples, the complete MPN test, but not gram staining, shall be performed on at least one water source for which results have been positive;

   iv. Laboratory pure water shall be analyzed by the

test for bactericidal properties for distilled water as set forth in Standard Methods, 16th Edition, p. 835, or Microbiological Methods - EPA, p. 200. Only satisfactorily tested laboratory pure water is permissible in preparing media, reagents, rinse, and dilution water. If the tests do not meet the requirements for laboratory pure water set forth in subparagraph viii. below, then corrective action, including but not limited to purchasing a fresh supply of laboratory pure water or purification of the existing supply and source of laboratory pure water, shall be taken immediately and the water shall be retested;

v. Laboratory pure water shall be analyzed for conductance, pH, chlorine residual, and standard plate count. If the test results for any of the substances exceed the standards set forth in subparagraph viii. below, then corrective action, including but not limited to purchasing a fresh supply of laboratory pure water or purification of the existing supply and source of laboratory pure water, shall be taken and the water shall be retested;

vi. The laboratory shall ensure that laboratory pure water does not come in contact with heavy metals. Laboratory pure water shall be analyzed for trace metals, with particular emphasis upon analysis to detect Pb, Cd, Cr, Cu, Ni, and Zn. If the test results show that the laboratory pure water does not meet the requirements set forth in subparagraph viii. of this section, then corrective action, including but not limited to purchasing a fresh supply of laboratory pure water or purification of the existing supply and source of laboratory pure water, shall be taken and the water shall be retested;

ii. Standard plate count procedure shall be performed on laboratory pure water as described in Standard Methods, 16th Edition, or Microbiological Methods - EPA. Plates shall be incubated at 35.0 $\pm$ 0.5 degrees centigrade for 48 hours.

viii. Requirements and monitoring frequency for laboratory pure water:

QUALITY OF PURIFIED WATER USED IN MICROBIOLOGY TESTING

Test	Monitoring Frequency	Limit
Chemical tests:		
Conductivity	Continuously or with each use	>0.5 megohms resistance or <2 umhos/cm at 25 degree centigrade
pH	With each use	5.5 - 7.5
Total organic carbon	Monthly	<1.0 mg/L
Heavy metals, single (Cd, Cr, Cu, Ni, Pb, & Zn)	Monthly	<0.05 mg/L
Heavy metals, total	Monthly	<1.0 mg/L
Ammonia/organic nitrogen	Monthly	<0.1 mg/L
Total chlorine residual	Monthly or with each use	<detection limit
Bacteriological tests:		
Heterotrophic plate count (See Section 907)*	Monthly	<1000 colonies/mL
Water quality test (See 3c1)*	Annually and for a new source	0.8 - 3.0 ratio
Use test (see 3d)*	Annually and for a new source	Student's t $\leq$ 2.78

*Refers to 16th Edition of Standard Methods Manual.

ix. Each laboratory should analyze one quality control sample per year, when available from the Department, for the parameter or parameters for which the laboratory has received certification or interim approval;

x. Each laboratory shall satisfactorily analyze one unknown performance sample per year, when available, for the parameter or parameters within the category or categories for which the laboratory has received certification or interim approval;

xi. Duplicate analyses should be run on known positive samples at least once per month, and the duplicates should then be run as a split sample by more than one analyst, with each split constituting a 50 mL sample;

xii. In the case of laboratories having more than one

analyst, each analyst should count the total coliform sheen colonies on a membrane from a polluted water source at least once per month, colonies on the membrane should be verified, and the analysts' counts should be compared to the verified count;

xiii. There shall be available at all times, in the immediate area of laboratory personnel engaged in examining samples and performing related procedures within a category, current laboratory manuals or other complete written descriptions and instructions relating to:

(1) The analytical methods to be used by those personnel, properly designated and dated to reflect the most recent supervisory reviews;

(2) Pertinent current literature references; and

(3) Such written descriptions and instructions may be supplemented by, but not replaced by, textbooks relating to the particular analytical methods and procedures employed by such personnel;

xiv. Only the laboratory director or supervisor shall make changes in laboratory procedures and those changes shall only be effective when put in writing; and

xv. Laboratories shall maintain an acceptable quality control program covering each method or procedure for testing and analysis performed by the laboratory in order to verify and assess accuracy, measure precision, and detect errors in the results of such tests and analyses.

2. The following procedures shall be followed in performing quality control checks of laboratory media, equipment, and supplies:

i. Each pH Meter shall be cleaned immediately after each use period and calibrated prior to each use period using a minimum of two pH buffer standards and records of each calibration shall be maintained; buffer aliquots shall not be used more than once; and commercial buffer solutions shall be dated at the time of initial use; buffer standards should bracket the value to be measured;

ii. Top loader or pan balances shall be checked monthly against class "s" weights, and a record shall be made of each calibration check;

iii. The accuracy of all thermometers used to monitor temperatures shall be verified by comparing the readings of such thermometers with readings of a certified thermometer. A record shall be made containing the identification number of each thermometer, the temperatures displayed on the certified thermometer and the thermometer being verified, correction factors when applicable, dates on which the quality control checks were performed, and the name and of the analyst performing such checks. Glass thermometers shall be verified yearly and metal thermometers shall be verified quarterly.

iv. The temperature of air or water-jacketed incubators, water baths, and incubator rooms shall be either recorded continuously or recorded twice daily from in-place thermometers immersed in liquid and placed on the top and bottom shelves in use;

v. Date, time, and temperature shall be either recorded continuously or recorded individually during each sterilization cycle of the autoclave;

vi. Each hot air oven shall be equipped with a thermometer, the bulb of which shall be placed in sand, or with a temperature recording device, and records shall be maintained showing the date, time and temperature of each sterilization cycle;

vii. Laboratories shall use only membrane filters that have been recommended by the manufacturer for use in the analysis of water;

viii. The temperature of each refrigerator shall be either recorded continuously or recorded daily from an in-place thermometer immersed in liquid and placed on at least one of the shelves in use;

ix. Washing processes shall be adequate to provide clean glassware with no stains or spotting, and at the time of initial use of a detergent or washing product and whenever the brand or type of washing product is changed, the rinsing process shall demonstrate that the detergent or washing product provides glassware free of toxic material by the

inhibitory residue test as set forth in Standard Methods, 16th Edition, p. 853, or Microbiological Methods - EPA, p. 199;

(1) Test each batch of clean glassware for acid or alkaline residue by adding bromthymol blue indicator or representative items;

x. At least one sample bottle from each batch of sterilized sample bottles shall be checked by adding approximately 25 mL of sterile LTB to the bottle or bottles and then incubating the preparation at 35 degrees ± 0.5 degrees centigrade for 24 hours, at the end of which time the bottle or bottles shall be checked for growth;

xi. Service contracts or internal protocols approved by the Quality Assurance Office shall be maintained on balances, autoclave, water still, and any other equipment requiring periodic servicing, and records of actual servicing shall be entered in a log book;

xii. Records of preparation of each batch of sterilized media shall be made available for inspection and shall show the lot number of the batch, date of preparation, sterilization time and temperature, final pH of each batch, and the preparing technician's name;

xiii. Both positive and negative cultures should be used, and should be tested to determine recovery and performance compared to a previous acceptable lot of medium;

xiv. Media should be ordered on the basis of estimated needs for the next 12 month period. Bottles shall be dated upon receipt by the laboratory and upon initial opening. Except for large volume uses, media should be purchased in 1/4-lb bottles. Bottles of media should be used within six months after opening; however, in no case should opened media be used after one year. Opened bottles should be stored in a desiccator to extend storage time beyond six months. Shelf life of unopened bottles is two years;

xv. Testing should be carried out on membranes to determine recovery and performance as compared to a previously acceptable lot of membranes;

xvi. The lot number of packages of membrane filters and date of receipt by the laboratory shall be recorded;

xvii. Heat sensitive tapes, spore strips, or spore ampoules should be used during sterilization, and it is recommended that a maximum registering thermometer be used;

xviii. All reagents and solutions shall be labeled to indicate identity and, when applicable, strength or concentration, recommended storage requirements, preparation or expiration date, and other information pertinent to identification;

xix. Materials of substandard reactivity and deteriorated materials shall not be used; and

xx. All outdated material shall be discarded immediately.

3.8 Records and Data Reporting

(a) Each laboratory shall maintain records and report data in accordance with the requirements set forth in this section.

(b) Records of microbiological analysis shall be kept by the laboratory for not less than five years. This requirement is equally applicable to all raw data, quality control data, chain of custody forms and laboratory reports.

(c) The following information shall be kept by the laboratory as part of the records of all bacteriological analyses, although such information need not be included in the report to the person requesting the laboratory analysis unless otherwise required:

1. Laboratory number or other form of identification of the sample;

2. Date, time, and specific location of sampling, as well as the name of the person who collected the sample or the laboratory which submitted the sample;

3. Identification of the sample source.

4. The date and time of receipt of the sample by the laboratory; whether the sample, when received, was preserved or unpreserved;

5. The date and time of analysis of the sample;

6. The person or persons who performed analysis of the sample;

7. The type of analysis performed and the specific analytical method or methods employed;

8. The results of the analysis; and

9. The name and address of the laboratory to which the sample was forwarded, if the analysis was not performed at the laboratory which received the sample.

(d) If the chain of custody information is reported on the chain of custody form, a copy of the chain of custody form shall be attached to the sample report form.

(e) The results of each analysis shall be calculated and entered on the sample report form which is to be forwarded to the person requesting the analysis of the sample. A careful check shall be made to assure that each result entered on the bench sheet and once the check is completed the supervisor shall initial the bench sheet.

(f) The original or true duplicate of the results of the tests or analyses shall be sent promptly to the person who requested such tests or analyses, and shall be signed by the laboratory director or a designee whose designation is in writing and has been submitted to the Department.

(g) Whenever a certified or interimly approved laboratory refers samples to another laboratory for analyses, the person requesting the analyses or tests shall receive the original laboratory report or a true duplicate of that report on the form of the laboratory that performs the tests or analyses.

(h) All results shall be reported immediately to the person requesting the analyses.

(i) If results are entered into a computer storage system, a printout of the data should be verified with the raw data.

Section 4 Criteria and Procedures for Chemical Testing and Analysis

4.1 Scope

This section establishes the Department's requirements which a certified laboratory or a laboratory seeking certification shall continually meet and follow when performing chemical analyses.

4.2 Laboratory Facilities and Safety

(a) Laboratory space and facilities shall be adequate to properly carry out the services performed in, or offered by, the laboratory.

(b) Laboratory work areas shall be arranged so as to minimize problems in transportation and communication.

(c) Workbench space within the laboratory shall be ample for the tests or analyses to be performed, and shall be well-lighted and convenient to a sink, and such water, gas, suction and electrical outlets as are necessary to properly carry out the specific tests or analyses performed in, or offered by, the laboratory.

(d) The laboratory shall be adequately ventilated to exhaust the gases produced by the tests or analyses performed by and the types of materials handled by the laboratory.

(e) The temperature and humidity within the laboratory shall be maintained within limits required for the proper performance of each test or analysis and for the proper operation of instruments which may be affected by temperature variations.

(f) Volatile or corrosive chemicals and flammable solvents shall be stored in accordance with the Federal Occupational Safety and Health Act and attendant Regulations.

(g) Adequate fire precautions shall be taken, including but not limited to having readily available a fire extinguisher rated for the types of fires that may reasonably be foreseen given the types of testing and analyses performed by and the types of materials handled by the laboratory.

(h) Appropriate occupational safety and health laws shall be posted and observed.

4.3 Specifications for Laboratory Equipment and Instrumentation

(a) Laboratories which have received certification or are seeking certification to perform any of the required chemical analyses, shall have on the premises and under the control of the laboratory director, all of the equipment and instruments necessary to analyze each parameter in which the laboratory is certified, or is seeking certification and such equipment and instruments shall meet the following specifications:

1. Analytical balance shall meet and be operated in accordance with the following specifications:

   i. Each analytical balance shall have a sensitivity of 0.1 mg;

   ii. The analytical balance should be mounted on a heavy, shockproof table. The balance level should be checked frequently and shall be adjusted as necessary;

   iii. The analytical balance should be located in an area that is not near laboratory traffic and is protected from sudden drafts and humidity changes;

   iv. The balance temperature shall be equilibrated with room temperature;

   v. Special precautions should be taken to avoid spillage of corrosive chemicals on the pan or inside the balance case, and the interior of the balance housing should be kept scrupulously clean.

   vi. Two class "S" weights shall be available for checking the analytical balance.

2. The photometers shall meet and be operated in accordance with the following specifications:

   i. Spectrophotometers:

      (1) The maximum spectral bandwidth shall be no more than 20 nm;

      (2) Wavelength accuracy shall be within 0 $\pm$ 2.5 nm;

      (3) The spectrophotometer should be capable of using several sizes and shapes of absorption cells to provide a sample path from 1 to 5 cm.

      (4) All cells shall be kept clean and free of

scratches, fingerprints, smudges, and evaporated film residues; and

(5) An exterior, high-capacity, constant voltage transformer is recommended for general laboratory analyses;

ii. Filterphotometers:

(1) Isolation of relatively broad bands (10 to 75 nm) of this radiant energy is achieved by use of filters at or near the maximum absorption of the colorimetric methods; and

(2) Filterphotometers should be capable of using several sizes and shapes of absorption cells to provide a sample path from 1 to 5 cm.

3. The magnetic stirrer shall have variable speeds, and a Teflon coated stirring bar.

4. The pH meter shall meet and be operated in accordance with the following specifications:

i. The accuracy of the pH meter shall be within ± 0.05 units;

ii. The scale readability of the pH meter shall be ± 0.1 units;

iii. Both glass and calomel electrodes shall be rinsed well with laboratory pure water after each reading, and shall be either rinsed or dipped several times into the next sample to be tested before the final reading is taken;

iv. Weakly buffered samples should be stirred during measurement;

v. Glass electrodes should be either immersed in distilled water or stored according to the manufacturer's recommendations during periods of inactivity.

5. Specific ion meter shall be readable and accurate to ± 5 mv.

6. The atomic absorption spectrophotometer shall meet and be operated in accordance with the following requirements and minimum specifications:

i. The atomic absorption spectrophotometer shall be a single channel, single or double beam instrument having a grating monochromator, photomultiplier detector, adjustable slits, a wavelength range of 190 to 800 nm, and provisions for interfacing with either a strip chart recorder or a digital printout unit;

ii. The lamps of the spectrophotometer should be dated when first used;

iii. The pressure inside the acetylene tank should always be greater than 75 psi;

iv. Proper ventilation shall be maintained above the burner head;

v. A moisture trap should be incorporated into the flow system between the air source and the atomic absorption spectrophotometer itself;

7. Laboratories performing atomic absorption analysis shall have a digital print out or strip chart recorder for use with the atomic absorption spectrophotometer. The strip chart recorder shall have a chart width of 10 inches (25 cm), a full scale response time of 0.5 second or less, 10 or 100 mv input to match the instrument, and variable chart speeds of 5 to 50 cm/min, or an equivalent chart speed. A digital printout unit may be substituted for the recorder.

8. Laboratories performing gas chromatographic and/or mass spectrometry analysis:

i. Laboratories performing gas chromatographic analysis may use either a commercial or custom-designed gas chromatograph (with appropriate detectors), but in either case the gas chromatograph shall have a column oven capable of isothermal temperature control to within $\pm$ 0.2 degrees centigrade. The system should be equipped with accurate needle-valve gas flow controls and should accept 1/4 in. glass columns with the option of direct on-column injection.

ii. Laboratories performing mass spectrometry analysis - Reserved.

9. Laboratories performing gas chromatographic analysis shall have a strip chart recorder for use with the gas chromatograph. The strip chart recorder shall have at a

minimum a chart width of 10 in (25 cm), a full scale response time of one second or less, a 1 mv (-0.05 to 1.05) signal to match the instrument, and variable chart speeds of 5 to 50 cm/minute or an equivalent chart speed. A digital/integrator plotter may be substituted for the recorder.

10. The conductivity meter, suitable for checking laboratory pure water quality, shall be readable in ohms-cm or mhos/cm, have a range of 2 to 2,500,000 ohms-cm or equivalent mhos-cm (± 1 percent), and have a sensitivity of 0.33 percent or better. The conductivity meter should be equipped with platinum electrodes.

11. Gravity or mechanical convection drying ovens shall have a selectable temperature control ranging from room temperature to 180 degrees centigrade or higher. A long stem thermometer which has been calibrated against a certified thermometer shall be inserted through a center ceiling port, and the bulb of the thermometer shall be inserted into a cylinder filled with sand.

12. Desiccators with the appropriate indicator desiccant shall be either glass or plastic as appropriate to the particular task being performed.

13. Hot plates shall have selectable temperature controls.

14. For storage of aqueous reagents and samples, a standard household refrigerator may be used. However, for storage of organics and flammable materials, an explosion-proof refrigerator should be used. The refrigerator shall maintain an internal temperature of 1 degree to 4.4 degrees centigrade (34 degrees to 40 degrees Farenheit).

15. Laboratory glassware should be made of borosilicate glass that is resistant to damage by heat, chemicals, and repeated use. When applicable, Class A volumetric glassware shall be used and need not be calibrated before use. The following criteria and procedures apply to laboratory glassware:

    i. Unless otherwise specified, borosilicate bottles shall be used for the storage of reagents and standard solutions;

    ii. Polyethylene bottles may be used instead of borosilicate bottles for the storage of reagents and standard solutions;

    iii. Serological or Mohr-type pipets are not volumetric

pipets and shall not be used in tests or analyses requiring quantitative sample transfer and measurement;

16. The stirred water bath for nitrate analysis shall have a temperature range from ambient temperature to 100 degrees centigrade, the bath shall have a gable lid and it shall be stirred by a stirring device.

17. Temperature monitoring devices shall meet the following requirements:

    i. Glass or metal thermometers shall be graduated in 0.5 degree centigrade increments;

    ii. Continuous temperature-monitoring devices shall be sensitive to 0.5 degrees centigrade;

    iii. The liquid column of glass thermometers shall have no separation; and

    iv. Laboratories shall have available at least one certified thermometer.

4.4 Sample Collection, Handling and Preservation

(a) Sample collection, handling, and preservation techniques recommended in 40 CFR 136 shall be followed for WVPDES unless stated otherwise, samples requiring preservation shall be preserved at the time of collection.

(b) Sample collection, handling and preservation techniques found with the analytical methods shall be followed for the organic parameters analyzed by those methods.

(c) Additional information on sampling for pesticide and herbicide analysis may be found in the following publications:

1. "The Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples", USEPA, Health Effects Research Laboratory, Research Triangle Park, N.C. 27711, 1979 (hereinafter referred to as PEST);

2. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories", USEPA, EMSL, Cincinnati, Ohio 45268, EPA 600/4-79-1979 (hereinafter referred to as EPAQC); and

3. Standard Methods, 16th Edition.

(d) The sample report form shall be completed immediately after collection and shall state the sampling location, date and time of collection, collector's name, and any remarks.

(e) The following chain of custody procedures shall be employed in collecting and handling samples and the information shall be reported on the sample report form or a chain of custody form.

1. Decontaminated containers shall be used for sampling.

2. Tie-on or affixed labels with an identification number shall be used for labeling all samples.

3. After the sample has been collected, the appropriate information as to identity of the sample shall be written on the label. If the label has been removed, it shall be reaffixed before removing the label from any other container.

4. After collecting the sample, the label shall remain affixed to the sample container and shall not be removed until the required analyses have been completed and the surplus sample has been discarded.

5. Immediately upon delivery of the sample to the laboratory, the sample collector shall complete the appropriate chain of custody section of the sample report form or chain of custody form.

6. The chain of custody information reported on the sample report form or the chain of custody form shall list at a minimum the following information:

i. Sample number;

ii. Description of samples;

iii. Specific location of sample collection;

iv. Identity of person collecting the sample;

v. Date and time of sample collection;

vi. Date and time of custody transfer to laboratory (if the sample was collected by a person other than laboratory personnel);

vii. Identity of person accepting custody (if the

sample was collected by a person other than laboratory personnel);

viii. Date and time of initiation of analysis;

ix. Name of laboratory performing the analysis;

7. Prior to accepting custody of the sample, the laboratory personnel who will accept the sample shall be reasonably assured that the sample has met the preservation requirements. If the sample fails to meet those requirements, the chain of custody section of the sample report form or the chain of custody form shall so indicate and the sample shall be refused.

8. The laboratory personnel accepting responsibility for the sample as well as all other laboratory personnel performing analysis on that sample shall sign the form containing the chain of custody information.

9. When it is necessary to send samples by mail, bus, courier service, or private shipping, the chain of custody form shall be completed by the sampler prior to the shipping of the sample and shall accompany the sample during shipping. Upon receipt of the sample in the laboratory, steps (e) 6 thru 8 above shall be followed.

4.5 Methodology

(a) Test procedures identified in 40 CFR 136 shall be utilized for the analysis of National Pollutant Discharge Elimination System compliance monitoring parameters.

(b) All procedures other than those set forth in subsection (a) are considered alternative analytical methods as described in 40 CFR 136. Laboratories shall make special application to the Department for the use of alternative analytical methods and such application shall include a showing of acceptable comparability data.

(c) All laboratories which have previously been granted approval to use an alternate analytical method by the USEPA shall be allowed to continue using such method after it submits written proof of the approval to the Department.

4.6 General Laboratory Practices

(a) Laboratories utilizing visual comparison devices shall calibrate the standards incorporated into such devices at

least once every four months. The laboratory shall make and maintain records of the date and method of each such calibration. Directions for preparing temporary and permanent type visual standards are specified in the applicable sections of Standard Methods, 16th Edition. By comparing standards of known concentrations to the sealed, permanent visual standard and plotting the comparison on graph paper, a correction factor shall be derived, documented, and applied to all future results.

(b) Distilled and deionized water shall have at a minimum, resistivity values between 0.5 to 2.0 megohms-cm (2.0 to 0.5 micromhos/cm.) at 25 degrees centigrade. Preferably, distilled and deionized water should have resistivity values greater than 1.0 megohms-cm (less than 1.0 micromhos/cm) at 25 degrees centigrade. When purchasing distilled or deionized water, laboratories should request a list of quality specifications for the water purchased. Containers of distilled or deionized water should be capped when not in use and should be capped immediately after each use.

(c) Analytical reagent grade (AR) chemicals should be used for most analyses. Detailed information on reagent grades is set forth in Standard Methods, 16th Edition, section 102, pages 4-6. Individual analytical procedures in Standard Methods, 16th Edition, and the EPA's "Methods for Chemical Analysis of Water and Wastes" 600/4-79-020, Environmental Protection Agency, Office of Technology Transfer, Washington D.C. 20460, 1979, specify requirements for the reagents to be used. In addition, laboratory chemicals and reagents shall meet the following requirements:

1. Stock and working standard solutions shall be checked regularly for signs of decomposition, including but not limited to discoloration, formation of precipitates, and concentration change due to evaporation;

2. All solutions shall be properly labeled with identification of the compound, concentration, solvent, date, and analyst who prepared the solution;

3. All standards used for atomic absorption analyses shall be of high purity;

4. All chemicals, solutions, and standards, shall be dated upon receipt by the laboratory; and

5. Compressed gases used for atomic absorption analyses may be of commercial grade; and

6. Special purity solvents and reagents may be required for

specific organic analysis.

(d) All glassware should be washed in a warm detergent solution and thoroughly rinsed first in tap water and then in distilled water. This cleaning procedure is sufficient for most analytical needs, but the individual methods should be referred to for more elaborate precautions to be taken against contamination of glassware. It has been found advantageous to maintain a separate set of glassware, suitably prepared, for the nitrate, mercury, phosphate, lead, pesticide and herbicide methods due to the potential for contamination from the laboratory environment. All glassware used in pesticide and herbicide analysis should be cleaned and stored as outlined in section 3A of "The Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples", USEPA, Health Effects Research Laboratory, Research Triangle Park, N.C. 27711, 1979.

4.7 Quality Control Program

(a) All quality control data and records required by this section shall be retained for a period of five years by the laboratory and shall be made available for inspection by the Office of Quality Assurance. Such retained data shall include, but shall not be limited to, the results of and the raw data generated by Performance Evaluation Sample analyses.

(b) Each laboratory shall develop a detailed written description of the laboratory's current quality control program, and such written description shall include, but need not be limited to, the following:

1. Procedures which the laboratory will use in meeting the quality control requirements of section 4.7 pertaining to laboratory equipment and instrumentation, and the frequency with which such procedures will be performed.

2. Procedures which the laboratory will use in meeting the quality control requirements of section 4.6 pertaining to general laboratory practices and the frequency with which such procedures will be performed.

3. Procedures which the laboratory will use in meeting the quality control requirements of subsection (e) below, and the frequency with which such procedures will be performed.

(c) Each laboratory shall develop a written laboratory procedures manual which shall set forth, in detail, the methods which

the laboratory will use in chemical analyses for all parameters for which the laboratory is seeking certification, and such methods shall comply with the criteria and procedures set forth in section 4.5.

(d) Each laboratory shall record and retain all raw data and calculations derived from analyses and quality control procedures in a manner that shall provide easy verification of the data and calculations during on-site inspections.

(e) Laboratories shall perform the following internal quality control checks:

1. Each analytical balance shall be checked and adjusted annually by a service person employed by the laboratory or by a balance consultant. The accuracy of each analytical balance shall be checked on each day of use using at least two class "S" weights, one in the gram range and one in the milligram range. The weights used, weight detected to nearest 0.1 mg, dates on which checks were performed, analyst, and other pertinent information shall be recorded in a log book.

2. The wavelength setting of the spectrophotometer shall be checked yearly by comparing the wavelength setting to the absorption maxima of colored standards or filters such as didymium glass. The wavelength observed, dates on which checks were performed, analyst and other pertinent information shall be recorded in a log book.

3. Each pH meter electrodes shall be cleaned immediately after each use period and calibrated prior to usage with two pH buffer standards bracketing the value to be measured and the calibration recorded. A daily check shall be made of the pH meter after calibration by setting the meter to pH 7.00 with a buffer standard and then without further adjustment, reading pH buffer standards of pH 4.00 and 10.00 and recording the actual readings.

4. Conductivity meters equipped with conductivity cells having platinum electrodes shall be checked over the range of interest using at least five concentrations of a standard potassium chloride solution. Conductivity cells not having platinum electrodes shall be checked against a conductivity meter equipped with platinum electrodes. This check shall be performed annually and the raw data, cell constant, and results shall be recorded in a log book.

5. A daily record of the temperature of the drying oven

shall be maintained for each day on which the drying oven is in use.

6. The temperature of each refrigerator and each incubator shall be either recorded continuously or recorded daily from inplace thermometers immersed in liquid and placed on one of the shelves being used.

7. The accuracy of all thermometers used to monitor temperatures shall be verified by comparing the readings of such thermometers with the readings of a certified thermometer. A record shall be made containing the identification number of each thermometer, the temperatures displayed on the certified thermometer and the thermometer being verified, correction factors when applicable, dates on which quality control checks were performed, and the name of the analyst performing such checks. Glass thermometers shall be verified yearly and metal thermometers shall be verified quarterly.

8. Standard curves used in analysis of parameters in the Limited Chemistry category shall be prepared as follows:

   i. Standard curves consisting at a minimum of one reagent blank and five standards shall be prepared with each analysis. The absorbance or transmittance reading for each prepared standard shall be based upon the average of three replicate readings of each standard; or

   ii. A standard curve consisting at a minimum of a reagent blank and five standards shall be prepared and shall be used with each subsequent analysis provided the standard curve is verified by using at least one reagent blank and one standard at or near, in the case of analyses under the WVPDES program, the concentration levels normally encountered in such analyses. The absorbance or transmittance reading for each prepared standard shall be based upon the average of three replicate readings of each standard. A new standard curve shall be prepared whenever a new reagent is changed. Such verifications shall be considered satisfactory if, and only if, the results are within ± 10 percent of the original curve. All data used (on) in drawing the curve, shall be so indicated on the curve, and a record shall be made of the verification containing the dates on which such verifications were performed, the results of the verification, and the name of the analyst who performed the check.

9. Standard curves used in the analysis of parameters in the Atomic Absorption category shall be prepared as stated above in paragraph 8 except that a minimum of one reagent blank and four standards are required.

10. Laboratories which analyze 20 or more samples per day shall verify the working standard curve by running an additional standard, for analyses under the WVPDES program, at the concentration level normally encountered in such analyses. The frequency of such analyses shall be one verification analysis after the analysis of each set of 20 samples. Such checks shall be satisfactory only if the results are within ± 10 percent of the original documented reagent curve.

11. In all cases where possible, replicate sample analyses shall be conducted for parameters in the Limited Chemistry and Atomic Absorption categories to verify the precision of the method and such verification shall be performed at one of the two following frequencies:

    i. Laboratories which analyze twenty or more samples per month of any one parameter shall verify the precision of such analyses on at least 5 percent of the samples analyzed and shall document the result, the dates on which such verification analyses were performed, the method of verification, and the name of the analyst performing such verifications; or

    ii. Laboratories which analyze an average of less than twenty samples per month of any one parameter shall verify the precision of the analysis once per analysis batch, and shall make a record of such verification in accordance with subparagraph i above.

12. In all cases where possible, spiked sample analyses shall be conducted to verify the accuracy of the method at the same frequency as set forth in paragraph 11 of this subsection for the applicable parameters in the Limited Chemistry and Atomic Absorption categories. Documentation shall be made in accordance with the requirements of that paragraph.

13. In all cases where possible, standard deviations shall be calculated and documented for all applicable measurements being conducted in the Limited Chemistry and Atomic Absorption categories and such calculations and documentation shall be done in accordance with the following criteria and procedures:

i. Standard deviations shall be calculated for control samples which have been prepared or at the concentration level normally encountered in the analysis for all parameters;

ii. Once the standard curve has been prepared or verified, the control sample shall be analyzed;

iii. After 20 such determinations have been obtained, using one control sample per run, the standard deviation shall be calculated;

iv. Standard deviations shall be obtained for all parameters;

v. The theoretical value, mean value, and the range of acceptable values derived from two standard deviations, shall be recorded; and

vi. Standard deviations shall be documented in either tabular form or, preferably on control charts.

14. Spiked reference materials (SPRM's) shall be analyzed for all organic methodologies requiring the use of a gas chromatograph at the following frequency:

i. For laboratories ten or less samples per day, one SPRM shall be analyzed during that time of analysis and documented; or

ii. For laboratories analyzing more than ten samples per day, each 10th sample shall be a SPRM.

15. Information pertaining to SPRM may be found in section 3 of "The Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples", USEPA, Health Effects Laboratory, Research Triangle Park, N.C. 27711, 1979.

16. A record shall be maintained for each gas chromatograph and shall contain the following information:

i. Date of installation and serial number of each detector installed;

ii. Background current (BGC) profiles obtained at the time of installation of each detector and subsequent profiles (column identity notations should be made); and

iii. Date of change of pyrometer batteries, if used.

17. A record shall be maintained on each gas chromatographic column used and shall contain the following information:

    i. Column identification number;

    ii. Date of packing or purchase;

    iii. Liquid phase identity and lot number of precoated column packing;

    iv. Conditioning temperature, flow rate and number of hours;

    v. Length and shape of column.

    vi. Background current on newly installed column and subsequent background current profiles during the life of the column;

    vii. Date of each silylation of column; and

    viii. When applicable, compound conversion data; with dates monitored, and percentage of compound breakdown.

18. A record shall be maintained on the preparation of pesticide and herbicide standards and shall include, but not be limited to, the following information:

    i. The identification number of the concentrated stock standard solution, date of preparation, chemist who prepared the solution, all chemical compounds in the solution, lot number, purity, gross weight, tare weight, net weight, adjusted net weight (corrected for purity of primary standard), dilution volume and concentrations in ng/ul;

    ii. The identification number of the intermediate concentration standard solution, date of preparation, chemist who prepared the solution, all chemical compounds in the solution, identification number of the concentrated stock, strength of concentrated stock in ng/ul, aliquot of concentrated stock, dilution volume, and final concentration in ng/ul; and

    iii. The identification number of the working standard solution, date of preparation, chemist who prepared the solution, all chemical compounds in the solution, identification number of the

intermediate concentration standards, concentration of intermediate standards, aliquot volumes, dilution volumes, and final concentrations in pg/ul.

iv. Additional information on preparation of standards may be found in "The Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples", USEPA, Health Effects Research Laboratory, Research Triangle Park, N.C. 27711, 1979, pp 59-67.

19. All quality control procedures cited in the gas chromatography methodologies shall be performed and documented.

20. All reagents and solutions shall be labelled to indicate identity and, when applicable, titer, strength, or concentration, recommended storage requirements, preparation or expiration date, and any other pertinent information. Materials of substandard reactivity and deteriorated materials shall not be used. All outdated material shall be discarded immediately.

21. There shall be available at all times, in the immediate area of personnel engaged in the examination of samples and related procedures within the chemical category, the most current laboratory manuals or other complete written descriptions and instructions relating to:

i. The analytical methods to be used by such personnel, properly designated and dated to reflect the most recent supervisory reviews;

ii. Pertinent current literature in the field for use as reference materials including the appropriate Federal regulations; and

iii. Textbooks may be used to supplement such written descriptions, but may not be used in lieu thereof.

22. Only the laboratory director or supervisor shall make changes in laboratory procedures and those changes shall only be effective when put in writing.

23. Dissolved oxygen meters shall be checked weekly using the Winkler method and the results recorded.

4.8 Records and Data Reporting

(a) Records of chemical analyses, including but not limited to

all raw data, calculations, quality control data, and laboratory reports, shall be kept by the laboratory for at least five years.

(b) The following information shall be retained by the laboratory as part of the records of chemical analysis and the records of chain custody:

1. The laboratory number or other form of identification of the sample;

2. The date, time, specific site of sampling, and the name of the person who collected the sample or the laboratory which submitted the sample;

3. The date and time when the laboratory received the sample, whether the sample was received preserved or unpreserved,

4. The date and time of analysis of the sample;

5. The person or persons who performed the analysis;

6. The type of analysis performed and the analytical method employed;

7. The results of the analysis and the raw data generated by the analysis; and

8. The name and address of the laboratory to which the sample was forwarded, if the analysis was not performed at the laboratory which first received the sample.

(c) If the chain of custody information is reported on a chain of custody form, a copy of the chain of custody form shall be attached to the sample report form.

(d) The results of each analysis shall be calculated and entered on the sample report form which is to be forwarded to the person requesting the analysis of the sample. A careful check shall be made to assure that each result entered on the sample report form is the same as the result entered on the bench sheet and once the check is completed the supervisor shall initial the bench sheet.

(e) The original or true duplicate of the results of the test or analysis shall be sent promptly to the person who requested such tests or analysis, and shall be signed by the laboratory director or a designee whose designation is in writing and has been submitted to the Department.

(f) Whenever a laboratory refers samples to another laboratory, the person ordering the examination shall receive the original laboratory report or a true duplicate of that report on the form of the laboratory that actually performed the test or analysis.

Section 5. CRITERIA AND PROCEDURES FOR RADIOLOGICAL TESTING AND ANALYSIS

5.1 Scope

This section establishes the Department's requirements which a certified laboratory or a laboratory seeking certification shall continually meet and follow when performing radiological analyses.

5.2 Laboratory Facilities

(a) Laboratory facilities shall meet the following minimum requirements:

1. The counting instruments required for measurement of specific radionuclides shall be located in a room other than the one in which samples and standards are prepared or in which other types of chemical analyses are being performed. The temperature of the room in which counting instruments are located shall not exceed 27 degrees centigrade. Temperature variation under normal operation conditions shall not exceed 3 degrees centigrade.

2. All instruments shall be properly grounded, and a regulated power supply, either external or internal, shall be available for use with each instrument.

3. In areas in which radioactive standards are being prepared, care shall be taken to minimize contamination of surfaces and personnel. Either bench surfaces of an impervious material covered with absorbent paper, or trays constructed of stainless steel, plastic, or fiberglass and lined with absorbent paper may be used.

4. Laboratory space and facilities shall be adequate to properly carry out the services performed in, or offered by, the laboratory. There should be at least 100 to 150 square feet of floor space per analyst. This space should contain no less than 15 linear ft. of bench space and the following equipment:

   i. A sink with hot and cold running water;

   ii. Electrical outlets with a carrying capacity at 150 V a.c. and such outlets shall be grounded;

   iii. A source of distilled or deionized water;

   iv. A supply of natural gas or liquefied petroleum, or

a propane cylinder with proper attachments in the case of laboratories performing limited amounts of analytical work;

v. A vacuum line, pump, or aspirator; and

vi. An exhaust hood.

5.3 Specifications for Laboratory Equipment and Instruments

(a) Laboratories performing radiological tests and analyses shall have on the premises and under the control of the laboratory director the equipment and instruments listed in this section necessary for the preparation and analysis of the specific standards and samples for which the laboratory is seeking certification or is certified. Such instruments, when required, shall meet the following specifications:

1. The following are specifications for general instrumentation and equipment:

i. The analytical balance shall have a precision of ± 0.5 mg, and minimum scale readability of 0.1 mg;

ii. The pH meter shall have an accuracy of ± 0.1 units;

iii. The specific ion meter shall have an expanded scale millivolt capability, and shall be readable to 0.1 mv and accurate to ± 0.5 mv.;

iv. The conductivity meter shall be readable in ohms or mhos, shall have a range of 2 to 2.5 million ohms-cm or micromhos/cm ± 1 percent, and shall have a sensitivity of 0.33 percent or better;

v. A gravity convection drying oven which shall be capable of maintaining stable temperatures;

vi. Either glass or plastic dessicators may be used as appropriate for the particular task being performed;

vii. Either a large or small hot plate may be used however hot plates shall have temperature controls;

viii. Laboratory glassware shall be constructed of borosilicate glass, and all volumetric glassware

shall be marked Class A, denoting that it meets Federal specifications, thereby eliminating the need for calibration prior to use;

ix. The muffle furnace shall be automatically controlled and shall have a chamber capacity of at least 2,200 cc (10 x 9. 5 x 23) and a maximum operating temperature of 1,000 degrees centigrade continuous and 1,100 degrees centigrade intermittent;

x. A general purpose table-top centrifuge which shall have a maximum speed of at least 3,000 rpm and a loading option of 4 x 50 mL; and

xi. The fluorometer shall be capable of detecting 0.0005 ug of uranium.

(b) The types of radiation counting systems needed to comply with this chapter are described in 40 CFR 141. Laboratories shall have on the premises and under the control of the laboratory director those instruments needed to analyze for those activities or specific radionuclides for which the laboratory is certified. Such instruments shall meet the following specifications:

1. A liquid scintillation system is required if the laboratory is to be certified for measurement of tritium in water samples. The system shall be such that the sensitivity shall meet or exceed the requirements of 40 CFR 141.25.

2. A gas-flow proportional counting system shall be used for the measurement of gross alpha and gross beta activities, radium-226, radium-228, strontium-89, strontium-90, cesium-134, and iodine-131 as described in the reference cited in 40 CFR 141.25 (a). The detector shall be either a "windowless" internal proportional counter or a "thin window" type. A minimum shielding equivalent to 5 cm of lead shall surround the detector. A cosmic (guard) detector should be operated in anticoincidence with the main detector. The gas-flow proportional counting system shall be such that the sensitivity of the radiological analysis of the water sample will meet or exceed the requirements of 40 CFR 141.25.

3. For measurement of gross activities and radium-226, a scintillation system designed for alpha counting may be substituted for the gas-flow proportional counter described in paragraph 2 above. When a scintillation system is used for counting, a Mylar disc coated with a

phosphor (silver-activated zinc sulfide) shall be placed either directly on the sample or on the face of a photomultiplier tube, and the disc and sample or tube shall then be enclosed within a light-tight container along with the appropriate electronics, including but not limited to a high voltage supply, amplifier, timer, and scaler.

4. For the specific measurement of radium-226 by the radon emanation method, a scintillation system designed to accept scintillation flasks ("Lucas cells") shall be used. This type of scintillation system consists of a light-tight enclosure capable of accepting the scintillation flasks, a detector (phototube), and the appropriate electronics which includes but is not limited to a high voltage supply, amplifier, timers, and scalers. The flasks (cells) required for this measurement shall be either purchased from commercial suppliers or shall be constructed by laboratory personnel in accordance with the specifications set forth in Lucas, H.F., "Improved Low-Level Alpha Scintillation Counter for Radon". Rev. Sci. Instrum., 28:680, 1967).

5. Gamma spectrometer systems shall have either a sodium iodide (NaI(Tl)) crystal detector or a solid state lithium drifted germanium (Ge(Li)) detector connected to a multichannel analyzer if the gamma spectrometer system is to be used for analyses of manmade photon emitters, and the detector shall meet the criteria and specifications set forth in either subparagraph i or subparagraph ii below:

   i. If a sodium iodide detector is used, such detector shall meet the following criteria and specifications:

      (1) A 10 cm x 10 cm NaI cylinder crystal should be used, but, at a minimum, a 7.5 cm x 7.5 cm crystal shall be used;

      (2) The detector shall be shielded with at least [illegible]cm of iron or the equivalent thereof;

      (3) The distance from the center of the detector to other part of the shield should be at least 30 cm;

   ii. A system with a lithium drifted germanium (Ge(Li)) detector may be used for measurement of manmade photon emmitters provided the following requirements are met:

(1) The efficiency of the detector shall be such that the sensitivity of the gamma spectrometry system meets the minimum detectable activity requirements cited in 40 CRF 141.25;

(2) The detector shall be shielded with at least 10 cm of iron or the equivalent thereof; and

iii. The multichannel analyzer, in addition to appropriate electronics, shall have a memory of at least 200 channels for NaI and 2000 channels for Ge(Li) and shall have at least one readout device.

5.4 Preservation of Samples, Methodology, and Major Instrumentation

(a) Table I below gives the minimum requirements for sample handling including preservation, methodology, and major instrumentation.

Table I - Sample handling, preservation, methodology (1) and major instrumentation (minimum requirements)

Parameter	Preservative(2)	Container(3)	Instrumentation(4)
Gross alpha	Conc. HCl or HNO to pH 2(5)	P or G	A or B
Gross beta	Conc. HCl or HNO(3) to pH 2(5)	P or G	A
Strontium-89	Conc. HCl or HNO(3) to pH 2	P or G	A
Strontium-90	Conc. HCl or HNO(3) to pH 2	P or G	A
Radium-226	Conc. HCl or HNO(3) to pH 2	P or G	A, B or D
Radium-228	Conc. HCl or HNO(3) to pH 2	P or G	A
Cesium-134	Conc. HCl to pH 2	P or G	A or C
Iodine-131	None	P or G	A
Tritium	None	G	E
Uranium	Conc. HCl to HNO(3) to pH 2	P or G	F
Photon emitters	Conc. HCl to HNO(3) to pH 2	P or G	C

(1) 40 CFR 141.

(2) It is recommended that the preservative be added to the sample at the time of collection unless suspended solids activity is to be measured. However, if the sample must be shipped to a laboratory or storage area, acidification of the sample (in its original container) may be delayed for a period not to exceed 5 days. A minimum of 16 hours must elapse between acidification and analysis.

(3) P = Plastic, hard or soft; G = Glass, hard or soft.

(4) A = Low background proportional system; B = Alpha scintillation system; C = Gamma spectrometer (NaI(Tl) or Ge(Li)); D = Scintillation cell (radon) system; E = Liquid scintillation system (section C.2.a); F = Fluorometer (section C.1.i).

(5) If HCl is used to acidify samples which are to be analyzed for gross alpha or gross beta activities, the acid salts must be converted to nitrate salts before transfer of the samples to planchets.

5.5 Methodology

(a) Laboratories shall use the analytical procedures specified in 40 CFR 141.

(b) All procedures other than those set forth in subsection (a) of this section are considered alternative analytical methods as described in 40 CFR 141.27. Laboratories shall make special application to the Department for the use of alternative analytical methods and such application shall include a showing of acceptable comparability data.

5.6 General Laboratory Practices

(a) Laboratory practices shall meet the following requirements:

1. All glassware shall be washed in a warm detergent solution and shall be thoroughly rinsed in tap water. A distilled or deionized water rinse shall follow the tap water rinse. When necessary for proper performance of specific analytical methods, more detailed procedures for ensuring cleanliness of glassware shall be employed.

2. All water used in preparation of reagents, standards, and samples shall have resistance values that are between 0.5 to 2.0 megohms-cm (2.0 to 0.5 micromhos/cm) at 25 degrees centigrade. If such high quality water is not available in the laboratory, it shall be purchased from commercial suppliers; the laboratory shall request a list of quality specifications for water purchased from the supplier and the laboratory shall periodically check the actual quality of the purchased water against these specifications.

3. Analytical reagent grade (AR) chemicals shall be used for all analyses, unless otherwise required for an individual

analytical procedure.

4. Radioactive standards and radioactive wastes shall be stored in an enclosed and properly labeled area, either within the analytical laboratory or in a separate room or facility. Standards, samples, and radioactive wastes shall be safely stored in suitable containers.

5. Standards and samples shall be prepared in an area of the laboratory specifically designated for and exclusively used for the preparation of radioactive standards and samples. Adequate precautions shall be taken in this area to ensure against radioactive contamination. Provisions shall be made for safe storage and disposal of radioactive wastes and for monitoring of the work area for radioactivity.

6. All reagents and solutions shall be labeled with pertinent information. Materials of substandard reactivity and deteriorated materials shall not be used.

5.7 Quality Control

(a) Laboratories shall develop and implement quality control procedures meeting the following minimum requirements:

1. Each laboratory shall develop and have on file and available for inspection a written description of the current laboratory quality control program. Such quality control program shall cover all types of tests and analyses performed by the laboratory and shall provide for verification and assessment of the accuracy, measurement of precision, and detection of error. Management, supervisors, and analysts should participate in the quality control program. Each participant within the laboratory should have a copy of the quality control program and detailed guidelines for implementation of the participant's responsibility.

2. Quality control data and records shall be available for inspection.

3. There shall be available at all times, current laboratory manuals or other complete written descriptions and instructions relating to:

    i. The analytical methods to be used by those personnel, properly designated and dated to reflect the most recent supervisory reviews;

ii. Operating manuals and calibration protocols for counting instruments shall be available to analysts and technicians;

iii. Such manuals, written descriptions, protocols, and instructions may be supplemented by, but not replaced by, textbooks relating to the particular analytical methods and procedures employed by such personnel.

4. Permanent records shall be maintained of preventive maintenance, periodic inspection, testing, and calibration for the proper operation of radiation instruments and analytical balances; validation of methods; evaluation of reagents and volumetric equipment; surveillance of results; and remedial actions taken in response to detected defects. Such records shall be kept on file by the laboratory for a period of at least five years.

5. The following minimum daily quality control measures shall be performed by the laboratory:

i. To verify internal laboratory precision duplicate analyses equal to ten percent of sample analyses shall be performed. The differences between duplicate measurements shall be less than twice the standard deviation of the specific analysis as described in "Environmental Radioactivity Laboratory Intercomparison Studies Program" (EPA-600/4-77-001). If the differences exceed two standard deviations, the prior measurements shall be considered to be suspect, all calculations and procedures for that day shall be examined, and all samples shall be reanalyzed when necessary.

ii. Laboratories performing 20 or more specific analyses each day, shall measure at least one calibration standard and at least one background sample along with each group of 20 samples.

iii. Laboratories performing less than 20 specific analyses in any one day, shall measure one calibration standard and one background sample along with the samples measured on that day.

iv. Quality control performance charts, performance records, and raw data used in the verification procedure set forth in this paragraph shall be maintained for a period of at least five years.

6. Balances shall be checked periodically using Class "S" weights, and laboratories shall have current service contracts in effect for balances.

7. Laboratories shall have current factory servicing contracts for repair of laboratory instruments.

8. Only the laboratory director or supervisor shall make changes in laboratory procedures and those changes shall only be effective when put in writing.

5.8 Records and Data Reporting

(a) The laboratory shall maintain records which are adequate and appropriate for the services offered.

(b) Work records of quantitative tests shall be maintained for at least five years and shall indicate final results together with all corresponding instrument readings and calculations. Where instrumentation produces tracings or printouts, such tracings or printouts may serve as the work record.

(c) A record shall be maintained for at least five years of the daily receipt of samples. Each such record shall be numbered or otherwise appropriately identified and shall contain the following information:

1. The laboratory number or other identification of the sample.

2. Identification of the sample source, water system, discharger, or permit number.

3. The name of the person or laboratory which submitted the sample.

4. The date, time, and specific location of sample collection.

5. The date and time of receipt of the sample by the laboratory.

6. The type of test or analysis performed, method of testing or analysis, and date of analysis.

7. The results of the test or analysis, including raw data, and the name of the analyst or analysts.

8. The name and address of the laboratory to which the sample was forwarded, if the testing or analysis was not

performed by the laboratory which initially received the sample.

(d) The original or a true duplicate of the results of the tests or analyses shall be sent promptly to the person who requested such tests or analyses, and shall be signed by the laboratory director or a designee whose designation is in writing and has been submitted to the Department.

(e) Whenever a certified laboratory refers samples to another certified laboratory for analysis, the person requesting the analyses or tests shall receive the laboratory report or a true duplicate of that report on the form of the laboratory that performs the tests or analyses.

(f) Laboratories shall follow the chain of custody procedures set forth in sections 4.4(d), 4.8(c) and 4.8(d).

(g) Records of radiological analyses shall be kept by the laboratory for not less than five years. This includes but is not limited to all raw data, calculations, quality control data, and reports. In addition, actual laboratory reports shall be kept for not less than five years. However, all data may be transfered to tabular summaries provided that the following information is included:

1. The date, specific place, and time of sampling.

2. The name of the person who collected the sample.

3. Identification of sample as a routine sample, check sample, raw water sample, or other special purpose sample.

4. The date of receipt of the sample by the laboratory and the date of analysis.

5. The name of the laboratory and the person or persons who performed the analysis.

6. The analytical technique or method used.

7. The results of the test or analysis, including the raw data generated during the test or analysis.

Section 6. CRITERIA AND PROCEDURES FOR BIOASSAY TESTING AND ANALYSIS

6.1 Scope

This section establishes the Department's requirements which a certified laboratory or a laboratory seeking certification shall continually meet and follow when performing bioassay analysis.

6.2 Definitions

For the purpose of this section the following definitions in addition to those found in section 1.7 are applicable.

"Acclimation" means an organism's physiological adjustment to environmental changes including, but not limited to, changes in temperature and salinity.

"Acute toxicity" means causing death or severe damage to an organism by poisoning during a brief exposure period, normally 96 hours or less.

"Bioassay" means a determination of the concentration or dose of a given material necessary to affect a test organism under stated conditions. This term is commonly used interchangably with the term "Toxicity test".

"Biomonitoring" means all testing methods which utilize a biological system or any of its parts for assessing the presence or effects of one or more pollutants and/or environmental factors, either alone or in combination. For purposes of this section biomonitoring refers to acute toxicity bioassays.

"Composite sample" means a sample composed of several discrete samples collected at equal time intervals, or collected proportionally to the flow rate of the discharge, over the compositing period.

"Control" means an exposure chamber which receives only dilution water samples and is used in conjunction with an effluent bioassay.

"Definitive test" means a full-scale bioassay consisting of a minimum of five different concentrations of effluent in a logarithmic or geometric series with each concentration and control being tested against a minimum of 20 organisms of a species designated by the Department.

"Dilution water" means the unpolluted water of desired quality to be used for preparing the different test concentrations of the effluent and the controls. Dilution water is usually collected from a point upstream of the effluent as close as possible, but not within, the mixing zone influenced by the effluent. Laboratory water of known quality may be suitable as dilution water in cases where upstream waters have been determined to be unacceptable or inappropriate.

"Discharge" means the releasing, spilling, leaking, pumping, pouring, emitting, emptying, or dumping of a pollutant into the waters of the State or onto land or into wells from which the pollutant might flow or drain into said waters, and shall include the release of any pollutant into a municipal treatment works.

"Effluent" means an outflow from a point source.

"Flow-through bioassay" or "Continuous flow-through bioassay" means a bioassay test technique which permits test solutions to flow into and out of the test chambers on a once through basis for the duration of the test.

"Grab sample" means an individual sample collected over a time period of less than 15 minutes.

"Incipient lethal level" or "incipient LC50 means the concentration at which acute toxicity ceases, that is, the concentration at which 50 percent of the test organism's population can live for an indefinite time.

"Laboratory grade water" is water which is suitable for culturing and holding bioassay organisms. Natural or artificial sources may be used. Laboratory grade water shall be law in turbidity, high in dissolved oxygen, low in B.O.D., and of a pH favorable to the maintenance of the organism.

"LC50" means the concentration of a toxicant which is lethal to 50

percent of the organisms of a particular species under a given set of conditions in a specified length of time (i.e., 24, 48, or 96 hours).

"Mixing zone" means a localized area of surface waters, as may be designated by the department, into which wastewater effluents may be discharged for the purpose of mixing, dispersing, or dissipating such effluents without creating nuisances or hazardous conditions in compliance with the Surface Water Quality Standards.

"Methods for Measuring Acute Toxicity - EPA" means "Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms," (Third Edition), USEPA, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, EPA-600/4-85-013.

"Modified static daily-renewal bioassay" means a test in which the aquatic organisms are exposed to a test solution which is changed once every 24 hours for the duration of the test period. Fresh samples of the effluent and dilution water are obtained daily, fresh concentrations of test solution are prepared, and the test organisms are transferred daily to the new test solution.

"Permit" a WVDNR permit issued pursuant to 20-5A Part III of the Water Pollution Control Act.

"Point source" means any discernable, confined, and discrete conveyance from a mobile or stationary source, including, but not limited to, any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock, concentrated animal feeding operation, vessel or other floating craft, from which pollutants are or may be discharged.

"Pollutant" means any dredged spoil, solid waste, incinerator residue, filter backwash, sewage, garbage, refuse, oil, grease, sewage sludge, munition, chemical wastes, biological materials, radioactive materials, thermal waste, wrecked or discarded equipment, and construction waste or runoff or other residue discharged to the land, groundwaters or surface waters of the state.

"Range-finding test" means a short-term (i.e., 8-24 hours) flow-through or, more often, static bioassay used for determining the approximate concentrations, above and below the LC50, to be used in the definitive test when the toxicity of a waste is unknown. In

this test, groups of five test organisms are exposed to three to five widely-spaced effluent dilutions, such as 1%, 10%, 50% and a control.

"Salinity" means the total amount of dissolved salts in water in parts per thousand (0/00 or ppt) by weight when all the carbonate has been converted to oxide, the bromide and iodine have been replaced to chloride, and all organic matter has been completely oxidized.

"Sampling point" means a particular site whose location may be specified in a permit, or otherwise, and from which effluent samples are to be collected for testing and evaluation.

"Screening test" means an abbreviated bioassay with a single (100%) effluent concentration plus a control. The duration of the screening test is usually 24 hours. However, if mortality occurs within the first few hours of exposure, the test may be terminated at 6-8 hours. If lethality is observed in the screening test, a definitive test may be required.

"Total length" means the straight line measurement from the tip of the snout of a fish to the extreme tip of the fish's caudal fin.

"Toxicity test" means the determination of the concentration of a mixture of substances necessary to affect a test organism under stated condition. This term is commonly used interchangably with the term "bioassay."

"Volume percent" is (100 x volume of effluent) divided by (volume of effluent + volume of dilution water).

"Waters of the State" means the same as defined at 20-5A-2(e) of the State Water Pollution Control Act, all springs, streams and bodies of surface or groundwater, whether natural or artificial, within the boundaries of this state.

"Year class" means fish which originate from the same annual brood or spawning.

6.3 Laboratory Facilities and Safety

(a) Laboratory space and facilities shall be adequate to properly carry out the services performed in, or offered by the laboratory.

(b) Workbench space within the laboratory shall be ample for the tests or analyses to be performed, and shall be well-lighted and convenient to a sink, and such water, gas, suction, and electrical outlets as are necessary to properly carry out the specific tests or analyses performed in, or offered by, the laboratory.

(c) The temperature, photoperiod, and humidity within the laboratory shall be maintained within the limits required for the proper performance of each test or analysis and for the proper operation of instruments which may be affected by temperature variations.

(d) Adequate fire precautions shall be taken, including but not limited to, having readily available a fire extinguisher rated for the types of fires that reasonably may be foreseen given the types of tests and analyses performed by the laboratory.

(e) Appropriate occupational safety and health laws shall be posted and observed.

6.4 Laboratory Equipment, Supplies and Materials

(a) Laboratories performing bioassay tests and analyses shall have under the control of the laboratory director the equipment and instruments listed in this section necessary for the analysis of samples for which the laboratory is seeking certification or is certified. Such instruments, when required, shall meet the following specifications:

1. Air supply for aeration of tanks shall either be from compressors equipped with seals designed to prevent contamination of air lines from oil, or from compressed air or oxygen tanks or from commercially available pumps designed specifically for aeration of fish tanks.

2. Bioassay testing systems may consist of temperature control units, pipes, valves and fittings, diluter, pumps, mixing equipment, tanks, and exposure chambers and shall meet the following requirements:

   i. All components of the bioassay testing system shall be constructed of lead-free glass, No. 316 stainless steel, silicone sealant and tubing, unplasticized polyethylene or polypropylene,

Teflon, Tygon, nylon, fiberglass or any other materials proven to be nontoxic in the test organisms;

ii. Tubing, connectors and screens made of materials known to absorb significant amounts of trace organic compounds shall be used once and discarded; and

iii. All components that are reused shall withstand cleaning, without significant degradation, by the procedures cited in section 6.7 (e).

3. For flow through bioassays, a dilutor system is required for the accurate measuring, mixing, and delivery of sample and dilution water to the exposure chambers. A variety of methods may be used, however, the proportional dilutor is the preferred system for routine effluent toxicity tests. Detailed descriptions of dilutor systems may be found in Standard Methods - 16th Edition and Methods for Measuring Acute Toxicity - EPA. All dilutor systems shall meet the following requirements:

i. Dilutor system shall provide an adequate supply of dilution water to maintain a 24 hour continuous operation. This supply shall be provided by the usage of a large dilution water reservoir or by direct continuous pumping from the source of the water.

ii. The dilutor system shall be capable of metering the flow of dilution water and sample into a mixing chamber for the determination of concentrations. Metering of dilution water and sample may be accomplished by using a constant head box or metering pumps.

iii. Mixing chambers shall be used to ensure complete mixing of dilution water and sample prior to dispensing of solutions into the exposure chambers.

iv. Separate delivery tubes shall be used for transmission of the dilution water and sample from the flow splitters, after the mixing chambers, to each of the duplicate exposure chambers.

v. The flow rate through the exposure chambers shall be sufficient to maintain adequate dissolved oxygen in the exposure chambers, according to section 6.6 (s), but no less than five water

volume changes every 24 hours.

vi. The flow rate through the exposure chambers shall not vary by more than ± 10 percent between all exposure chambers or ± 5 percent within any given exposure chamber throughout the duration of the test.

vii. The dilutor system shall maintain the test concentration in each exposure chamber within ± 5 percent of the starting concentration for the duration of the test.

viii. The dilutor system shall have heating and cooling equipment designed to maintain a constant temperature in the exposure chambers to within ± 2 degrees centigrade of the specified test temperature.

ix. If the supply of dilution water to the mixing chamber is interrupted, the dilutor system shall be designed to automatically curtail the delivery of the sample to the mixing chambers.

x. The exposure chambers shall have an overflow system designed to prevent the test organisms from entering the outlets.

xi. The dilutor system shall be capable of maintaining a minimum of five separate effluent dilutions and a control containing dilution water at any necessary flow rate, required by section 6.4 (a) 3v. with duplicate exposure chambers.

xii. The dilutor system shall be capable of, but not limited to, providing concentrations at 10^{-1} and 10^{-2} of the logarithmic series of concentration, 5.6, 10.0, 18.0, 32.0, 56.0, and 100 percent effluent by volume.

4. Holding, acclimating, and culturing chambers shall meet the following requirements:

i. Chambers shall be constructed of non-toxic materials and shall meet the requirements set forth in section 6.4 (a) 2.

ii. Chambers shall either be constructed so as to include devices for temperature control, or be located in a temperature controlled room. Chambers shall also be equipped with aeration devices.

iii. Chambers shall be constructed for ease of cleaning and the prevention of waste material build-up.

iv. The interior surface of the chambers shall be smooth to facilitate cleaning, reduce risk of injury to test organisms, and prevent accumulation of material in cracks or corners.

v. Chambers shall be shielded from outside disturbances. Materials used for shielding shall meet the requirements set forth in section 6.4 (a) 2i.

5. Laboratories shall have available top-loader or pan balances which shall meet the following requirements:

i. Balances shall be clean, not corroded, and shall be provided with appropriate weights of good quality; and

ii. Balances shall tare out and detect a weight of at least 100 mg accurately.

6. Temperature monitoring devices shall meet the following requirements:

i. Glass or metal thermometers shall be graduated in 0.5 degrees centigrade increments;

ii. Continuous temperature recording devices shall be sensitive and accurate within $\pm$ 0.5 degrees centigrade;

iii. The column of liquid in glass thermometers shall have no separation; and

iv. A certified thermometer shall be available for use by the laboratory.

7. Air or water-jacketed incubators, incubator rooms, and water baths shall meet the following requirements:

i. Incubators, incubator rooms, and water baths shall be of sufficient size to accommodate periods of peak work load;

ii. Incubators must maintain internal temperatures at the desired level to within $\pm$ 0.5 degrees centigrade;

iii. Whenever an air incubator is in use, a calibrated

thermometer with its bulb immersed in liquid shall be placed on one of the shelves in use within the incubator; and

iv. The temperature within an incubator shall be recorded daily, or a recording thermometer, sensitive to temperature of ± 0.5 degrees centigrade shall be used and the recording tape shall be checked daily.

v. Incubator shall be illuminated to provide photoperiod if required.

8. The refrigerator shall be of sufficient size to accept the required sample volumes, and shall maintain an internal temperature of 1 degree to 4.4 degrees centigrade.

9. Laboratories shall have at least one low power magnification device, preferably a binocular microscope with up to 10x magnification, for working with invertebrates.

10. Laboratory glassware, plastic-ware, and metal utensils not previously specified, shall meet the following requirements:

i. Glassware and metal utensils shall be resistant to the effects of corrosion, high temperatures, and vigorous cleaning operations;

ii. Volumetric containers should be Class A and need not be calibrated before use;

iii. Plastic items shall be of inert, nontoxic materials;

iv. Metal utensils shall be made of stainless steel.

11. Dilution water sample containers shall meet the following requirements:

i. Either wide-mouthed lead-free glass or unplasticized plastic containers that are equipped with glass stoppers, screw caps or an equivalent closure.

(1) After each use, containers shall be cleaned in accordance with the procedures set forth in section 6.7 (e).

12. Effluent sample containers shall meet the following requirements:

    i. Either wide-mouthed lead-free glass, or disposable unplasticized plastic containers that are equipped with glass stoppers, screw caps or equivalent closure shall be used.

        (1) After each use, only reusable containers shall be cleaned in accordance with procedures set forth in section 6.7 (e).

        (2) Disposable containers shall be discarded after use and shall not be cleaned and reused.

    ii. Glass-stoppered containers shall be stored to prevent contamination.

    iii. Screw caps shall have leakproof non-toxic liners.

6.5 Sample Collection, Handling and Preservation

(a) Dilution water samples shall be collected, handled and preserved as follows:

1. Dilution water shall be deemed acceptable for use in a bioassay provided healthy test organisms survive in it through the acclimation period without showing any signs of stress, including but not limited to, abnormal behavior or discoloration.

2. Dilution water samples shall be representative of the receiving water system which the effluent is discharged into. Samples shall be collected in the following manner:

    i. Dilution water samples shall be collected from a location as close as possible to, but upstream of, the effluent mixing zone. Water used to make dilutions should be collected immediately prior to the test, but never more than 96 hours before test initiation.

    ii. The sampling location shall be such that the salinity of the sample shall be within the salinity range for receiving water immediately outside of the effluent mixing zone.

    iii. When samples are collected from streams or rivers,

it is recommended that an integrated sample be collected. This is a sample that is collected from bottom to top of the water column so that the sample collected is proportional to flow. If only a grab sample can be taken then it should be collected at mid-depth in midstream.

iv. When samples are collected from reservoirs or lakes, the effects of seasonal stratification, runoff, and previous rainfall upon the chemical/physical characteristics of the water should be considered.

3. If the receiving water is influenced immediately upstream of the effluent outfall by other point sources of pollution so as to disqualify its use as dilution water, then the dilution water sample(s) shall be obtained from a location just above the other point sources.

4. If acceptable dilution water cannot be obtained from the receiving water at any location because of upstream pollution, then some other unpolluted water, meeting the following requirements, shall be used:

   i. Another surface water or groundwater having a natural quality similar to that of the receiving water prior to its pollution may be used; or

   ii. Reconstituted or artificial freshwater having a natural quality similar to that of the receiving water prior to its pollution may be used;

   iii. A substitute dilution water shall have a total hardness, total alkalinity, and specific conductance within 25 percent and a pH within 0.2 units of the receiving water prior to its pollution.

5. Modification of the salinity of a dilution water sample in order to comply with section 6.6 (n) 11 or the preparation of reconstituted fresh water shall follow the procedures given in Standard Methods, 16th Edition, pp. 699-701 and Methods for Measuring Acute Toxicity - EPA.

6. Except for aeration under the procedures stated in section 6.6 (s) and for salinity under the procedures described in section 6.5 (a) 5, the only other permissible treatment of the dilution water shall be filtration through screening made of a non-toxic material as specified in section 6.4 (a) 2. This screening shall have a mesh of 2mm or larger.

7. Dilution water collection and transport containers shall meet the requirements listed in section 6.4 (a) 12. Prior to sample collection, containers shall be pre- rinsed with the dilution water and then filled so that there is little or no air in the container neck or cap.

8. Dilution water sample storage shall be in covered containers constructed of non-toxic materials as specified in section 6.4 (a) 2.

9. Dilution water shall not be stored for more than 36 hours and should be collected as close as possible to the time of testing.

(b) Effluent samples shall be collected, handled and preserved as follows:

1. Unless otherwise specified by the Department, the effluent sampling location shall be the same as the specified in the WVPDES permit. An alternate sampling location may be specified when the following conditions prevail:

   i. When there is better access to the effluent at a point located between the final treatment and the discharge outfall; or

   ii. When the processed waste is chlorinated prior to discharge and the purpose of the test is to determine the toxicity levels of the unchlorinated effluent. In this case, the sampling point shall be located prior to the effluent's contact with the chlorine.

2. Samples shall be representative of the discharge, taking into account the plant operating conditions and the retention time of the effluent in the wastewater treatment plant.

3. For flow-through bioassays the following sampling procedures shall be adhered to in order to insure a representative effluent sample:

   i. If the facility discharges continuously, the effluent shall be pumped directly and continuously from the discharge line to the dilutor system for the duration of the test; or

   ii. If the facility discharges continuously but the effluent cannot be pumped directly and continuously to the diluter system, then the

following procedure shall be employed:

(1) Where the calculated retention time of the effluent is less than 14 days, as determined from flow metering devices or dye studies, two grab samples are collected daily (e.g., 8:00 a.m. and 4:00 p.m.). The freshly collected effluent should not be combined with the effluent remaining from the previously collected sample. The remaining part of the previously collected sample is discarded and the container is refilled with the fresh effluent.

(2) Where the calculated retention time of the effluent is 14 days or greater, as determined from flow metering devices or dye studies, a single grab sample of sufficient volume to supply the dilutor for 24 h is collected daily. Here again, the volume of sample remaining from the previous day is discarded and replaced by the fresh sample.

iii. If the facility discharge is intermittent, one of the following procedures is used:

(1) Where a continuous discharge occurs during a single eight-hour work shift or two successive eight-hour work shifts, at least one grab sample of sufficient volume to supply the dilutor for 24 h is collected daily, midway during the discharge period.

(2) Where the facility retains the wastewater during an eight-hour work shift, and then treats and releases it in a batch discharge, a single grab sample of sufficient volume to last 24 h is collected daily during the test period.

(3) Where the facility discharges wastewater to an estuary only during an outgoing tide (usually during the four hours following slack high tide), a single grab sample of sufficient volume to last 24 h is collected during one discharge period every 24 h for the duration of the test. An alternate sampling method would be to place the effluent sampling pump in the final waste lagoon adjacent to the discharge pipe so that a continuous source of effluent would be

available for the test.

4. In order to insure the collection of a representative effluent sample for a static or modified static bioassay, the following sampling procedures shall be followed:

   i. If the facility discharge is continuous, but the calculated retention time (as determined from flow metering devices or dye studies) of a continuously discharged effluent is less than 14 days and the variability of the waste is unknown, one of the following approaches is used:

      (1) Static tests: a minimum of four separate grab samples are collected at evenly-spaced (6-h) intervals over the first 24-h period and used in four separate tests to determine the variability in toxicity.

      (2) Modified static daily renewal tests: a minimum of four separate grab samples are collected at evenly-spaced (6-h) intervals over the first 24-h period and used in four separate tests begun on the first day and renewed daily with samples collected at the appropriate time.

   ii. If the calculated retention time of a continuously discharged effluent is greater than 14 days (as determined from flow metering devices or dye studies) or if it can be demonstrated that the wastewater does not vary in chemical composition or concentration regardless of holding time, one of the following approaches is used:

      (1) Static tests: a minimum of one grab sample is collected and used in a single test.

      (2) Modified static daily renewal tests: a minimum of one grab sample is collected each day and used to renew the test solutions.

   iii. If the facility discharge is intermittent, one of the following approaches is used:

      (1) Where the effluent is continuously discharged during a single eight-hour work shift or two successive eight-hour work shifts, a minimum of one grab sample is collected midway during the discharge period and used in a static test, or a grab sample may be collected daily

for a modified static.

(2) Where the facility retains the wastewater during an eight-hour work shift, then treats and releases the wastewater as a batch discharge, a grab sample is collected for a static test, or a grab sample is collected daily for a modified static test.

(3) Where the facility discharges wastewater to an estuary only during an outgoing tide (usually during the four hours following slack high tide), a grab sample is collected during a discharge period for use in a static test, or a grab sample is collected daily for a modified static test.

(4) At the end of the shift, clean up activities may result in the discharge of a slug of toxic waste. It would be advisable, therefore, to consider collecting a sample at that time and conducting a separate toxicity test.

5. Alteration of samples shall be limited to filtration through Teflon or No. 316 austenitic stainless steel screening having a mesh of 2mm or larger and/or introduction of dry, disease free, artificial salts for the purposes of adjusting the effluent salinity according to the procedures specified in section 6.6 (q) 2. Screening constructed of unplasticized polyethylene or polypropylene may be substituted provided the screens are discarded upon the completion of a bioassay.

6. Composite or grab sample collection and handling containers shall meet the requirements listed in section 6.4 (a) 13. Prior to sample collection, containers shall be pre-rinsed with the effluent and then filled so that there is no air space in either the neck or cap.

7. Effluent samples for on-site, flow-through tests shall be stored in covered, unsealed containers constructed of non-toxic materials as specified in section 6.4 (a) 2.

8. Unless the purpose of the bioassay is to ascertain the persistance of the toxicity of an effluent, testing shall begin within 24 hours of the collection of an effluent.

9. If samples are collected for offsite testing, the samples should be immediately placed on ice and shipped iced to the central laboratory and stored at 4.4 degrees

Centigrade. Samples that are to be tested two or more hours after collection should be kept chilled at between 0 degrees - 4 degrees centigrade.

(c) The following chain of custody procedures shall be employed in collecting and handling composite or grab samples:

1. Only clean or new containers, as specified in section 6.4 (a) 12 and 13, previously rinsed with laboratory pure water or the material being sampled shall be used for taking composite or grab samples.

2. Tie-on affixed labels with an identification number shall be used for labeling all samples.

3. After a sample has been collected, the appropriate information as to identity of the sample shall be written on the label and the label affixed. The label shall remain affixed until the test has begun and the surplus sample has been discarded.

4. Immediately upon delivery of a sample to the laboratory, the sample collector shall complete the appropriate chain of custody section of the sample report form or chain of custody form.

5. The chain of custody form shall list at a minimum the following information:

   i. Sample number;

   ii. Description of samples;

   iii. Specific location of sample collection;

   iv. Identity of person collecting the sample;

   v. Date and time of sample collection;

   vi. Date and time of custody transfer to laboratory (if the sample was collected by a person other than laboratory personnel);

   vii. Identity of person accepting custody (if the sample was collected by a person other than laboratory personnel);

   viii. Date and time of initiation of analyses;

   ix. Name of laboratory performing the analyses.

6. Prior to accepting custody of a sample, the laboratory personnel accepting the sample shall be reasonably assured that the sample has met the collection and handling requirements. If the sample fails to meet those requirements, the chain of custody section of the sample report form or the chain of custody form shall so indicate and the sample shall be refused.

7. The laboratory personnel accepting responsibility for the sample shall sign the form containing the chain of custody information.

6.6 Methodology

(a) The following aquatic organisms shall be used for bioassay testing:

1. Either the fathead minnow, Pimephales promelas, Daphnia pulex and Daphnia magna, or the bluegill, Lepomis macrohirus shall be used as the bioassay test organisms when the effluent's receiving water has a salinity of less than or equal to 1 ppt.

2. For receiving waters, whose salinity is greater than 1 ppt., any of the following organisms shall be used as the bioassay test organism:

i. Atlantic silverside, Menidia menidia.

ii. Sheepshead minnow, Cyprinodon variegatus.

iii. Grass shrimp, Palaemonetes pugio.

iv. Opossum shrimp, Mysidopsis bahia.

(b) Bioassay test organisms should either be cultured in the laboratory or obtained from commercial or Federal government hatcheries. Collecting bioassay test organisms from the field is not recommended.

(c) Culturing of fathead minnows in the laboratory shall be in accordance with the methods contained in Methods for Measuring the Acute Toxicity.

(d) Culturing of Daphnia spp., in the laboratory shall be in accordance with the methods contained in Methods for Testing Acute Toxicity.

(e) Culturing of bluegills in the laboratory shall be in accordance with the methods contained in U.S. EPA,

"Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnow, Channel Catfish, and Bluegills," May 1975.

(f) Culturing of the mysid shrimp in the laboratory shall be in accordance with the methods contained in Methods for Measuring the Acute Toxicity.

(g) Culturing of the atlantic silverside and the sheepshead minnow in the laboratory shall be in accordance with Standards Methods section 810A 2-5, pp. 802-810.

(h) Culturing of the grass shrimp in the laboratory shall be in accordance with the methods contained in U.S. EPA-1978.

(i) Culturing of the opossum shrimp in the laboratory shall be in accordance with the methods contained in U.S EPA-1978.

(j) Fathead minnows, bluegills, atlantic silversides or sheepshead minnows to be used as test organisms shall meet the following requirements:

1. All fish shall be actively feeding young one to ninety days old which have not been used previously in any bioassay or other test procedure;

2. All fish used in a bioassay shall be from the same source;

3. The total length of the longest fish of a group shall not be more than 1 1/2 times that of the shortest fish of the same group to be used for any test; and

4. The net weight of each fish should be within 0.1 to 1.5 grams.

5. The maximum difference in age should not exceed $\pm$ 3 days.

(k) Daphnia spp. to be used as test organisms shall meet the following requirements.

1. All Daphnia should be less than 24 hours old and have not been used previously in any bioassay or other test procedure.

2. All Daphnia used in a bioassay shall be from the same source and of the same species.

(l) Grass shrimp to be used as test organisms shall meet the following requirements:

1. Immature (larval) stages should be used whenever

possible, but post larvae are permissible. All shrimp shall not have been used previously in any bioassay or other test procedure;

2. All shrimp used in a bioassay shall be from the same source;

3. Organisms should be as uniform in size as possible. The maximum size shall be 10-20 mm rostrum to telson length.

(m) Opossum shrimp to be used as test organisms shall meet the following requirements:

1. Only newly hatched juvenile opossum shrimp up to five days old shall be used; and

2. All adult opossum shrimp from which the juveniles are obtained shall be from the same source.

3. The maximum difference in age should not exceed $\pm$ 24 hours.

(n) The holding and handling of the organisms shall be as follows:

1. While being transported to the laboratory, organisms shall not be overcrowded, the dissolved oxygen shall be maintained at or above 60 percent of saturation, and the temperature shall not change by more than 3 degrees centigrade in any 12 hour period;

2. When first brought into the laboratory, the organisms shall be quarantined for at least 10 days in order to observe them for parasites and diseases. If more than 10 percent of the quarantined organisms die after the second day or are heavily diseased or parasitized, and the problem cannot be controlled, that lot shall be destroyed, the holding tanks and equipment cleaned and sterilized, and another batch of organisms obtained;

3. After the quarantine period is over and the healthy organisms have been transferred to the stock holding tanks, the organisms shall be gradually acclimated to the laboratory holding conditions. The organisms shall not be subjected to water temperature changes of more than 3 degrees centigrade or salinity changes of more than three ppt., in any 12 hour period, nor to the total change of more than 6 degrees centigrade or six ppt. salinity during the entire transportation, quarantine, and acclimation period;

4. Organisms that touch dry surfaces, are dropped, have obvious physical deformities, or are injured during handling shall be discarded;

5. Organisms shall be handled as little as possible. When handling is required, the following equipment shall be used:

   i. Large organisms shall be handled with dipnets of the appropriate size and mesh; and

   ii. Small organisms, such as juvenile invertebrates and fish fry, shall be handled by pipeting with smooth glass tubes of approximately 4 to 8mm I.D.;

6. The preferred type of holding tank is one of flow through design which allows for a flow rate of at least two tank volumes per day. If flow-through tanks are not feasible, then holding tanks with a closed-recirculating water system, where the water is filtered through charcoal, shall be used;

7. Only laboratory grade water as specified in section 6.7 (a) shall be used to hold organisms in the laboratory; unless the organisms are to be acclimated to the dilution water for subsequent use in a bioassay immediately after the quarantine period. Under those circumstances dilution water, as specified in section 6.5 (a), shall be used.

8. Dissolved oxygen levels in holding tanks shall be kept above 60 percent of saturation at all times. If necessary, the use of aeration is acceptable;

9. Photoperiods and light intensities favorable to the organisms as specified in Standard Methods, 16th Edition, pg. 714 should be used;

10. For seasonal bioassays, the environment the organisms are maintained in should follow the natural seasonal variations. Temperature should be as follows:

    i. Depending upon the season, fathead minnows should be held at temperatures between 10 degrees - 25 degrees centigrade; and

    ii. Depending upon the season, Daphnia spp. should be held at temperatures between 10 degrees - 25 degrees centigrade; and

    iii. Depending upon the season, bluegill should be held

at temperatures between 10 degrees - 25 degrees centigrade; and

iv. Depending upon the season, opossum shrimp should be held at temperatures between 18 degrees - 28 degrees centigrade; and

v. Depending upon the season, grass shrimp should be held at temperatures between 18 degrees - 25 degrees centigrade;

vi. Depending upon the season, sheepshead minnows should be held at temperatures between 10 degrees - 30 degrees centigrade; and

vii. Depending upon the season, atlantic silversides should be held at temperatures between 10 degrees - 25 degrees centigrade.

11. Depending upon the procedure selected from section 6.6 (q) estuarine/marine test organisms shall either be held at a salinity within their optimal range or to a series of salinities, ranging from 5-35 ppt. and including at least 10, 20, and 30 ppt.

i. Optimal salinity for atlantic silversides is between 24 and 30 ppt.

ii. Optimal salinity for sheepshead minnows is between 15 and 20 ppt.

iii. Optimal salinity for grass shrimp is between 10 and 20 ppt.

iv. Optimal salinity for opossum shrimp is between 10 and 27 ppt.

12. Organisms shall not be fed for at least 24 hours prior to the test, except for opossum shrimp which shall be fed ad libitum up to and during a test. Daphnia spp. must be fed at least every 48 hours.

(o) Test organisms shall be taken from groups whose mortality while held was less than 10 percent for the seven day period prior to the bioassay.

(p) The test organisms shall be acclimated to the dilution water and the test temperature in accordance with the following requirements:

1. Fathead minnows and bluegills shall be acclimated by

gradually changing from 100 percent holding water to 100 percent dilution water over at least a 24 hour period. Temperature changes shall not exceed the criteria given in section 6.6 (n) 3.

2. All test organisms shall be exposed to 100 percent dilution water at the required test temperature for a minimum of 24 hours before they are used in the bioassay.

3. If more than five percent of the test organisms die during the 48 hour acclimation period, immediately preceding the test, the organisms shall not be used and the following procedures shall be used:

   i. The entire group of test organisms shall be discarded and a new group obtained;

   ii. The new group of organisms shall be transported, held, and acclimated in accordance with the procedures contained in section 6.6 (a) through (p); and

   iii. If more than five percent of the second group of test organisms die within 48 hours, an alternate source of dilution water shall be used.

(q) Effluents can, from time to time, consist of adulterated freshwater. Therefore, when the effluent is mixed with a saline dilution water the salinity of the mixture will be inversely proportional to the percent volume of effluent. Obviously a 100% concentration of a freshwater (1 ppt. salinity) effluent cannot be used with estuarine/marine test organisms. One of the two following procedures shall be used to deal with this situation.

1. Hold and acclimate the test organisms to a series of salinities, ranging from 5-35 ppt., staying within the natural salinity tolerances of the organism. The salinities chosen will be dependent upon the salinity of the effluent, the salinity of the dilution water, and the test concentrations of effluent used; or

2. Using dry artificial salts, adjust the salinity of the effluent to that of the dilution water so that the test concentrations will all be at one salinity. Acclimate test organisms to the dilution water at that salinity.

(r) The Standardized test temperature for all test organisms specified must be 20 degrees centigrade $\pm$ 2 degrees centigrade, unless seasonal bioassays are performed. If seasonal bioassays are performed the test temperatures used

shall be the highest average monthly temperature for the receiving water during each season. Both dilution water and effluent sample shall be warmed in a water bath to test temperature prior to test initiation.

(s) The dissolved oxygen levels in the exposure chambers shall be maintained at or above 40 percent of saturation for the fathead minnow, bluegill, sheepshead minnow, atlantic silverside, grass shrimp, and opossum shrimp by the following methods:

1. In static and modified static bioassay tests, a depression of dissolved oxygen below 45 percent of saturation shall necessitate aerating of all exposure chambers. Aeration may be accomplished by bubbling air through a 1 ml pipet at a rate of no more than 100 bubbles/min, using an air valve to control the flow. The turbulence caused by aeration should not result in a physical stress to the organism.

2. In flow-through bioassay test, if the dissolved oxygen becomes depressed below 50 percent of saturation, first increase the flow rate to the maximum, if necessary. If the increased flow does not prevent a continued decrease in dissolved oxygen then, aerate the dilution water prior to the addition of the effluent. If those two measures fail to maintain adequate dissolved oxygen, then also aerate all the exposure chambers, as described in 6.6(s)1. In the case of the 100 percent effluent concentration, aeration shall be the second measure taken after increasing flow.

(t) Short-term range-finding bioassays should be used to determine the approximate range of effluent concentrations that should be used in a subsequent short-term definitive or flow-through test. The range-finding methodology shall meet the following requirements:

1. Test duration shall be 24 hours;

2. Test type shall be either static or flow-through with the following specifications;

    i. Exposure chamber loading for fish and grass shrimp shall not exceed 2.5 grams per liter in flow-through tests and 0.4 grams per liter for static tests at temperatures above 20 degrees centigrade. Exposure chamber loading for fish and grass shrimp shall not exceed 5 grams per liter in flow-through tests and 0.8 grams per liter for static tests at temperatures of 20 degrees centigrade or

less.

3. Test organisms shall be exposed to:

   i. Three to five widely spaced effluent concentrations such as 1%, 10%, 50%; or

   ii. Progressive bisections of intervals on the logarithmic scale as described in Standard Methods, 16th Edition, pg. 712; and

   iii. A control.

4. Effluent concentrations shall be expressed as percent effluent by volume;

5. Five test organisms shall be exposed to each effluent concentration and the control;

6. Water temperature in the exposure chambers shall be maintained for the duration of the test, to with ± 2 degrees centigrade of the specified test temperature;

7. If the lowest concentration used kills all the test organisms, another test shall be set-up using a series of concentrations which starts at the lowest concentration previously tested;

8. All effluent solutions for a concentration series shall be prepared from the same sample of effluent; and

9. Any undissolved material in the effluent sample shall be dispersed uniformly by gentle agitation prior to withdrawal and aliquots of both effluent and dilution water shall be mixed well in the exposure chambers. Test organisms shall be added within 30 minutes of test initiation.

(u) Short-term definitive bioassay tests shall be used to determine the acute toxicity of an effluent. The test methodology shall meet the following requirements:

1. Test duration shall be at least 48 hours, but, if required by the Department, the test shall be continued until the toxicity curve shows that the threshold toxicity, called the Incipient LC50, has been reached;

2. Test type shall be either static, modified static (daily renewal), or flow-through with the same specifications on test organism loading as listed in section 6.6 (t) 2;

3. Test organisms shall be exposed to at least five effluent concentrations, the range of which will have been determined previously by a range-finding bioassay based on progressive bisections of intervals on the logarithmic scale as described in Standard Methods, 16th Edition, pg. 712, or a geometric series as described in Methods of Measuring Acute Toxicity - EPA, page 32, and a control. Concentrations shall be expressed as percent effluent by volume;

4. The test shall be conducted in replicate with at least twenty organisms exposed to each effluent concentration and the control. Replicates shall be true replicates with no direct water connections between them;

5. Exposure chambers shall be randomly assigned to either an effluent concentration or the control, and the test organisms shall be randomly assigned to the exposure chambers;

6. Water temperature maintenance shall be as specified in section 6.6 (t) 6;

7. Test organisms shall be acclimated to the dilution water in accordance with the procedures listed in section 6.6 (p) prior to their use in a test;

8. All effluent solutions for a concentration series shall be prepared from the same sample of effluent;

9. The following required methods apply only to conducting modified static (daily-renewal) tests:

    i. The test organisms shall be exposed to fresh solution of the same concentration of effluent every 24 hours either by transferring the test organisms from one test chamber to another or by replacing the effluent solutions in the exposure chambers;

    ii. The modified static test procedure used for opossum shrimp should follow the method described in U.S. EPA-1978, pp. 61- 63;

    iii. The procedure described in section 6.6 (t) 9 shall be followed for setting-up and beginning a modified static test;

10. The following required methods apply only to conducting flow-through tests:

i. The diluter system of the flow-through apparatus shall be in operation for at least 24 hours prior to the addition of the organisms and the beginning of the test. During this time, the water temperature, flow rate through the exposure chamber, and the effluent concentrations in the exposure chambers shall be adjusted to the test requirements.

ii. After adjustments but prior to beginning the test, there shall be at least one tank volume exchange. Flow rate through the exposure chambers shall be sufficient to maintain a minimum dissolved oxygen concentration of 40 percent of saturation and provide no less than five tank water volume changes every 24 hours.

(v) Observations of test organisms in the exposure chambers shall be made at least once every 24 hours for the duration of the test. It is suggested that observations be made of each exposure chamber at 1.5, 3, 6, 12, and 24 hours after the beginning of the test and twice a day thereafter.

(w) In short-term acute toxicity bioassays, death is the adverse effect which shall be quantified. The criterion for death shall be no movement which will include respiratory movement in fish, no movement of antenna, mouth parts or other organs in invertebrates, and no reaction to gentle prodding.

(x) Effects such as erratic swimming, loss of reflex, hyperventilation, curved spine, hemorrhaging, discoloration, changes in behavior, excessive mucus production, molting and cannibalism shall be reported.

(y) During short term tests, deaths in the controls should be virtually absent. For all fish, a control mortality of greater than or equal to 10 percent shall invalidate the test. When using grass or opossum shrimp, control mortalities of greater than or equal to 15 percent shall invalidate the test.

(z) Dissolved oxygen, pH, specific conductivity, total alkalinity, total hardness, and, when applicable, salinity shall be measured in the exposure chambers and recorded initially and at least once every day thereafter for the duration of the test.

(aa) The lengths and weights of the test organisms (when fish are used) shall be determined by sacrificing and measuring a representative sample of the stock organisms before the test and by measuring all of the test organisms after the

completion of the test. This may be accomplished by preserving both the surviving and dead fish. An acceptable alternative shall be to measure a representative sample of the test organisms, consisting of both surviving and dead fish, instead of measuring all of the test organisms.

(ab) The calculation and reporting of the results of any bioassay shall meet the following requirements:

1. The results of all bioassays are to be expressed in terms of their median lethal concentration, or LC50 for a specified time period.

2. Range-finding bioassays shall be analyzed by using the graphical interpolation method for estimating the LC50 presented in Methods for Measuring Acute Toxicity - EPA, pp. 57-58.

3. Definitive bioassays shall be analyzed by any of the following methods and with the following requirements:

   i. The LC50 values for the 24 and 48 hour exposure times, depending upon the duration of exposure, shall be estimated by methods described in Methods for Measuring Acute Toxicity - EPA, pp. 57-78, or by probit analysis or Finney's method of formal probit analysis as described in Standard Methods, 16th Edition, pgs. 715 and 716.

   ii. The 95 percent confidence or fiducial limits for the 96 hour or Incipient LC50's shall be calculated. The simplified nomographic methods of Litchfield and Wilcoxon, Methods for Measuring Acute Toxicity-EPA, are acceptable.

   iii. In order to estimate an LC50 for a definitive test by any of the aforementioned methods, partial mortalities must have occurred in at least two of the test concentrations. If these conditions are not met, the LC50 shall be estimated by the graphical interpolation method referenced in section 6.6 (ab) 2.

   iv. If the highest effluent concentration does not kill more than 65 percent of the test organisms exposed to it, the percentage of organisms killed by various concentrations of the effluent shall be reported.

   v. A toxicity curve shall be plotted using the LC 50's for each of the observation times according

to the methodology presented in Standard Methods, 16th Edition, section 801 F. 1 d pg 717. The presence or absence of an Incipient LC50, as estimated from the toxicity curve, shall be reported along with its value.

4. If the responses from two or more exposure chambers deviate from the expected trend in such a manner that a lower effluent concentration shows a more toxic response than a higher effluent concentration, then the test should be considered invalid, no estimation of the LC50 made, and the test should be repeated.

5. LC50 values shall not be estimated for any bioassay if a test is invalid under the definition given in section 6.6 (y).

(ac) The analysis of all parameters, excluding salinity, as required by this subchapter shall be conducted in accordance with the requirements set forth in 40 CFR 136 and section 4.

(ad) The determination of salinity as required in this section shall be computed from chlorinity, electrical conductivity, refractive index, or some other property whose relationship is well established.

(ae) All procedures other than those set forth in this section are considered alternative analytical methods. Laboratories shall make special application to the Department for the use of alternative analytical methods and such application shall include a showing of comparability data.

6. General Laboratory Practices

(a) Laboratory grade water shall meet the following requirements:

1. Natural or artificial sources of water may be used, but natural sources are preferred.

2. Natural sources shall be free of pollution, low in turbidity, high in dissolved oxygen, low in B.O.D., and the pH shall be favorable to the maintenance of the organisms.

3. Fresh water shall meet the following requirements:

i. Fresh water shall be constant in quality and shall not contain more than the designated amounts of the following:

(1) 20 mg/l of suspended solids;

(2) 10 mg/l of chemical oxygen demand;

(3) 20 ug/l of un-ionized ammonia;

(4) 50 ng/l of total organophosphorus pesticides;

(5) 50 ng/l of total organochloride pesticides plus PCB's; and

(6) Water shall be considered of constant quality if the monthly ranges of total alkalinity, total hardness, specific conductivity, TOC or COD, and salinity are less than 10 percent of the respective averages.

ii. Municipal water supplies are acceptable. Water from a municipal source must be passed through a filter to remove orgnic chemicals and chlorine before use.

4. Saltwater shall meet the following requirements:

i. Natural saltwater shall be from a source free of pollution, and having a pH and salinity favorable to the organism (see section 6.6 (n) 11.); and

ii. If adjustments to the salinity of natural saltwater are necessary they shall be made either by adding laboratory pure water to reduce salinity or a strong natural brine (Standard Methods, 16th Edition, pg. 701) or dry artificial sea salts to raise the salinity. Prior to use, the saltwater shall be filtered through a 20 micron filter; and

iii. Artificial saltwater may be substituted for natural saltwater if an acceptable supply of the latter is unavailable. It shall be prepared according to the methods listed in Standard Methods, 16th Edition, pg. 706, or obtained from a commercial source.

(b) The food and feeding of the test organisms shall be as follows:

1. Fish shall be fed at least once a day a combination of natural foods, either live or frozen, and any of several prepared dried foods.

2. Grass and opossum shrimp shall be fed ad libitum live

freshly hatched brine shrimp nauplii or commercial fish foods, or both.

3. Daphnia shall be fed as outlined in Methods of Measuring Acute Toxicity - EPA - pg. 97.

(c) Treatment of diseased or parasitized organisms shall be in accordance with the procedures given in Standard Methods, 16th Edition, pg. 706, and Methods for Measuring Acute Toxicity-EPA.

(d) Organisms treated for disease or parasites shall not be used in bioassays for at least 10 days after treatment.

(e) Cleaning of all chambers and equipment shall be in accordance with the following procedure:

1. Soak 15 minutes, and scrub with detergent in tap water, or clean in an automatic dishwasher.

2. Rinse twice with tap water.

3. Rinse once with fresh, dilute (20%, V:V) nitric acid or hydrochloric acid (add 20 mL of concentrated acid to 80 mL of distilled water) to remove scale, metals, and bases.

4. Rinse twice with tap water.

5. Rince once with full-strength acetone to remove organic compounds.

6. Rinse well with tap water.

7. Rinse well with dilution water or laboratory grade water.

When feasible, the above cleaning procedure must also be used for other equipment that comes in contact with the effluent, such as the dilutor system, pumps, tanks, etc. All test chambers and equipment must be thoroughly rinsed with the dilution water immediately prior to use in each test.

(f) When measuring sample volumes of more than 10 ml, graduate cylinders having an accuracy within 2.5 percent tolerance shall be used.

(g) A laboratory that has received either certification or interim approval shall accept only samples that are properly labeled and for which reasonable assurance is given that the samples have been collected, preserved, processed, stored and

transported in a manner that will assure both the identity of the sample and that the sample is sufficiently stable to be used in the requested tests or analyses. If the identity or stability of the sample has not been assured, both the chain of custody form and the laboratory report shall clearly state that the result may be invalid due to the possible misidentification or instability of the sample.

6.8 Quality Control Program

(a) An acceptable degree of precision for definitive bioassays shall be that the 95 percent confidence or fiducial intervals be within less than ± 30 percent of the 48 hour or incipient LC50 value.

(b) Each laboratory shall develop and have on file and available for inspection a written description of the current laboratory quality control program. The written description shall outline the procedures which the laboratory will use in meeting the quality control requirements set forth in sections 4.6 and 4.7. A record of analytical control tests and quality control checks on equipment and materials shall be prepared by the laboratory and retained for at least five years.

1. Laboratories shall perform the following analytical quality control tests to ensure that general laboratory practices and methodology are in compliance with the requirements of this subchapter:

   i. A reference toxicant test shall be performed to establish the validity of effluent toxicity data generated by bioassay laboratories.

      (1) Sodium dodecylsulfate, sodium pentachlorophenate, or cadmium chloride shall be used as reference toxicants. These chemicals are available from the Environmental Protection Agency's Environmental Monitoring and Support Laboratory in Cincinnati. Instructions for their use and the expected LC50 values are provided with the samples.

      (2) If the laboratory does not have an ongoing culturing program and obtains the test organisms periodically in large numbers (batches) from an outside source, the sensitivity of each batch of test organisms must be evaluated with a reference toxicant.

(i) The reference toxicant test must be conducted within the seven days immediately preceding an effluent bioassay or concurrently with the bioassay.

(ii) Laboratories that obtain test organisms from outside sources and conduct less than one bioassay per month may run all reference toxicity tests concurrently with effluent bioassays.

(3) If the laboratory maintains breeding stock, the sensitivity of the offspring should be checked with a reference toxicant at least once each quarter. This reference toxicant test may be run concurrently with an effluent bioassay.

(4) If the LC50 of reference toxicant does not fall in the expected range for the test organisms, the sensitivity of the test system are suspect. In this case, the test procedure should be examined for defects, and a different batch of test organisms should be employed in repeating the reference toxicant and effluent toxicity test.

(5) A control chart, as described in Methods for Measuring Acute Toxicity-EPA, pg. 8, should be prepared for each reference toxicant/organism combination, and successive LC50s plotted and examined to determine if the results are within prescribed limit.

(i) Data generated should be stored following the procedures described in Section 6.9.

ii. Laboratory pure water shall be analyzed for and meet the following requirements:

QUALITY OF PURIFIED WATER USED IN BIOASSAY TESTING

Test	Monitoring Frequency	Limit
Chemical tests:		
Conductivity	Continuously or with each use	>0.5 megohms resistance or < 2 umhos/cm at 25 degrees centigrade
pH	With each use	5.5 - 7.5
Total organic carbon	Monthly	<1.0 mg/L
Heavy metals, single (Cd, Cr, Cu, Ni, Pb, & Zn)	Monthly	<0.5 mg/L
Heavy metals, total	Monthly	≤1.0 mg/L
Ammonia/organic nitrogen	Monthly	<0.1 mg/L
Total chlorine residual	Monthly or with each use	<detection limit
Bacteriological tests:		
Heterotrophic plate count (See Section 907)*	Monthly	<1000 colonies/mL
Water quality test (See 3c1)*	Annually and for a new source	0.8 - 3.0 ratio
Use test (See 3d)*	Annually and for a new source	Student's t ≤ 2.78

*Refers to 16th Edition of Standards Methods Manual.

iii. Laboratory pure water checks shall be performed on a frequency as specified in the above table and documented.

iv. Laboratory grade fresh water shall be analyzed at least twice annually for the materials specified in section 6.7 (a) (3) (i);

v. There shall be available at all times, in the immediate area of laboratory personnel engaged in examining samples and performing related procedures within a category, current laboratory manuals or other complete written descriptions and instructions relating to:

(1) The analytical methods to be used by those personnel, properly designated and dated to reflect the most recent supervisory reviews;

(2) Pertinent current literature references; and

(3) Such written descriptions and instructions may be supplemented by, but not replaced by, textbooks relating to the particular analytical methods and procedures employed by such personnel;

vi. Only the laboratory director or supervisor shall make changes in laboratory procedure and those changes shall only be effective when put in writing.

vii. The following procedures shall be followed in performing quality control checks of laboratory media, equipment, and supplies:

(1) Each pH meter shall be cleaned immediately after each use period and calibrated prior to usage using two pH buffer standards and records of each calibration shall be maintained; buffer aliquots shall not be used more than once; and commercial buffer solutions shall be dated at the time of initial use; it is recommended that the buffer standards bracket the value to be measured;

(2) Top loader or pan balances shall be calibrated annually, calibration shall be checked monthly against class "s" weights, and a record shall be made of each calibration check;

(3) The accuracy of all thermometers used to monitor temperature shall be verified by comparing the readings of such thermometers with the readings of a certified thermometer. A record shall be made containing the identification number of each thermometer, the temperature displayed on the certified thermometer and the thermometer being verified, correction factors when applicable, dates on which quality control checks were performed, and the name of the analyst performing such checks. Glass thermometers shall be verified yearly and metal thermometers shall be verified quarterly.

(4) The temperature of air or water-jacketed incubators, aluminum block incubators, water

baths, and incubator rooms shall be either recorded continuously or recorded daily from in-place thermometers immersed in liquid and placed on at least one of the shelves in use.

(5) Date, time and temperature shall be either recorded continuously, or recorded individually during each sterilization cycle of the autoclave;

(6) Each hot air oven shall be equipped with a thermometer, the bulb of which shall be placed in sand, or with a temperature recording device, and records shall be maintained showing the date, time and temperature of each sterilization cycle;

(7) The temperature of each refrigerator shall be either recorded continuously or recorded daily from an in-place thermometer immersed in liquid and placed on at least one of the shelves in use;

(8) All reagents and solutions shall be labelled to indicate identity and, when applicable, titer, strength or concentration, recommended storage requirements, preparation or expiration date, and other information pertinent to identification;

(9) Materials of substandard reactivity and deteriorated materials shall not be used; and

(10) All outdated material shall be discarded immediately.

(c) The temperature, dissolved oxygen concentrations, flow rate through the exposure chambers and the maintainance of effluent concentrations, shall be checked initially, daily during the duration of the test, and upon completion of the test, adjusted as necessary, and documentation of these adjustments and measurements shall be made.

6.9 Records and Data Reporting

(a) Each laboratory shall maintain records and report data in accordance with the requirements set out in this section.

(b) Records of bioassay analysis shall be kept by the laboratory for not less than five years. This requirement is equally

applicable to all raw data, quality control data, quality assurance, data chain of custody forms and laboratory reports.

(c) The following information shall be kept in a bound notebook by the laboratory as part of the daily log of feeding, behavioral observations, and mortality of organisms during holding and acclimation:

1. Water temperature of holding tanks;

2. Air temperature in culturing/holding room;

3. Mortalities of organisms per holding tank;

4. Analysis of laboratory grade water as specified in sections 6.8 (b) and 6.7 (a) (3) (i);

5. Food and feeding schedule; and

6. General observations of behavior and condition.

(d) A sample report form shall be completed immediately after collection of either dilution water or effluent composite or grab samples and shall state the sampling location, date and time of collection, chlorine residual, collector's name, and any remarks.

(e) The bioassay experimental results shall be reported in accordance with the specifications given in Methods for Measuring Acute Toxicity-EPA, pp. 24-25, and;

1. The incipient LC50 shall be reported if applicable.

2. A figure showing the toxicity curve shall be included.

(f) The original or true duplicate of the results of the bioassay shall be sent promptly to the person who requested such test and shall be signed by the laboratory manager or a designee whose designation is in writing and has been submitted to the Department.

Section 7 - Criteria and Procedures for Solid and Hazardous Waste Testing and Analysis.

7.1 Scope

This section establishes the Department's requirements which a certified laboratory or a laboratory seeking certification shall

continually meet and follow when performing solid and hazardous waste analyses.

The Department incorporates from the latest U.S. EPA SW-846 manual all the standards, criteria, sample and analytical procedures and methodology, quality assurance and quality control specified for evaluation and certification purposes under this section.