

recommendations on disallowed costs resulting from the final audit.

§ 1210.72 Subsequent adjustments and continuing responsibilities.

(a) The closeout of an award does not affect any of the following.

(1) The right of the NHPRC to disallow costs and recover funds on the basis of a later audit or other review.

(2) The obligation of the recipient to return any funds due as a result of later refunds, corrections, or other transactions.

(3) Audit requirements in § 1210.26.

(4) Property management requirements in §§ 1210.31 through 1210.37.

(5) Records retention as required in § 1210.53.

(b) After closeout of an award, a relationship created under an award may be modified or ended in whole or in part with the consent of the NHPRC and the recipient, provided the responsibilities of the recipient referred to in § 1210.73(a), including those for property management as applicable, are considered and provisions made for continuing responsibilities of the recipient, as appropriate.

§ 1210.73 Collection of amounts due.

(a) Any funds paid to a recipient in excess of the amount to which the recipient is finally determined to be entitled under the terms and conditions of the award constitute a debt to the Federal Government. If not paid within a reasonable period after the demand for payment, the NHPRC may reduce the debt by:

(1) Making an administrative offset against other requests for reimbursements;

(2) Withholding advance payments otherwise due to the recipient; or

(3) Taking other action permitted by statute.

(b) Except as otherwise provided by law, the NHPRC shall charge interest on an overdue debt in accordance with 4 CFR Chapter II, "Federal Claims Collection Standards."

Appendix A to Part 1210—Contract Provisions

All contracts, awarded by a recipient including small purchases, shall contain the following provisions as applicable:

1. Equal Employment Opportunity—All contracts shall contain a provision requiring compliance with E.O. 11246, "Equal Employment Opportunity," as amended by E.O. 11375, "Amending Executive Order 11246 Relating to Equal Employment Opportunity," and as supplemented by regulations at 41 CFR part 60, "Office of Federal Contract Compliance Programs, Equal Employment Opportunity, Department of Labor."

2. Copeland "Anti-Kickback" Act (18 U.S.C. 874 and 40 U.S.C. 276c)—All contracts and subgrants in excess of \$2,000 for construction or repair awarded by recipients and subrecipients shall include a provision for compliance with the Copeland "Anti-Kickback" Act (18 U.S.C. 874), as supplemented by Department of Labor regulations (29 CFR part 3, "Contractors and Subcontractors on Public Building or Public Work Financed in Whole or in Part by Loans or Grants from the United States"). The Act provides that each contractor or subrecipient shall be prohibited from inducing, by any means, any person employed in the construction, completion, or repair of public work, to give up any part of the compensation to which he is otherwise entitled. The recipient shall report all suspected or reported violations to the Federal awarding agency.

3. Davis-Bacon Act, as amended (40 U.S.C. 276a to a-7)—When required by Federal program legislation, all construction contracts awarded by the recipients and subrecipients of more than \$2,000 shall include a provision for compliance with the Davis-Bacon Act (40 U.S.C. 276a to a-7) and as supplemented by Department of Labor regulations (29 CFR part 5, "Labor Standards Provisions Applicable to Contracts Governing Federally Financed and Assisted Construction"). Under this Act, contractors shall be required to pay wages to laborers and mechanics at a rate not less than the minimum wages specified in a wage determination made by the Secretary of Labor. In addition, contractors shall be required to pay wages not less than once a week. The recipient shall place a copy of the current prevailing wage determination issued by the Department of Labor in each solicitation and the award of a contract shall be conditioned upon the acceptance of the wage determination. The recipient shall report all suspected or reported violations to the Federal awarding agency.

4. Contract Work Hours and Safety Standards Act (40 U.S.C. 327-333)—Where applicable, all contracts awarded by recipients in excess of \$2,000 for construction contracts and in excess of \$2,500 for other contracts that involve the employment of mechanics or laborers shall include a provision for compliance with Sections 102 and 107 of the Contract Work Hours and Safety Standards Act (40 U.S.C. 327-333), as supplemented by Department of Labor regulations (29 CFR part 5). Under Section 102 of the Act, each contractor shall be required to compute the wages of every mechanic and laborer on the basis of a standard work week of 40 hours. Work in excess of the standard work week is permissible provided that the worker is compensated at a rate of not less than 1½ times the basic rate of pay for all hours worked in excess of 40 hours in the work week. Section 107 of the Act is applicable to construction work and provides that no laborer or mechanic shall be required to work in surroundings or under working conditions which are unsanitary, hazardous or dangerous. These requirements do not apply to the purchases of supplies or materials or articles ordinarily available on the open

market, or contracts for transportation or transmission of intelligence.

5. Rights to Inventions Made Under a Contract or Agreement—Contracts or agreements for the performance of experimental, developmental, or research work shall provide for the rights of the Federal Government and the recipient in any resulting invention in accordance with 37 CFR part 401, "Rights to Inventions Made by Nonprofit Organizations and Small Business Firms Under Government Grants, Contracts and Cooperative Agreements," and any implementing regulations issued by the awarding agency.

6. Clean Air Act (42 U.S.C. 7401 *et seq.*) and the Federal Water Pollution Control Act (33 U.S.C. 1251 *et seq.*), as amended—Contracts and subgrants of amounts in excess of \$100,000 shall contain a provision that requires the recipient to agree to comply with all applicable standards, orders or regulations issued pursuant to the Clean Air Act (42 U.S.C. 7401 *et seq.*) and the Federal Water Pollution Control Act as amended (33 U.S.C. 1251 *et seq.*). Violations shall be reported to the Federal awarding agency and the Regional Office of the Environmental Protection Agency (EPA).

7. Byrd Anti-Lobbying Amendment (31 U.S.C. 1352)—Contractors who apply or bid for an award of \$100,000 or more shall file the required certification. Each tier certifies to the tier above that it will not and has not used Federal appropriated funds to pay any person or organization for influencing or attempting to influence an officer or employee of any agency, a member of Congress, officer or employee of Congress, or an employee of a member of Congress in connection with obtaining any Federal contract, grant or any other award covered by 31 U.S.C. 1352. Each tier shall also disclose any lobbying with non-Federal funds that takes place in connection with obtaining any Federal award. Such disclosures are forwarded from tier to tier up to the recipient.

8. Debarment and Suspension (E.O. 12549 and E.O. 12689)—No contract shall be made to parties listed on the General Services Administration's List of Parties Excluded from Federal Procurement or Nonprocurement Programs in accordance with E.O. 12549 and E.O. 12689, "Debarment and Suspension." This list contains the names of parties debarred, suspended, or otherwise excluded by agencies, and contractors declared ineligible under statutory or regulatory authority other than E.O. 12549. Contractors with awards that exceed the small purchase threshold shall provide the required certification regarding its exclusion status and that of its principal employees.

Dated: August 31, 1995.

John W. Carlin,

Archivist of the United States.

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ENVIRONMENTAL PROTECTION AGENCY**40 CFR Part 136**

[WH-FRL-5308-7]

RIN 2040-AC54

Whole Effluent Toxicity: Guidelines Establishing Test Procedures for the Analysis of Pollutants**AGENCY:** Environmental Protection Agency (EPA).**ACTION:** Final rule.

SUMMARY: This final rule amends the "Guidelines Establishing Test Procedures for the Analysis of Pollutants," 40 CFR part 136, to add whole effluent toxicity (WET) testing methods to the list of Agency approved methods in Tables IA and II, under the Clean Water Act. This action amends 40 CFR 136.3 (Tables IA and II) by adding methods for measuring the acute and short-term chronic toxicity of effluents and receiving waters.

This rulemaking was initiated at the request of the States. The overall benefit of today's rulemaking is that it will reduce costs and eliminate the confusion caused by the multiple versions of any one test method currently in use. For example, currently, an industry with facilities in six different states may be required to conduct six different versions of the same test method. EPA estimates that standardizing these approved methods could save the regulated community up to 20% of the current test method costs, which range from \$160.00-\$2240.00, depending upon the test method. This rulemaking will also reduce the current resource burden in the States because they will no longer need to justify the inclusion of WET monitoring or WET limits in National Pollution Discharge Elimination System (NPDES) permits on a case-by-case basis.

This rule incorporates three technical documents, by reference, thereby dramatically reducing the number of pages included in today's Federal Register. A listing of these documents and where they can be viewed or obtained can be found in section VIII of the preamble.

Methods for measuring mutagenicity (changes in genes or chromosomes) or for monitoring viruses in wastewaters and sludges that were included in the December 1989 proposal are not included in this final rule. When better scientific methods for measuring mutagenicity and viruses become available, the Agency will evaluate them for possible inclusion in 40 CFR part

136. Finally, the methods for marine chronic toxicity in today's rule do not apply to discharges into marine waters of the Pacific Ocean. Methods addressing such discharges will be proposed at a later date.

EFFECTIVE DATE: This final rule becomes effective November 15, 1995. The incorporation by reference of certain publications listed in this regulation is approved by the Director of the Office of Federal Register on November 15, 1995.

In accordance with 40 CFR 23.2, this rule shall be considered issued for the purposes of judicial review October 26, 1995, at 1 p.m. eastern daylight time. Under section 509(b)(1) of the Clean Water Act, judicial review of these amendments can be obtained only by filing a petition for review in the United States Court of Appeals within 120 days after they are considered issued for the purposes of judicial review. Under section 509(b)(2) of the Clean Water Act, the requirements of these amendments may not be challenged later in civil or criminal proceedings to enforce these requirements.

ADDRESSES: The public record and all supporting materials pertinent to the development of this final rule, including response to comments received on the December 1989 proposal, are available for inspection at the Water Docket located at the U.S. Environmental Protection Agency, 401 M Street SW., Washington, DC 20460. For access to the Docket materials, call (202) 260-3027 between 9 a.m. and 3:30 p.m. A listing, of where to view or obtain copies of the three manuals incorporated by reference in today's rulemaking, can be found in section VIII of the preamble.

FOR FURTHER INFORMATION CONTACT: Ms. Margarete A. Heber, Health and Ecological Criteria Division, Office of Science and Technology, (Mail Code 4304) U.S. Environmental Protection Agency, 401 M St. SW., Washington, DC 20460 or call (202) 260-0658; or Ms. Teresa Norberg-King, Environmental Research Laboratory, U.S. Environmental Protection Agency, 6201 Congdon Boulevard, Duluth, MN 55804.

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 - I. Authority
 - EPA is promulgating this rule under the authority of sections 301, 304(h), and 501(a) of the Clean Water Act ("CWA" or the "Act"), 33 U.S.C. 1251 et seq., 33 U.S.C. 1311, 1314(h), 1361(a). Section 301 of the Act prohibits the discharge of any pollutant into navigable waters unless the discharge complies with certain requirements of the Act, including a requirement for a National Pollutant Discharge

Elimination System ("NPDES") permit issued pursuant to CWA section 402. Section 304(h) of the Act requires the Administrator to "promulgate guidelines establishing test procedures for the analysis of pollutants that shall include the factors which must be provided in any certification pursuant to (CWA section 401) or permit applications pursuant to (CWA section 402)." 33 U.S.C. 1314(h). Section 501(a) authorizes the Administrator "to prescribe such regulations as are necessary to carry out his function under the Act." 33 U.S.C. 1361(a).

II. Regulatory Background

A. Analytical Methods Under 40 CFR Part 136

The CWA establishes two principal bases for the incorporation of effluent limitations in NPDES permits. Effluent limitations implement both technology-based and water quality-based requirements of the Act. Technology-based limitations represent the degree of control that can be achieved using various levels of pollution control technology. In addition to the technology-based effluent limitations, the Act directs the states, with federal approval and oversight, to establish water quality-based standards to assure protection of the quality of state waters. The state standards designate uses for navigable waters and establish water quality criteria to protect such uses. If necessary to achieve compliance with applicable water quality standards, NPDES permits must contain water quality-based limitations more stringent than the applicable technology-based standards.

To ensure compliance with these effluent limitations, EPA has promulgated regulations providing nationally-approved testing procedures in 40 CFR part 136. Approved analytical test procedures also must be used for the analysis of pollutants in permit applications, discharge monitoring reports, state certification under CWA section 401, as well as determining compliance with pretreatment standards issued under CWA section 307. Test procedures have previously been approved for 262 different parameters (Table 1, 40 CFR 136.3). Approved test procedures apply to the analysis of bacteriological, inorganic (metal, non-metal, mineral, nutrient, demand, residue) and physical, non-pesticide organic, pesticide, and radiological parameters. Today's rule adds methods to the list of nationally-approved methods. Regulations also provide a mechanism for the approval of alternate analytical methods at 40 CFR 136.4.

Under this regulation, the Administrator may approve alternate test procedures developed and proposed by dischargers or other persons.

Finally, there may be discharges that require limitations for certain parameters using test procedures not yet approved under 40 CFR part 136. Under 40 CFR 122.41(j)(4) and 122.44(i)(1)(iv) permit writers may include, through permit proceedings, parameters requiring the use of test procedures that are not approved part 136 methods. EPA also may include such parameters in accordance with the provisions prescribed at 40 CFR 401.13, "Test Procedures for Measurements." Many of the whole effluent toxicity testing methods, incorporated by reference in today's rulemaking, have been included in NPDES permits utilizing the provisions in 40 CFR 122.41(j)(4). Today's rulemaking will relieve the NPDES permit writers of having to include these test methods on a case-by-case-basis. By the same token, the test methods standardized in today's rule will replace unapproved test methods (or variations thereof) for NPDES permits issued after the effective date of today's rule. Existing NPDES permits need not be re-opened to include test methods from today's rule.

B. Toxicity Testing

Until recently, EPA programs for the control of toxic discharges were based largely on effluent limitations for individual chemicals. EPA has developed water quality criteria for many pollutants based on comprehensive testing and evaluation that, unlike whole-effluent testing, considers a variety of toxic endpoints, including human health impact and bioaccumulation. Once a water quality criterion is developed, it can be used to develop a state numeric criteria within a water quality standard (40 CFR 131.11(b)) and/or permit limit to ensure that the level of that toxicant in the discharge does not exceed the water quality standard (40 CFR 122.44(d)(1)(iii) & (iv)).

Data on the toxicity of substances to aquatic organisms, however, are available for only a limited number of elements and compounds. Effluent limitations on specific compounds, therefore, do not necessarily provide adequate protection for aquatic life when the toxicity of effluent components is not known, effects of effluent components are additive, synergistic, or antagonistic, and/or when an effluent has not been chemically characterized. In such situations, EPA and the States can use biological methods to examine the

whole effluent toxicity, rather than attempt to identify all toxic pollutants, determine the effects of each pollutant individually, and then attempt to assess their collective effect.

When whole effluent toxicity testing is used, toxicity itself is a pollutant parameter. The toxicants creating that toxicity need not be specifically identified to limit the effluent's toxicity. An analogy between effluent toxicity and biochemical oxygen demand (BOD) can be drawn. Both are measurements of a biological effect. Both can be quantified. In neither case are the causative agents of the biological effect specifically identified. Thus, whole effluent toxicity is like BOD in that it is a useful parameter for characterizing an undesirable effect caused by the discharge of a complex mixture of waste materials.

The Declaration of Goals and Policy at Section 101(a)(3) of the Act states that "it is the national goal that the discharge of toxic pollutants in toxic amounts be prohibited." Section 502 (13), describes toxic pollutants as "* * * those pollutants, or combinations of pollutants, including disease-causing agents, which after discharge and upon exposure, ingestion, inhalation or assimilation into any organism, either directly from the environment or indirectly by ingestion through food chains, will, on the basis of information available to the Administrator, cause death, disease, physiological malfunctions, behavioral abnormalities, physical deformation, birth defects, genetic mutations, and cancer." Today's rule establishes procedures to measure some of these effects. Owners or operators of NPDES facilities may be required as a permit application or permit condition to perform one or more of these tests methods to assure compliance with relevant water quality standards. Both the D.C. and Ninth Circuit Courts of Appeals have recently upheld EPA's authority to set and measure limits on toxicity without regulating specific toxic pollutants (*NRDC v. EPA* 859 F.2d 156 (D.C. Cir. 1988); *NRDC v. EPA* 863 F.2d 1426 (9th Cir. 1988)).

C. EPA's Whole Effluent Toxicity (WET) Policy

To achieve the goals of the Federal water pollution control legislation, extensive effluent toxicity screening programs were conducted during the 1970s by the EPA regional and state programs and permittees. Acute toxicity tests (USEPA, 1975, Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians, National Water Quality Research

Laboratory, Duluth, Minnesota; USEPA, 1978, Environmental Monitoring and Support Laboratory, USEPA, Cincinnati, Ohio, EPA/600/4-78/012) were used to measure effluent toxicity and to estimate the effects of toxic effluents on aquatic life in receiving waters. During this period, short-term inexpensive methods were not available to detect the more subtle, low-level, long-term (chronic), adverse effects (such as reduction in growth and reproduction, and occurrence of terata) of effluents on aquatic organisms. Rapid developments in toxicity test methods since 1980, however, have resulted in the availability of several methods that permit detection of the low-level, adverse effects (chronic toxicity) of effluents to freshwater and marine organisms in nine days or less.

As a result of the increased awareness of the value of effluent toxicity test data for toxics control in the water quality program and the NPDES permit program, EPA issued a national policy statement entitled, "Policy for the Development of Water Quality-Based Permit Limitations for Toxic Pollutants," in the Federal Register (49 FR 9016, Mar. 9, 1984). This policy statement was updated in a document entitled, "Whole Effluent Toxicity (WET) Control Policy," published by EPA in July 1994 (EPA 833-B-94-002).

The policy recommended the use of toxicity data to assess and control the discharge of toxic pollutants to the nation's waters through the NPDES permits program. The policy stated: "Biological testing of effluents is an important aspect of the water quality-based approach for controlling toxic pollutants. Effluent toxicity data, in conjunction with other data, can be used to establish control priorities, assess compliance with state water quality standards, and set permit limitations to achieve those standards."

The policy also addressed the technical approach for assessing and controlling the discharge of toxic pollutants to the nation's waters through the NPDES permit program, and discussed the application of chemical and biological methods for assuring the regulation of effluent discharges in accordance with federal and state requirements. The policy stated that "EPA will use an integrated strategy consisting of both biological and chemical methods to address toxic and non-conventional pollutants from industrial and municipal sources. In addition to enforcing specific discharge limits for toxic pollutants, EPA and the States will use biological techniques and available data on the biological effects of chemicals to assess toxicity

impacts and human health hazards based on the general standards of 'no toxic materials in toxic amounts'."

Additional guidance on the implementation of biomonitoring and the use of effluent and receiving water toxicity data is available in a technical support document published by the EPA Office of Water ("Technical Support Document for Water Quality-Based Toxics Control," March 1991, EPA/505/2-90/001; PB91-127415).

Since the 1984 Agency policy, the use of effluent toxicity tests has increased steadily within the EPA and State NPDES programs to identify toxic discharges, and by permittees as a self-monitoring tool (USEPA, 1979, Interim NPDES Compliance Biomonitoring Inspection Manual, Washington, DC). Regulatory authorities must now establish whole effluent toxicity limits where necessary to meet the requirements of 40 CFR 122.44(d) (54 FR 23868, Jun. 2, 1989). The 1989 rule, which clarified EPA's Surface Water Toxics Control Program, defined "whole effluent toxicity" and described procedures for determining whether an NPDES permit must include a water quality-based effluent limitation. The regulation also addressed procedures for deriving effluent limits from state narrative or numeric water quality criteria. At that time, EPA noted that protocols and guidance documents used to perform toxicity tests were only recommended. With today's rule, when NPDES permits require whole effluent toxicity limits, testing must be conducted according to the toxicity test protocols described in the test manuals cited in Table IA, 40 CFR part 136, as amended (except for chronic toxicity limitation for discharges into marine waters of the Pacific Ocean).

The Environmental Monitoring Systems Laboratory—Cincinnati (EMSL-Cincinnati) developed standard test procedures and published standardized acute and chronic toxicity tests methods to minimize intralaboratory and interlaboratory variability in toxicity tests conducted by EPA regional and state programs and NPDES permittees.

D. Proposed Rule Published December 4, 1989

On December 4, 1989, EPA proposed at 54 FR 50216 to add the following methods to Table IA, 40 CFR part 136: (1) Methods to measure the acute toxicity of effluents and receiving waters to freshwater and marine organisms, (2) short-term methods to estimate the chronic toxicity of effluents and receiving waters to freshwater, estuarine, and marine organisms, (3) methods to measure the mutagenicity

(genotoxicity) of wastewaters, sludges, and surface waters, and (4) methods to recover, enumerate, and identify human enteric viruses in wastewater, sludges, and surface waters. Changes were also proposed for Table II, on sample preservation and holding times. EPA provided a 60-day public comment period.

In response to the Proposed Rule, comments were received from a broad cross-section of public and private agencies, including major trade organizations, large industries, large environmental consulting firms, universities, state and interstate water pollution control agencies, and other Federal agencies. A summary of the major comments concerning acute and chronic testing for freshwater and marine organisms, and EPA's responses to them, are addressed below. Responses to the remainder of the comments are contained in the Supplementary Information Document (SID) portion of the rulemaking record. The entire Water Docket is available for inspection from 9 to 3:30 p.m. at 401 M St SW., Washington DC 20460. Call (202) 260-3027 for an appointment.

In addition, the Agency decided not to finalize the test methods proposed to measure the mutagenicity (genotoxicity) of wastewaters, sludges, and surface waters; and methods to recover, enumerate, and identify human enteric viruses in wastewater, sludges, and surface waters. In the mid 1980s, the Agency believed that a simple test like the Ames test could be used as a predictor of chronic health effects (*i.e.* carcinogenicity). However, this test produces many false results, and, thus, could potentially confuse or mislead regulators. Presently, the Agency is working on different methods to recover, enumerate, and identify human enteric viruses, and so the methods proposed are no longer representative of the best available science.

III. Biological Methods Included in the Final Rule

A. Basis for Approval

Many of the comments received on the proposed rule were helpful in identifying ambiguities and minor inconsistencies in the aquatic toxicity test methods which had been published at different times during the seven years preceding the proposal. This was particularly true with regard to the comment received from numerous commenters to reformat the three manuals to make them both consistent with each other and easier to use. The biological methods added to Table IA, 40 CFR part 136, in this final rule are

described below, and are included in the rulemaking docket.

The tests have been validated in a number of studies conducted by EPA, state programs, and universities. The methods are well established and are currently being implemented in a number of NPDES permits. Furthermore, each of the methods has extensive guidance on quality assurance and routine quality control activities.

Information on the single laboratory precision of the methods is included in the respective short-term test manuals in the rulemaking docket. The methods in this rule have precision profiles comparable to previously established part 136 methods. The Agency stands behind the conclusion that the biological methods in this rule are applicable for use in NPDES permits.

B. Summary of Methods to Measure the Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms

The three aquatic toxicity test manuals cited at 54 FR 50216 have been revised as a result of public comment on the proposed rule. The revised editions, discussed below, are as follows: (1) USEPA. 1993. *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, Fourth Edition, EPA/600/4-90/027F; (2) USEPA. 1994. *Short-term Methods to Estimate the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, Third Edition, July 1994, EPA/600/4-91/002; and (3) USEPA. 1994. *Short-term Methods to Estimate the Chronic Toxicity of Effluents and Receiving Waters to Estuarine and Marine Organisms*, Second Edition, July 1994, EPA/600/4-91/003.

1. Methods to Measure the Acute Toxicity of Effluents and Receiving Waters to Freshwater Estuarine and Marine Organisms

This rule includes methods to measure the acute toxicity of effluents and receiving waters to freshwater and marine fish and invertebrates, as described in the EPA methods manual, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* (EPA/600/4-90/027F). This methods manual represents the fourth edition of the acute toxicity test manual first published by EMSL-Cincinnati in 1978 (EPA/600/4-78/012). The methods, developed with the assistance of the Agency's Toxicity Assessment Subcommittee of the Biological Advisory Committee, are periodically updated, expanded, and republished.

Any such changes, however, will be published in the Federal Register prior to their effective date for regulatory purposes. The most recent (third) edition was published in 1985 (EPA/600/4-85/013).

The current manual (EPA/600/4-90/027F) describes tests for effluents and receiving waters, and includes guidelines for the following areas: Laboratory safety; quality assurance; facilities and equipment; effluent sampling and holding times; dilution water; test species selection, culturing, and handling; data collection, interpretation and utilization; report preparation; and dilutor and mobile toxicity test laboratory design.

The acute toxicity tests in the manual generally involve exposure of any of 20 test organisms to each of five effluent concentrations and a control water. The test duration depends on the objectives of the test and the test species, and ranges from 24-96 hours. The manual includes a list of freshwater and marine test organisms, and specified test conditions for 10 commonly used freshwater and marine organisms—*Ceriodaphnia dubia*, *Daphnia magna*, *Daphnia pulex*, fathead minnows (*Pimephales promelas*), rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), mysids (*Mysidopsis bahia* and *Holmesimysis costata*), Bannerfish shiners (*Notropis leedsii*), sheepshead minnows (*Cyprinodon variegatus*), and silversides (*Menidia menidia*, *M. beryllina*, and *M. peninsulae*). The organisms and test conditions are selected by the user (e.g. permitting authority for NPDES permits) depending on the objectives of the test and the effluent and receiving water characteristics.

The tests are used to determine the effluent concentration, expressed as a percent volume, that within the prescribed test period causes death in 50% of the organisms (LC50), or whether survival in a given (single) concentration of effluent, or in receiving water, is significantly different than in controls. Where death is not easily detected, e.g., with some invertebrates like *Ceriodaphnia* and *Daphnia*, immobilization is considered equivalent to death. Procedures for determining the LC50 include the graphical method, the Probit method and the trimmed Spearman-Kärber method. Where survival in a single effluent concentration or in receiving water is compared to survival in the control to determine if they are significantly different, a hypothesis test, Dunnett's Test, is used. Copies of computer programs for statistical analysis of the

data referred to in the manual are available from EMSL-Cincinnati.

End-of-the-pipe effluent toxicity data are used to predict potential acute and chronic toxicity of effluents in the receiving water, based on the LC50 and appropriate dilution, application, and persistence factors. The tests can be conducted as a part of self-monitoring permit requirements, compliance evaluation inspections, compliance biomonitoring inspections, compliance sampling inspections, toxics sampling inspections, performance audit inspections, and special investigations. The tests can be performed in a central test laboratory or on-site by the regulatory agency or the permittee. Acute toxicity tests can be used in toxicity reduction evaluations to identify toxic waste streams within plants, to aid in the development and implementation of toxicity reduction plans, and also can be used to compare and control the effectiveness of various treatment technologies for a given type of industry, irrespective of the receiving water (49 FR 9016, Mar. 9, 1984).

Several types of acute toxicity tests are described, including static non-renewal, static renewal, and flow-through. The selection of the test type will depend upon the objectives of the test, available resources, requirements of the test organisms, and effluent characteristics, such as fluctuations in effluent toxicity. Special environmental requirements of some organisms (such as flowing water, or fluctuating water levels) may preclude the use of static tests.

Static tests include: (1) Non-renewal tests in which the test organisms are exposed to the same effluent solution or receiving water for the duration of the test, and, (2) renewal tests in which the organisms are exposed to a fresh test solution every 24 hours or other prescribed interval, either by transferring the test organisms from one test chamber to another or by replacing all or a portion of the effluent solution in the test chambers. Sample renewal reduces some of the possible effects of factors which may affect the apparent toxicity of the effluent, such as toxicant adsorption on the walls of the test chambers, biodegradation and/or chemical transformation of the toxicants, volatilization, and uptake and metabolism of toxicants by test organisms.

Two types of flow-through tests are described: (1) Effluent is pumped continuously from the sampling point directly to the dilutor system; and (2) effluent grab or composite samples are collected periodically, placed in a tank adjacent to the test laboratory, and

pumped continuously from the tank to the dilutor system. The flow-through method employing continuous effluent sampling is the preferred method for on-site tests. Because of the large volume (often 400 L/day) of effluent normally required, flow-through tests are generally considered too costly and impractical to conduct at off-site laboratories.

Parameters and Units:

The results of the test are reported as the LC50 (Lethal Concentration—50), which is the concentration of effluent causing death (or immobilization, or other adverse effect) in 50% of the test organisms or, in the case of single concentration tests, a statistically significant increase in lethality in the effluent sample as compared to the control.

Precision:

Data on single laboratory precision (intra-) and multi-laboratory (inter-) precision from tests with reference toxicants are provided in the manual (EPA/600/4-90/027F).

2. Short-Term Methods to Estimate the Chronic Toxicity of Effluents and Receiving Waters to Freshwater, Estuarine, and Marine Organisms

Today's rule includes two sets of short-term chronic toxicity test methods: (1) Four methods for freshwater organisms and (2) six methods for estuarine and marine organisms, found in the EPA methods manuals, Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Third Edition (EPA/600/4-91/002) July 1994, and Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Estuarine and Marine Organisms, Second Edition (EPA/600/4-91/003) July 1994, respectively. The tests are used to estimate one or more of the following: (1) The chronic toxicity of effluents collected at the end of the discharge pipe and tested with a standard dilution water; (2) the chronic toxicity of effluents collected at the end of the discharge pipe and tested with dilution water consisting of receiving water collected upstream or beyond the influence of the outfall, or with other uncontaminated surface water or standard dilution water having approximately the same hardness or salinity as the receiving water, depending on the nature of the receiving water (fresh or saline) and test organisms; (3) the toxicity of diluted effluent in the receiving water downstream or at increasing distance from the outfall; and (4) the effects of

multiple discharges on the quality of the receiving water. The tests may also be useful in developing site-specific water quality criteria.

The use of short-term, subchronic, and chronic toxicity tests in the NPDES Program is recommended in the 1984 EPA policy on water-quality based permit limits, and subsequently can be required under 40 CFR 122.44(d). The short-term chronic methods are more effective analytical tools because they provide a more comprehensive prediction of the effects of toxic effluents on aquatic life in receiving waters than is provided by acute toxicity tests, at a greatly reduced level of effort compared to earlier chronic toxicity test methods (i.e. fish full-life-cycle chronic and 30-day early life-stage tests, and the 21- to 28-day invertebrate life-cycle tests). The endpoints generally used in chronic tests are survival, growth, and reproduction. The effects include the synergistic, antagonistic, and additive effects of all the chemical, physical, and biological components that adversely affect the physiological and biochemical functions of the test organisms.

(a) Short-Term Chronic Toxicity Test Methods for Freshwater Organisms. The approved toxicity test methods for freshwater organisms are found in the manual, Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Third Edition (EPA/600/4-91/002) July 1994. The manual describes four- to seven-day methods for estimating the chronic toxicity of effluents and receiving waters to three species: (1) The fathead minnow, *Pimephales promelas*; (2) the cladoceran, *Ceriodaphnia dubia*; and (3) the alga, *Selenastrum capricornutum*.

Guidelines are also included on laboratory safety, quality assurance, facilities and equipment, dilution water, effluent sampling and holding, data analysis, report preparation, and organism culturing and handling. Copies of computer programs for statistical analysis of the data referred to in the manual, are available from EMSL-Cincinnati. The approved short-term chronic tests are:

METHOD 1000.0:

Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test. Larvae (preferably less than 24 hours old) are exposed in a static renewal system to a control water and at least five concentrations of effluent, or to receiving water for seven days. Test results are determined on the survival and weight of the larvae in test solutions, compared to the controls.

METHOD 1001.0:

Fathead Minnow (*Pimephales promelas*) Embryo-larval Survival and Teratogenicity Test. Fathead minnow embryos are exposed in a static renewal system to a control water and at least five different concentrations of effluent, or to receiving water, from shortly after egg fertilization to hatch, and the larvae are exposed an additional four days posthatch (total of eight days). Test results are determined on the combined frequency of both mortality and gross morphological deformities (terata) in test solutions, compared to the controls. The test is useful for screening for teratogens because organisms are exposed during embryonic development.

METHOD 1002.0:

Ceriodaphnia dubia Survival and Reproduction test. *Ceriodaphnia* neonates are exposed to a control water and at least five different concentrations of effluent, or to receiving water, in a static renewal system until 60% of control females have three broods of young, or a maximum of 8 days. Test results are based on survival and reproduction in test solutions, compared to the controls.

METHOD 1003.0:

Algal (*Selenastrum capricornutum*) Growth Test. A *Selenastrum* population is exposed to a control water and to at least five different concentrations of effluent, or to receiving water, in a static system, for 96 hours. The test results are determined by the population responses in test solutions in terms of changes in cell density (cell counts per milliliter), biomass, chlorophyll content, or absorbance, compared to the controls.

Toxicity Test Endpoints. The endpoints for the freshwater short-term chronic toxicity tests with effluents and receiving waters are summarized as: (1) The NOEC, which is the highest percent effluent concentration at which no adverse effect on survival, growth, or reproduction is observed, and (2) the IC25 (Inhibition Concentration, 25%), which is the effluent concentration at which growth or reproduction are reduced 25% from that of controls. Although both endpoints are permissible, EPA recommends the IC25 endpoint for regulatory use.

The precision of the freshwater chronic toxicity tests is discussed in the respective methods sections in the methods manual (EPA/600/4-91/002). NOECs from repetitive tests generally fall within one concentration interval of the median value, and when measured with the IC25, the precision is generally

in the range of 30–60%. Precision can be improved by decreasing the concentration interval around the median value. This is accomplished by adding more concentration on either side of the median value.

(b) Short-Term Chronic Toxicity Test Methods for Estuarine and Marine Organisms. The approved short-term chronic toxicity tests for estuarine and marine organisms are contained in the manual, Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Estuarine and Marine Organisms, Second Edition, July 1994 (EPA/600/4-91/003). This manual describes six short-term (one-hour to nine-day) methods for estimating the chronic toxicity of effluents and receiving waters to five species: The sheepshead minnow, *Cyprinodon variegatus*; the inland silverside, *Menidia beryllina*; the mysid shrimp, *Mysidopsis bahia*; the sea urchin, *Arbacia punctulata*; and the red macroalga, *Champia parvula*.

The marine chronic toxicity tests in today's rule do not apply to discharges into marine waters of the Pacific Ocean. Toxicity tests for such discharges will continue to be specified in NPDES permits on a case-by-case basis. EPA intends to propose standardized toxicity test methods based on the methods developed by the States and EPA laboratories on the Pacific Coast.

Guidelines are included on laboratory safety, quality assurance, facilities and equipment, dilution water, effluent sampling methods and holding times and temperatures, data analysis, report preparation, and organism culturing and handling. Copies of computer programs for statistical analysis of the data referred to in the manual are available from EMSL-Cincinnati. The approved short-term chronic tests are:

METHOD 1004.0:

Sheepshead Minnow (*Cyprinodon variegatus*) Larval Survival and Growth Test. Larvae (preferably less than 24 hours old) are exposed in a static renewal system to a control water and at least five concentrations of effluent, or to receiving water for seven days. Test results are determined on the survival and weight change of the larvae in test solutions, compared to the controls.

METHOD 1005.0:

Sheepshead Minnow (*Cyprinodon variegatus*) Embryo-larval Survival and Teratogenicity Test. Sheepshead minnow embryos are exposed in a static renewal system to a control water and at least five different concentrations of effluent, or to receiving water, from

shortly after fertilization of the eggs to hatch, and the larvae are exposed for an additional four days posthatch (total of nine days). Test results are determined based on the combined frequency of both mortality and gross morphological deformities (terata) in the test solutions, compared to the controls. The test is useful in screening for teratogens because organisms are exposed during embryonic development.

METHOD 1006.0:

Inland silverside (*Menidia beryllina*), Larval Survival and Growth Test Larvae (preferably 7–11 days old) are exposed in a static renewal system to a control water and at least five concentrations of effluent, or to receiving water for seven days. Test results are determined on the survival and weight change of the larvae in the test solutions, compared to the controls.

METHOD 1007.0:

Mysidopsis bahia Survival, Growth, and Fecundity Test. Seven-day old mysids are exposed in a static renewal system to a control water and at least five different concentrations of effluent, or to receiving water for seven days. Test results are determined on survival, growth, and egg production (fecundity) of the mysids in the test solutions, compared to the controls.

METHOD 1008.0:

Arbacia punctulata Fertilization Test. *Arbacia* sperm are exposed one hour in a static system to control medium and at least five concentrations of effluent, or to receiving water. Eggs are then added to the sperm and both are exposed for an additional 20 minutes. The response is measured in terms of the percent fertilization of the eggs compared to the control.

METHOD 1009.0:

Champia parvula Reproduction Test. Branches of male and female plants are placed together for 48 hours in a static system and exposed to a control medium and at least five concentrations of effluent, or in receiving water. The exposed plants are then transferred to control medium for a recovery period of 5–7 days. After the recovery period, the numbers of reproductive structures (cystocarps) that develop on the female plants as a result of fertilization in the test solutions are compared to the controls.

Test Endpoints. The endpoints for the estuarine and marine short-term chronic toxicity tests with effluents and receiving waters include: (1) The NOEC, which is the highest percent effluent concentration at which no adverse effect

on survival, growth, or reproduction is observed, and (2) the IC25 (Inhibition Concentration, 25%), which is the effluent concentration at which growth or reproduction are reduced 25% from that of controls. Although both endpoints are permissible, EPA recommends the IC25 endpoint for regulatory use.

The precision of the chronic toxicity tests is discussed in the respective methods sections in the manual (EPA/600/4-91/003). NOECs from repetitive tests generally fall within one concentration interval of the median value. The precision of these test methods is also given in the Technical Support Document (second edition) that provides additional data points.

IV. Summary of Response to Comments for Aquatic Toxicity Tests

This section of the preamble summarizes the changes to the three methods manuals and significant comments received. The rest of the comments are summarized in the Supplementary Information Document (SID) which is available in the Water Docket.

A. Summary of Changes

One of the most commonly mentioned comments in the proposal was to have all three manuals formatted similarly, so that the documents would be easier to use. The three documents incorporated by reference in this rulemaking are now formatted in the same way, and as a result, are more "user friendly".

With this rule, several technical and editorial changes are made in the manual, Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, to respond to public comments on the Proposed Rule, December 4, 1989, and to make certain technical and policy language consistent with the revised freshwater and marine short-term chronic toxicity test manuals (EPA/600/4-91/002, EPA/600/4-91/003). Most of the substantive method changes made pursuant to public comment were made in the acute toxicity manual. Changes to the chronic toxicity manuals were largely related to format and consistency between the manuals. Briefly the changes are explained below.

Two paragraphs have been added to the introduction. The first paragraph cautions against making unauthorized changes in the methods, and the second paragraph makes a statement about experience needed by users of the methods. In Section 7, on the selection of dilution water for tests, "ground water" is added as an acceptable

“natural” water. In Section 8, on sample collection and handling, the description of sample “holding time” was expanded, but holding conditions and limits on sample holding time were not changed. In Section 9, on toxicity test procedures, an explanation was added on how an increase in pH during a toxicity test can be reduced or avoided by using a static renewal or flow-through approach. In Section 9, on toxicity test procedures, one footnote was added to each of two tables of test summary conditions, listing an additional species that could be used with the test conditions. These changes were made in response to comments on the proposed rule.

B. Effluent and Receiving Water Toxicity Tests with Fish and Aquatic Life

1. Test Variability

Comment: Toxicity test results are too variable, and methods are not sufficiently well standardized or validated with round robin data to include in 40 CFR part 136.

Response: EPA agrees that methods approved under part 136 should be validated scientifically. Further, EPA recognizes that an interlaboratory study (round robin) provides a useful and desirable means of validating an analytical method. However, EPA does not consider such a study to be a requirement for approval under Part 136 for a variety of reasons. First, prior to each interlaboratory study conducted with aquatic toxicity tests methods, EPA conducted intralaboratory studies that demonstrated similar, satisfactory precision. Where the Agency does not have interlaboratory data for a species, adequate data on intralaboratory precision are available. Second, quality assurance and quality control procedures specified in the toxicity test methods manuals are designed to minimize any variability due to analyst error or stress in test cultures due to factors other than effluent toxicity. Finally, the toxicity test methods specify a procedure for a series of initial repetitive tests to ensure that laboratory results during any particular analysis establish a pattern of satisfactory performance and define that laboratory’s intralaboratory variability.

EPA does consider the precision of candidate methods in approving such methods under part 136. The essential criterion is that the precision of the methods fall within the approximate range of other Agency methods (including those in part 136), and that approved methods provide valid results. For some of the chemical-specific methods, e.g., for manganese, the

variability at the low end of the measurement detection range exceeds that of the toxicity test methods. Compare *Technical Support Document for Water Quality-based Toxics Control* at 3, Table 1–3 (EPA/505/2–90–001). A large amount of intra- and inter-laboratory precision data are available on the toxicity tests approved in today’s rule, and representative data sets are included in the methods manuals. On the basis of these data, EPA is comfortable with the conclusion that whole effluent toxicity tests are no more variable than chemical analytical methods in Part 136 and, therefore, stands behind the conclusion that toxicity tests in NPDES permits provide reliable indicators of whole effluent toxicity.

2. Quality Assurance/Quality Control (QA/QC)

Some commenters expressed the opinion that the Agency’s QA requirements were excessively time-consuming and costly, whereas other commenters stated that the requirements were too lenient. See the SID for additional QA/QC information, such as the requirements for five initial toxicity tests, cleaning labware and apparatus, and food quality. The major comments on QA were as follows:

a. Existence of QA Guidelines for Toxicity Tests

Comment: The proposed methods do not contain the necessary QA protocols.

Response: EPA disagrees. Each of the toxicity test methods manuals incorporated by reference into Table IA, 40 CFR part 136, contains separate, detailed, QA/QC guidelines, and each analytical method within these manuals discusses all aspects of the tests which relate to QA/QC.

b. Reference Toxicant Tests

Comment: The requirement for monthly chronic QA tests of the sensitivity of organisms cultured within the laboratory is excessive. Monthly acute tests, or monthly acute and quarterly chronic tests for such organisms should be sufficient.

Response: EPA believes that the condition of organisms produced in “in house” laboratory cultures can change rapidly, requiring monthly verification of test organism sensitivity with the appropriate acute and/or short-term chronic toxicity test(s), using reference toxicants. Without this assessment, changes in the cultures can lead to less precision in the tests. It is sufficient to use a single reference toxicant with one or all test species (e.g., sodium chloride, potassium chloride, sodium dodecyl

sulfate, or other suitable substance). The tests can be limited to acute toxicity tests if the laboratory performs only acute tests with effluents and receiving waters. However, EPA does not agree that acute tests can be used instead of short-term chronic tests for the monthly verification of the sensitivity of test organisms to be used in short-term chronic tests with effluents and receiving waters.

Comment: Where effluent and reference toxicant tests are performed concurrently with organisms from the same batch shipped to a laboratory, and only the reference toxicant test is invalid (e.g., for failure to meet acceptability criteria or control chart limits), the permittee should not be required to repeat both the effluent toxicity and reference toxicant tests.

Response: EPA believes that the probability that an effluent toxicity test could be valid when the side-by-side reference toxicant test does not meet acceptability criteria is very slight. Under these circumstances, therefore, the results of both tests are rejected and the tests must be repeated.

If the reference toxicant test meets the acceptability criteria but the results fall outside the control limits, the results of both the reference toxicant and effluent tests should be considered provisional and subject to careful review. Good laboratories that have developed very narrow control limits may be unfairly penalized if test results that fall outside the control limits are rejected. For this reason, the width of the control limits should be considered by the permitting authority in determining if the reference toxicant and effluent toxicity data should be rejected on the basis of the control chart limits.

The requirement for side-by-side reference toxicant tests with shipped organisms could be waived if the test organism supplier provides reference toxicant and control charts data from monthly tests conducted with young from the same source cultures during the previous five-month period, using the same reference toxicants and same toxicity test conditions.

Comment: EPA should provide guidance on the acceptable performance of each reference toxicant (e.g., as it has done with chemical QC samples).

Response: EPA believes that the laboratory conducting the WET tests should derive response data by conducting a range-finding test prior to the definitive test. Accuracy of toxicity test results cannot be ascertained, only the precision of toxicity can be estimated, therefore it is not appropriate to provide such information.

Comment: EPA should provide reference toxicants and standard test organisms.

Response: The Agency is currently divesting itself from the production and distribution of QC materials for chemical methods and transferring those tasks to the private sector under cooperative research and development agreements (CRADAs) authorized by the Federal Technology Transfer Act of 1986, (Pub.L. No. 99-502). However, biological QC materials, such as reference toxicants and reference *Artemia* cysts, are still available in limited quantity from the Quality Assurance Research Division, Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH 45268. Further information can be obtained by writing to the laboratory or calling 513-569-7325.

Adequate supplies of test organisms are currently available from the private sector, and the market place has and is expected to respond quickly to any increased demand for test organisms.

3. Sample Collection, Holding Time and Temperature

a. Sample Containers

Comment: Glass sample containers should be used instead of plastic containers because there is less adsorption of toxics from the samples. However, plastic sample containers would be acceptable if the users are warned of this problem.

Response: The use of plastic containers for collection and shipment of effluent samples is preferred over glass bottles, which are more easily broken during shipment. It must be recognized, however, that the loss of toxics from samples (and possible reduction in toxicity) by adsorption to plastic surfaces may be greater with plastic containers than with glass ones. Prolonged storage of samples in plastic containers before use, therefore, should be avoided to the extent possible.

b. Sample Holding Time and Temperature

Comment: The sample holding time (36 hours) prior to the start of the toxicity test is too restrictive.

Response: EPA believes that 36 hours provides sufficient time to deliver the samples to the performing laboratories in most cases. In the isolated cases where the permittee can document that this delivery time cannot be met, the permitting authority may allow an option for on-site testing, or a variance to extend the holding time. The request for a variance in sample delivery time

(directed to the Regional Administrator under 40 CFR 136.4 and 40 CFR 136.5) must include supportive data which show that the toxicity of the effluent sample is not reduced (e.g., because of biodegradation, chemical transformation, volatilization and/or sorption of toxics on the sample container surfaces) by extending the holding time beyond 36 hours. In no case should more than 72 hours elapse between collection and first use of the sample.

Comment: Current guidance on sample collection in the toxicity test manuals does not clearly indicate when sample holding time begins.

Response: EPA agrees and provides the following clarification in the manual. Sample holding time begins when the last grab sample in a series is taken (e.g., when a series of four grab samples are taken over a 24 hours period), or when a 24 hours composite sampling period is completed.

Comment: It is not possible to regularly maintain a sample temperature of 4 °C during sample shipment.

Response: EPA agrees that the requirement to maintain sample temperature at 4 °C may be difficult to achieve. However, the temperature requirement is important to minimize possible loss of toxicity due to chemical transformations and microbial degradation during transit and holding. Sufficient ice should be placed with the samples in the shipping container to ensure that ice is still present when the samples arrive at the laboratory. However, even if ice is present when a sample arrives at the laboratory, the analyst should measure and record the temperature of the samples to confirm that the 4 °C temperature maximum has not been exceeded. In the isolated cases where the permittee or the analyst can document that the 4 °C shipping temperature cannot be met, the permittee can be given the option of on-site testing or can request a variance in sample shipping temperature. The request for a variance must include supportive data to demonstrate that the toxicity of the effluent samples is not reduced when the holding temperature is increased to the level proposed.

4. Toxicity Testing Species

a. Addition of the MICROTOX^R Test System

Comment: Many commenters requested the inclusion of and provided information on a toxicity test known as the MICROTOX^R Luminescent Bacteria Toxicity Test using the organism, *Photobacterium phosphoreum*. Information supplied included

performance characteristics of the method and its use. Commenters urged inclusion of the test because of its alleged simplicity, cost effectiveness, reproducibility, and widespread use. One commenter suggested use of the method for compliance testing, toxicity reduction evaluations, and pretreatment evaluations.

Response: While EPA agrees that MICROTOX^R is a relatively rapid and simple test system that can provide data useful for in-plant toxicity screening, today's rule does not include *any* test methods to measure the toxic effect of effluent on bacteria. Consistent with the public notice in the proposed rule and the test manuals incorporated by reference therein, today's final rule only includes methods that measure toxicity to representative species from certain phylogenetic groups: i.e., fish, invertebrates, and algae. Information available to the Agency does not, at this time, indicate that the MICROTOX^R test system provides an acceptable, sensitive indicator of the toxic effects of effluents to the fish, invertebrates, or algae included in the test methods promulgated today.

The Agency hastens to add, however, that today's rule does not restrict the use of the MICROTOX^R test as an *additional* or supplemental test method for use in states with federally-approved NPDES programs. EPA also notes that tests such as MICROTOX^R may provide the permittee the additional benefit of a diagnostic tool for the purposes of in-plant toxicity screening for the protection of biological (microbial) treatment processes. Under EPA regulations, when a permittee conducts any testing required by the permit using an analytical method approved in 40 CFR part 136, all test results must be reported (40 CFR 122.41(l)(4)(ii)). Thus, a diagnostic test not included in 40 CFR part 136 provides permittees with the opportunity for internal effluent evaluation undisclosed to the permitting authority. The Agency notes, however, that results of any biological testing of "end-of-pipe" discharge or receiving waters must be reported in subsequent permit applications.

b. Indigenous (Feral) Test Organisms

Comment: The use of indigenous species from the receiving water should be allowed in effluent toxicity tests.

Response: The use of feral (feral indicates wild) indigenous species from the receiving water is not allowed due to lack of control in the quality of the test organisms, including such factors as range in age, possible previous exposure to contaminants, disease, and injury during collection, all of which might

significantly affect organism sensitivity to toxicants, and the precision and reproducibility of the test. However, the above discussion does not mean that EPA is adverse to persons developing credible toxicity methods based on other organisms, including methods based on organisms indigenous to specific surface waters. These toxicity methods would need to include QA/QC provisions that assure a proper level of precision and reproducibility, and would need to use test organisms cultured in a laboratory that are unaffected by environmental stresses. Such methods could be submitted for approval as an alternative test procedure (40 CFR 136.4 (a) and (d)).

c. Supplemental Species

Comment: Some commenters noted that some State laws prohibit the import of non-indigenous species. One commenter noted that the list of recommended test species in the acute toxicity test manual (EPA/600/4-90/027) did not include any test species indigenous to Pacific coastal waters. The commenter provided data from side-by-side testing (*Homesimysis costata*) suggesting that a west coast test species (that the commenter thought should be included) was at least as sensitive to toxicity as one of the test species recommended in the acute manual. The State of California expressed concern that test methods it had developed and has been including in NPDES permits would be displaced by today's rule.

Response: The species selected by EPA for effluent toxicity tests in the NPDES program represent a "performance standard" or indicator of sensitivity to toxicity for a given phylogenetic category. Therefore, to use a species other than the recommended species, the permittee or the permitting authority should provide data from side-by-side testing showing that the proposed substitute test species is at least as sensitive as the recommended test species for that phylogenetic category.

Toxicity test methods will not require use of non-indigenous test organisms when State law prohibits import of such species. However, the toxicity test manuals provide instructions for the disposal of test organisms and, if these instructions are followed, the use of non-indigenous organisms will not result in establishment of populations of these organisms in local waters that will threaten indigenous wildlife.

Appendix B in the acute toxicity test manual (EPA/600/4-90/027F) contains a list of "supplemental" test species that may be appropriate for use in acute

toxicity testing under certain test conditions. EPA accepts the use of *Notropis leedsii* (Bannerfish Shiner) in place of *Pimephales promelas* (Fathead Minnow), if the same test conditions are used, and the use of the mysid, *Homesimysis costata*, in place of *Mysidopsis bahia*, with the same test conditions except at a temperature of 12°C, instead of 20°C or 25°C, and a salinity of 32-34‰, instead of 5-30‰), where their use is required test organisms in discharge permits. However, other species on the list are not currently approved for use as recommended species.

California is correct in its conclusion that the standardization of methods by today's rule will displace unapproved methods (for NPDES permits issued after today's rule). In response to this concern, EPA is restricting the applicability of today's rule. The marine chronic tests in today's rule do not apply to discharges into marine waters of the Pacific Ocean. EPA seeks to minimize disruption in the administration of NPDES permit programs in those States with Pacific coastal waters. EPA intends to propose approval of marine chronic methods applicable to colder, Pacific coast waters in the near future. Marine acute west coast WET methods are included in the acute testing manual.

5. Test Conditions

See the SID for response to comments on the following: Dilution water, test temperature and pH, renewal of test solutions, age of test organisms, test duration, feeding before/during the tests, dilution factor, replication, dissolved oxygen and aeration, and the number of effluent concentrations used in tests.

6. Applicability of Tests

a. Criteria for Test Selection

Comment: In initially preparing, and subsequently revising, the toxicity test manuals, EPA failed to establish criteria for toxicity test selection. The toxicity tests proposed by the Agency did not satisfy the criteria for determining adequacy of testing methods.

Response: EPA believes the commenter refers to the criteria described in the EPA report to Congress entitled, "Availability, Adequacy, and Comparability of Testing Procedures for the Analysis of Pollutants Established Under Section 304(h) of the Federal Water Pollution Control Act," EPA/600/9-87/030, September 1988. In that document, EPA compared biological analyses to chemical analyses for the purpose of assessing the adequacy of a

given biological method. The document explained the attributes of biological tests that were significant for assessing adequacy: biological detection limits, precision, and applicability.

In toxicity tests, the detection limit is determined by the "sensitivity" of the test organisms. The sensitivity of organisms to pollutants is an intrinsic quality, which may vary greatly between species, but also varies somewhat among organisms within the same species, and is affected by the condition or "health" of the organisms. Because the sensitivity of the test organisms cannot be "calibrated" before each toxicity test, the tests must include standards to ensure data integrity. The final rule promulgated today includes the use of standard "reference" toxicants to maintain that integrity.

To assess the precision of biological tests, the EPA report indicated that the methods must account for inherent variability of response and natural variability of within-species sensitivity. The methods in the final rule account for that variability by use of replicate testing; the toxicity methods require that a series of controls be run concurrently with pollutant exposures. These methods also contain criteria for determining the acceptability of data from a toxicity test based on the performance of the control organisms.

The final attribute for assessing the adequacy of biological methods, as discussed in the EPA report, was applicability. The key criterion identified for determining biological test applicability was whether special conditions in the laboratory or a unique laboratory location is required to perform the test. For a test method to be applicable, it must be adaptable to a wide variety of laboratories. Applicability of a biological test depends on the ease with which the test can be performed on a routine basis and the consistency of availability of test organisms. The methods in this rule use readily available test organisms and can be competently performed by laboratories following the QA/QC guidelines described in the manuals.

EPA disagrees with the commenter's central proposition that to establish applicability, each method requires inter-laboratory validation. In validating each method, EPA considered intra-laboratory testing. For those tests for which EPA further relies on interlaboratory testing, comparable coefficients of variation (precision) were achieved. Based on the high degree of correlation between coefficients of variation between intralaboratory tests and interlaboratory tests, EPA is confident in its reliance on

intralaboratory studies to establish the applicability of the test methods to a wide variety of laboratories.

b. Ceriodaphnia Test

Comment: There are problems with the Ceriodaphnia dubia short-term chronic toxicity test as evidenced by the low rate of successful test initiation (61%) and test completion (56%) in the Battelle Columbus (1987) round robin.

Response: The Ceriodaphnia dubia short-term chronic toxicity test method (especially the diet) has been significantly improved since the Battelle round robin, as evidenced by the higher rates of successful test initiation and completion in a round robin supervised by EPA Region 4 in 1989 (EPA/505/2-90-001). In this inter-laboratory study, 36 (80%) of 45 tests were successfully completed. The endpoints (No Observed Effect Concentrations, or NOECs) of 35 of the 36 tests, fell on two adjacent concentrations. Also, an interlaboratory study of the Ceriodaphnia dubia 7-day chronic test conducted by the San Francisco Bay Regional Water Quality Control Board (Environ. Toxicol. Chem. 10:143-145, 1991), resulted in a coefficient of variation of 29%, demonstrating good precision.

c. Test Validation in Receiving Waters

Comment: The relationship between laboratory data on effluent toxicity and effects on aquatic life in receiving waters has not been established by the Agency.

Response: Numerous freshwater and marine site studies have been made to determine this relationship (see the Technical Support Document, EPA/505/2-90-001, 1991). These studies comprise a large data base specifically collected to determine the validity of toxicity tests to predict receiving water community impacts. The results of these studies clearly show the direct relationship between laboratory data on effluent toxicity and its adverse effect on aquatic life in receiving water.

d. Stage of Development of Toxicity Test Methods

Comment: EPA toxicity test methods are still in a developmental stage, and have not been properly peer reviewed.

Response: The acute toxicity tests have been widely used in the public and private sector for the past two decades, and the short-term chronic tests have been in general use in the NPDES permit program for six to nine years. The toxicity test manuals were widely distributed to expert peer reviewers in academia, major industries and trade organizations, consulting firms, and government agencies prior to

publication, and were subject to further review during the public comment period following issuance of the Proposed Rule. Codification of these methods was proposed December 4, 1989, because they were considered adequately standardized for use in the NPDES Program. Furthermore, these methods have been published in highly respected, peer reviewed journals.

e. Ability of Laboratories to Perform the Arbacia and Champia Tests

Comment: Few laboratories have the capability to perform some of the short-term chronic toxicity tests, such as the Champia and Arbacia tests.

Response: EPA agrees that the number of laboratories with the capability of conducting Champia and Arbacia tests is currently limited. However, as the requirements for use of these organisms in the NPDES permits program increases, EPA's past experience indicates that the resulting increase in market demand will result in an increase in the number of laboratories that are capable of performing these tests.

C. Statistical Analysis of Results of Toxicity Tests with Fish and Other Aquatic Life

Twenty-four sets of comments were received on statistical methods for toxicity data analysis. Some of the comments and responses are discussed below and the rest are in the SID.

Comment: The use of Coefficients of Variation (CVs) of point estimates, such as the LC50, and the range in NOEC's and/or LOEC's (Lowest Observed Effect Concentration) are an inappropriate measure of test precision. The use of the NOEC and LC50 endpoints for precision estimates is not consistent with the calculation of precision of chemical methods. Therefore comparison of toxicity test precision to chemical method precision is inappropriate.

Response: In the case of toxicity tests, test precision is a measure of agreement of successive test results. Toxicity results are expressed in terms of a point estimate, such as the LC1

(Concentration at which 1% of the organisms die), LC50, IC25, or a NOEC-LOEC pair derived from hypothesis testing. The CV is a widely used and acceptable method of expressing variability (precision) of point estimates from toxicity tests, such as LC50's, and is comparable to the calculation of precision of chemical methods. However, NOEC's and LOEC's are not point estimates, and it is not possible to express the precision of these values in terms of a similar statistic. In this case, precision can only be described by

listing the NOEC-LOEC interval for each test, and indicating the range in these values. For a more general discussion of statistical analysis using hypothesis testing versus point estimates, see page 11 of the "Technical Support Document for Water Quality-based Toxics Control", EPA/505/2-90-001, PB91-127415, March 1991.

Comment: The choice of statistical methods is not justified in the guidance documents.

Response: EPA recognizes that the statistical methods recommended in the toxicity test methods manuals are not the only possible methods of statistical analysis. In selecting the methods for the manuals, EPA statisticians evaluated and considered many other analyses. The methods finally selected were chosen, among other reasons, because there are: (1) Well tested and well documented; (2) applicable to most different toxicity test data sets for which they are recommended, but still powerful; (3) most easily understood by non-statisticians; and (4) amenable to use without a computer, if necessary.

Comment: Statistical analysis of toxicity test results is very complicated and should require the review and evaluation of a qualified statistician.

Response: The statistical analyses recommended in the three toxicity test manuals (acute, freshwater short-term chronic, and marine short-term chronic) cited in the proposed rule had been subjected to extensive peer review in the private and public sectors prior to their proposal. The reviewers included EPA statisticians, government contract statisticians, and statisticians from academia. EPA believes that this constitutes an objective peer review of the recommended statistical analyses by qualified statisticians. In addition, the methods have also been published in highly regarded peer reviewed journals. The manuals also provide detailed, stepwise guidance for the statistical analyses of individual test results.

Comment: It is not always obvious that an effect level that is determined to be statistically significant is also biologically significant.

Response: The implied question, concerning the "biological significance" of (threshold) "statistically significant" occurrences of adverse biological effects observed in toxicity tests, is an implementation question, and is not addressed in this rulemaking. However, in a related area, the Agency's water quality criteria for fish and other aquatic life are based on "safe concentrations" of toxicants which are defined as the highest concentration of toxicant not showing a "statistically significant" occurrence of an adverse biological

effect (NOEC) with the assumption that a "statistically significant" reduction in an important biological response will adversely affect the success of the organisms and, therefore, is a "significant" biological effect.

Comment: Only surviving adult females should be used for *Ceriodaphnia* reproduction analysis.

Response: The exclusion of reproduction data from females that do not survive to the end of the test would bias the results in favor of the organisms that are more tolerant to pollution. Therefore, EPA believes that it is best to use the reproduction data from all the test organisms in the analysis, except for those from test concentrations that have significantly greater mortality than the test controls. Data from the latter are not included in the determination of the reproductive endpoint.

Comment: More guidance is needed in selecting alternative statistical methods when replicate values are found to reflect wide variation in survival values.

Response: The freshwater and marine short-term chronic toxicity test methods manuals contain detailed flowcharts on the recommended statistical analyses. It is not possible to provide guidelines to cover all contingencies of toxicity data analysis. Therefore these recommendations were intended to cover most types of data that would occur in toxicity testing. As stated in the manuals, EPA advises analysts to consult with a qualified statistician for cases that are not covered by the recommended analyses.

Comment: The NOEC is not a meaningful endpoint and is too dependent upon the concentration intervals utilized in the test.

Response: EPA recognizes that the NOEC is dependent upon the concentration intervals used in a test, but disagrees that it is not a meaningful endpoint. The NOEC is the most commonly used endpoint in chronic toxicity tests and, prior to the development of the Linear Interpolation (or Inhibition Concentration) Method, was the only endpoint available for determination of "safe concentrations." The Agency's water quality criteria for fish and other aquatic life are based on "safe concentrations" of toxicants which are defined as the highest concentration of toxicant not causing a "statistically significant" difference in biological response (such as growth or reproduction). Use of the NOEC in effluent and receiving water toxicity tests is described in the Agency's "Technical Support Document for Water Quality-based Toxics Control", EPA/

505/2-90-001, PB91-127415, March 1991.

Comment: Statistical methods which require log or geometric dilution series should be discussed.

Response: The use of graphical method to determine the LC50 is recommended by EPA (EPA/600/4-90/027F) only when the response is "all or nothing," i.e., only two levels of response—zero mortality at lower test concentrations and 100% mortality at higher test concentrations. Results of this type occur in a high proportion (60% or more) of effluent toxicity tests. When such an all or nothing response occurs, the results are not amenable to statistical analysis. According to Finney, a leading authority on the analysis of acute toxicity data, a graphically-derived estimate of the LC50, which employs the known logarithmic relationship between toxicant concentration and mortality, is "the only reasonable approach" (Finney, D.J. 1985. Arch. Toxicol. 56:215-218). However, the graphical method is unable to provide confidence limits for the endpoints. When partial mortalities occur at one or more test concentrations, EPA recommends the use of the Trimmed Spearman-Kärber or Probit Analysis.

Comment: Regression (point estimation) should be used as an alternative tool for the data rather than exclusively using a "mean" system.

Response: The selection of the statistical analysis (in the two short-term chronic manuals) is dependant upon the intended use of the data. For example, in the NPDES permitting program, the recommended statistical procedure is the point estimate, because confidence intervals can be placed around the point estimate.

Comment: There must be an adequate concentration response or the test is of little value in calculation of a LC50 or EC50.

Response: Data from toxicity tests frequently show an all or nothing response, and in these instances the appropriate statistical procedure to estimate the LC50 are the Graphical Method and/or the Trimmed Spearman-Kärber. The alternative LC50 statistical procedures do require that the data show a dose response above and below the LC50 concentration.

D. Implementation and Miscellaneous Issues

Approximately 23 comments were related to the application and implementation of EPA Policy on the Water Quality-Based Toxics Control Program and other issues which were not specifically applicable to the

technical methods contained in this rulemaking. These comments are addressed in the SID which is part of the administrative record for this rulemaking.

VI. Regulatory Analyses

A. Unfunded Mandates Reform Act of 1995

Under section 202 of the Unfunded Mandates Reform Act of 1995 ("Unfunded Mandates Act"), signed into law on March 22, 1995, EPA must prepare a written statement to accompany rules where the estimated costs to State, local, or tribal governments, or to the private sector, will be \$100 million or more in any one year. Under section 205, EPA must select the most cost-effective and least burdensome alternative that achieves the objective of such a rule and that is consistent with statutory requirements. Section 203 requires EPA to establish a plan for informing and advising any small governments that may be significantly and uniquely affected by the rule.

EPA estimates that the costs to State, local, or tribal governments, or the private sector, from this rule will be less than \$100 million. This rulemaking should have minimal impact, if any, on the current regulatory burden imposed on NPDES permittees because the rulemaking merely standardizes methods (that are currently contained in guidance) to determine compliance with whole effluent toxicity limitations required under existing regulations. EPA has determined that an unfunded mandates statement therefore is unnecessary. Similarly, the standardized methods in today's rule do not establish any regulatory requirements that might significantly or uniquely affect small governments; any such requirements would have been established previously in NPDES regulations providing for inclusion of whole effluent toxicity limitations.

B. Regulatory Flexibility Act

Under the Regulatory Flexibility Act, 5 U.S.C. 601 et seq., EPA is required to determine whether a regulation will significantly affect a substantial number of small entities so as to require a regulatory analysis. The regulation requires no new reports beyond those now required. The analytical techniques approved here either can be handled by small facilities, or are widely available by contract at a reasonable price. Therefore, in accordance with 5 U.S.C. 605(b), I hereby certify that this rule will not have significant adverse economic

impact on a substantial number of small facilities.

C. Paperwork Reduction Act

This rule does not impose any additional information requirements on respondents, and consequently is not subject to the Paperwork Reduction Act, 44 U.S.C. 3501 et seq.

D. Executive Order 12866

Under Executive Order 12866, EPA must judge whether a regulation is "major" and therefore subject to the requirement of a "Regulatory Impact Analysis." This regulation is not major for the following reasons:

1. The rule only prescribes analytical methods and sample handling requirements that ensure a uniform measure of pollutants across all wastewater discharges within minimum acceptance criteria. The rule itself does not require that analyses actually be performed. Other existing rules require such analyses in certain circumstances. The purpose is to ensure that the quality of the environmental monitoring data meets certain minimum standards.

2. The impact of this regulation will be far less than \$100 million. The regulation affects unit monitoring cost for the NPDES programs, e.g., effluent guidelines regulations and the NPDES implementation regulations, and the pretreatment programs. However, the rule does not itself impose those costs. The monitoring costs for other programs are considered in the rulemaking for each program.

Under Executive Order 12866 The Office of Management and Budget waived review on October 26, 1994.

The range in cost for the acute and chronic methods, on a per test basis, is approximately \$200.00–\$2800.00. Clustered at the low end of the cost range estimate are the acute 96 hour test methods, and at the higher end the short-term chronic test methods. The majority of testing laboratories charged between \$200.00–\$1500.00 per test. EPA believes that the overall range of cost per test, particularly at the high end, will decrease as a result of promulgation of the methods. This is because the number of approved tests will be limited to those in the rule, as opposed to the many variations of each test method now being conducted. Experience has shown that the cost of the tests has decreased over time as the testing laboratories have become more competent in performing the different test methods. EPA estimates that the overall cost will drop by 20% (ranging from \$160.00–\$2240.00 for all labs, and \$160.00–\$1200.00 for the majority of labs) as a result of promulgation of this rule.

VII. Materials Incorporated by Reference Into 40 CFR Part 136

1. USEPA. 1993. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. Fourth Edition, August 1993. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio (EPA/600/4–90/027F). Table 1A, Note 7.

2. USEPA. 1994. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Third Edition, July 1994. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. (EPA/600/4–91/002). Table 1A, Note 9.

3. USEPA. 1994. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Second Edition, July 1994. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. (EPA/600/4–91/003). Table 1A, Note 10.

VIII. Public Availability of Materials To Be Incorporated by Reference

Copies of the documents incorporated by reference in today's rulemaking will be available to the general public from the following sources at no cost:

National Center for Environmental Publications and Information (NCEPI): available 24 hours a day, 7 days a week; (513) 489–8190, or FAX (513) 489–8695, identifying the name of the document or the publication number listed in section VII of this preamble. Available formats: paper copies and 3½ inch or 5 inch discs.

EPA Office of Water Resource Center: available 24 hours a day, 7 days a week; (202) 260–7786. Contract staff will assist caller in identifying a document from document title, publication number, or a description of the subject matter. Available formats: paper copies and 3½ inch or 5 inch discs.

EPA Regional Office Libraries: EPA has 10 Regional offices around the country, each with a publically accessible library. Copies of these documents can be viewed and copied at these EPA Regional libraries. EPA Region I, JFK Federal Building, One Congress Street, Boston, MA 02203, (617) 565–3420; EPA Region 2, 290 Broadway, New York, NY 10007–1866, (212) 637–3000; EPA Region 3, 841 Chestnut Building, Philadelphia, PA 19107, (215) 597–9800; EPA Region 4, 345 Courtland Street, NE., Atlanta, GA 30365, (404) 347–4727; EPA Region 5, 77 West Jackson Blvd., Chicago, IL 60604–3507, (312) 353–2000; EPA Region 6, First Interstate Bank Tower at Fountain Place, 1445 Ross Avenue, 12th Floor, Suite 1200, Dallas, TX 75202–2733, (214) 665–6444; EPA Region 7, 726 Minnesota Avenue, Kansas City, KS

66101, (913) 551–7000; EPA Region 8, 999 18th Street, Suite 500, Denver, CO 80202–2466, (303) 293–1603; EPA Region 9, 75 Hawthorne Street, San Francisco, CA 94105, (415) 744–1305; EPA Region 10, 1200 Sixth Avenue, Seattle, WA 98101, (206) 553–1200.

Internet. EPA operates a "public access server," also known as "Earth 1," through which EPA will include all of the ways that copies of the test methods manuals are available. The Office of Water will put the directions about electronic retrieval of the test methods manuals on EPA's Internet "homepage." By doing so, persons interested in electronic copies of the methods manuals may obtain copies either (1) retrieving the documents from EPA's file transfer protocol (FTP) site on the Internet at ftp.epa.gov or gopher.epa.gov (2) retrieving the documents by dial-in access at 919–558–0335, or (3) by requesting floppy disks from NCEPI, including requests through the Office of Water Resource Center. EPA would explain the limitations some users may encounter trying to print out diagrams, tables, charts and graphs, which would may require special "read" software. Later this year, the Office of Water will have its own Internet "homepage" which will include all Office of Water rules and information on how to obtain copies of all technical support documents.

By the end of 1995, EPA will be a participant in the Government Information Locator Service (GILS) consistent with Office of Management and Budget requirements. GILS is a "list of lists" on the Internet, of all U.S. Government publications, describing the publication and how to get it. The Office of Water will describe the means of electronic access to the whole effluent toxicity test methods manuals through the GILS system.

Public Libraries. A description of the whole effluent toxicity methods final rule and the test methods manuals has been placed in the combined catalogues of the Online Computer Library Center (OCLC) in Columbus, Ohio, available to all member libraries across the country (approximately 13,000). This summary will facilitate public access through interlibrary loans from the Regional EPA libraries. Through OCLC, EPA has placed the summary and access information in the Online Library System. Finally, EPA has provided the national association of public libraries with a summary of the whole effluent toxicity methods rule and the test methods manuals, as a way of emphasizing their availability through this means.

Copies of these documents will also be available for viewing and copying at the State Libraries: Alabama Library Association, 400 S. Union Street, Suite 255, Montgomery, AL 36104; Alaska Library Association, PO Box 81084, Fairbanks, AL 99708-1084; Arizona State Library Association, 13832 32d Street, Phoenix, AZ 85032; Arkansas Library Association, 1100 N. University, #109, Little Rock, AR 72204; California Library Association, 717 K. Street, Suite 300, Sacramento, CA 95814-3477; Colorado Library Association, 114 Pinecliffe Road, Pinecliffe, CO 80471; Connecticut Library Association, Box 1016, Hartford, CT 06360; Delaware Library Association, PO Box 816, Wilmington, DE 19903; District of Columbia Library Association, PO Box 14177, Benjamin Franklin Station, Washington, DC 20044; Florida Library Association, 1133 W. Morse Blvd., Suite 201, Winter Park, FL 32789-3788; Georgia Library Association, Young Harris College, PO Box 39, Young Harris, GA 30582; Guam Library Association, PO Box 22515 GFM, Barrigada, GU 96921; Hawaii Library Association, PO Box 4441, Honolulu, HI 96814-4441; Idaho Library Association, Boise State University, Boise, ID 83725; Illinois Library Association, 33 W. Grand Avenue, #301, Chicago, IL 60610; Indiana Library Federation 6408 Carrollton Avenue, Indianapolis, IN 46220-1615; Iowa Library Association, 823 Insurance Exchange Building, Des Moines, IA 50309; Kansas Library Association, South Central Kansas Library System, 901 N. Main, Hutchinson, KS 67501-4401; Kentucky Library Association, 1501 Twilight Tr., Frankfort, KY 40601; Louisiana Library Association, PO Box 3058, Baton Rouge, LA 70821; Maine Library Association, Community Drive, Augusta, ME 04330; Maryland Library Association, 400 Cathedral Street, 3d Floor, Baltimore, MD 21201; Massachusetts Library Association, Countryside Offices 707 Turnpike St., North Andover, MA 08145; Michigan Library Association, 1000 Long Blvd. Suite 1, Lansing, MI 48911; Minnesota Library Association, 1315 Lowrey Avenue, N. Minneapolis, MN 55411-1398; Mississippi Library Association, PO Box 20488, Jackson, MS 39209-1448; Missouri Library Association, 11306 Business 63 South, Suite B, Columbia, MO 65201; Montana Library Association, 507 Fifth Avenue, Helena, MT 59601-4359; Nebraska Library Association, 5302 S. 75th Street, Ralston, NE 68127-3903; Nevada Library Association, Elko County Public Library, 720 Court Street, Elko, NV 89801; New Hampshire Library

Association, Franklin Public Library, 310 Central Street, Franklin, NH 03235; New Jersey Library Association, 4 W. Lafayette, Trenton, NJ 08608; New Mexico Library Association, San Juan College Library, 4601 College Avenue, Farmington, NM 87401; New York Library Association, 252 Hudson Avenue, Albany, NY 12210; North Carolina Library Association, Southeastern Technical Asst. Center, 2013 Lejeune Blvd., Jacksonville, NC 28546-7027; North Dakota Library Association, University of North Dakota-Lake Region, 1800 N. College Drive, Devil's Lake, ND 58301; Ohio Library Council, 35 E. Gay Street, Columbus, OH 43215; Oklahoma Library Association, 300 Hardy Drive, Edmond, OK 73013; Oregon Library Association, 1270 Chemeketa Street, NE, Salem, OR 97301; Pennsylvania Library Association, 1919 N. Front Street, Harrisburg, PA 17110; Rhode Island Library Association, 300 Richmond Street, Providence, RI 02903; South Carolina Library Association, Rt 2, Box 139F, Denmark, SC 29042; South Dakota Library Association, PO Box 673, Pierre, SD 57501; Tennessee Library Association, Memphis State University Library, Memphis, TN 30152; Texas Library Association, 3355 Bee Cave Road, #401, Austin, TX 78746; Utah Library Association, 365 Emory, Salt Lake City, UT 84101; Vermont Library Association, Box 803, Burlington, VT 05402-0803; St. Thomas/St. John Library Association, University of Virgin Islands, St. Thomas, VI 00802; St. Croix Library Association, PO Box 306164, Veteran's Drive Station, Charlotte Amalie, VI 00803; Virginia Library Association, 669 S. Washington Street, Alexandria, VA 22314-4109; Washington Library Association, Ft. Vancouver Regional Library, 1007 E. Mill Plain Blvd. Vancouver, WA 98603-3504; West Virginia Library Association, West Virginia Library Community, Science and Culture Center, Charleston, WV 35305; Wisconsin Library Association, 4785 Hayes Road, Madison, WI 53704-2764; Wyoming Library Association, Sweetwater County Library, PO Box 550, Green River, WY 82935.

A limited number of copies will be available from the EPA Regional offices, and the State NPDES permitting offices. Finally, after the first printing, hard copies will be available from the National Technical Information Service (NTIS) in Springfield, Virginia for \$31.00, \$31.00, and \$45.00, respectively for "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Marine and

Estuarine Organisms, Second Edition" July 1994, EPA/600/4-91/003, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, Third Edition" July 1994, EPA/600/4-91/002, and "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fourth Edition" August 1993, EPA/600/4-90/027F. (NTIS is an organization within the U.S. Department of Commerce.)

EPA is also notifying the following groups of the availability of these documents: International Association of Environmental Testing Laboratories; American Society of Testing Materials; Society of Environmental Toxicology and Chemistry; American Chemical Society; Water Environment Federation; Association of Metropolitan Sewerage Agencies; Association of Analytical Chemists; and the Discharge Monitoring Requirement Quality Assurance Program.

IX. References

- Federal Register: U.S. Environmental Protection Agency. Policy for the Development of Water Quality-Based Permit Limitations for Toxic Pollutants, 49 FR 9016; Mar. 9, 1984.
- Anderson, S.L. and T.J. Norberg-King. 1991. Precision of Short-Term Chronic Toxicity Tests in the Real World. *Environmental Toxicology and Chemistry* 10(2):143-145.
- Finney, D.J. 1985. The Median Lethal Dose and its Estimation. *Arch. Toxicol.* 56:215-218.
- U.S. Environmental Protection Agency. July 1994. Whole Effluent Toxicity (WET) Control Policy. EPA 833-B-94-002.
- U.S. Environmental Protection Agency. 1991. Technical Support Document for Water Quality-Based Toxics Control, March 1991, EPA/505/2-90/001; PB91-127415.
- U.S. Environmental Protection Agency. September 1988. Report to Congress: Availability, Adequacy, and Comparability of Testing Procedures for the Analysis of Pollutants Established Under Section 304(h) of the Federal Water Pollution Control Act. EPA/600/9-87/030.

List of Subjects in 40 CFR Part 136

Environmental protection, Water pollution control, Incorporation by reference.

Dated: October 3, 1995.

Carol M. Browner,
Administrator.

For the reasons set out in the preamble, part 136 of title 40 of the Code of Federal Regulations is amended as follows:

PART 136—[AMENDED]

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

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

2. In § 136.3(a), Table IA is revised to read as follows:

§ 136.3 Identification of test procedures.
* * * * *

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS

Parameter and units	Method ¹	EPA	Standard methods, 18th Ed.	ASTM	USGS
Bacteria:					
1. Coliform (fecal), number per 100 mL.	Most Probable Number (MPN), 5 tube 3 dilution, or Membrane filter (MF) ² , single step ..	p. 132 ³ p. 124 ³	9221C E ⁴ 9222D ⁴	B-0050-85 ⁵
2. Coliform (fecal) in presence of chlorine, number per 100 mL.	MPN, 5 tube, 3 dilution, or MF, single step ⁶	p. 132 ³ p. 124 ³	9221C E ⁴ 9222D ⁴	
3. Coliform (total), number per 100 mL.	MPN, 5 tube, 3 dilution, or MF ² single step or two step	p. 114 ³ p. 108 ³	9221B ⁴ 9222B ⁴	B-0025-85 ⁵
4. Coliform (total), in presence of chlorine, number per 100 mL.	MPN, 5 tube, 3 dilution, or MF ² with enrichment	p. 114 ³ p. 111 ³	9221B ⁴ 9222(B+B.5c) ⁴	
5. Fecal streptococci, number per 100 mL.	MPN, 5 tube, 3 dilution MF ² , or Plate count	p. 139 ³ p. 136 ³ p. 143 ³	9230B ⁴ 9230C ⁴	B-0055-85 ⁵
Aquatic Toxicity:					
6. Toxicity, acute, fresh water organisms, LC50, percent effluent.	Daphnia, Ceriodaphnia, Fathead Minnow, Rainbow Trout, Brook Trout, or Bannerfish Shiner mortality.	Sec. 9 ⁷		
7. Toxicity, acute, estuarine and marine organisms, LC50, percent effluent.	Mysid, Sheepshead Minnow, or Menidia spp. mortality.	Sec. 9 ⁷		
8. Toxicity, chronic, fresh water organisms, NOEC or IC25, percent effluent.	Fathead minnow larval survival and growth Fathead minnow embryo-larval survival and teratogenicity.	1000.0 ⁸ 1001.0 ⁸		
9. Toxicity, chronic, estuarine and marine organisms, NOEC or IC25, percent effluent.	Ceriodaphnia survival and reproduction	1002.0 ⁸		
	Selenastrum growth	1003.0 ⁸			
	Sheepshead minnow larval survival and growth ..	1004.0 ⁹		
	Sheepshead minnow embryo-larval survival and teratogenicity.	1005.0 ⁹			
	Menidia beryllina larval and growth	1006.0 ⁹			
	Mysidopsis bahia survival, growth, and fecundity .	1007.0 ⁹			
	Arbacia punctulata fertilization	1008.0 ⁹			
	Champia parvula reproduction	1009.0 ⁹			

Notes to Table IA:

¹ The method must be specified when results are reported.

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

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

⁴ APHA. 1992. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 18th Edition. Amer. Publ. Hlth. Assoc., Washington, DC.

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

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

⁷ USEPA. 1993. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. Fourth Edition. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. August 1993, EPA/600/4-90/027F.

⁸ USEPA. 1994. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Third Edition. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency USEPA. 1994, Cincinnati, Ohio (July 1994, EPA/600/4-91/002).

⁹ Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Second Edition. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio (July 1994, EPA/600/4-91/003). These methods do not apply to marine waters of the Pacific Ocean.

3. Section 136.3(b) is amended by revising references (2), (6), and (11) and by adding references (34), (38), and (39) to read as follows:

§ 136.3 Identification of test procedures.
* * * * *

(b) * * *
References, Sources, Costs, and Table Citations

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(2) USEPA. 1978. Microbiological Methods for Monitoring the Environment, Water, and Wastes. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA/600/8-78/017. Available from: National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Publ. No. PB-290329/AS. Cost: \$36.95. Table IA, Note 3.
* * * * *

(6) American Public Health Association. 1992. Standard Methods for the Examination of Water and Wastewater. 18th Edition. Amer. Publ. Hlth. Assoc., 1015 15th Street NW, Washington, DC 20005. Cost: \$160.00. Table IA, Note 4.
* * * * *

(11) USGS. 1989. U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Laboratory Analysis, Chapter A4, Methods for

Collection and Analysis of Aquatic Biological and Microbiological Samples, U.S. Geological Survey, U.S. Department of the Interior, Reston, Virginia. Available from: USGS Books and Open-File Reports Section, Federal Center, Box 25425, Denver, Colorado 80225. Cost: \$18.00. Table IA, Note 5.
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(34) USEPA. 1993. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. Fourth Edition, December 1993. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio (EPA/600/4-90/027F). Available from: National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Publ. No. PB-91-167650. Cost: \$31.00. Table IA, Note 17. See changes in the manual, listed in Part V of this rule.
* * * * *

(38) USEPA. 1994. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Third

Edition. July 1994. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. (EPA/600/4-91/002). Available from: National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Publ. No. PB-92-139492. Cost: \$31.00. Table IA, Note 8.

(39) USEPA. 1994. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Second Edition, July 1994. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA/600/4-91/003. Available from: National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Publ. No. PB-92-139484. Cost: \$45.00. Table IA, Note 9.

4. In § 136.3(e), Table II is amended by revising the entry for "Table IA-Bacteria Tests:" and adding an entry for "Table IA-Aquatic Toxicity Tests:" and by revising footnote 1 and adding footnote 16 to read as follows:

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

Parameter No./name	Container ¹	Preservation ^{2,3}	Maximum holding time ⁴
Table IA—Bacteria Tests:			
1-4 Coliform, fecal and total	P,G	Cool, 4C, 0.008% Na ₂ S ₂ O ₃ ⁵	6 hours.
5 Fecal streptococci	P,G	Cool, 4C, 0.008% Na ₂ S ₂ O ₃ ⁵	6 hours.
Table IA—Aquatic Toxicity Tests:			
6-10 Toxicity, acute and chronic	P,G	Cool, 4C ¹⁶	6 hours.
* * * * *	* * * * *	* * * * *	* * * * *

¹ Polyethylene (P) or glass (G). For microbiology, plastic sample containers must be made of sterilizable materials (polypropylene or other autoclavable plastic).

² Sample preservation should be performed immediately upon sample collection. For composite chemical samples, each aliquot should be preserved at the time of collection. When use of an automatic sampler makes it impossible to preserve each aliquot, then chemical samples may be preserved by maintaining at 4C until compositing and sample splitting is completed.

³ When any sample is to be shipped by common carrier or sent through the United States Mails, it must comply with the Department of Transportation Hazardous Materials Regulations (49 CFR Part 172). The person offering such material for transportation is responsible for ensuring such compliance. For the preservation requirements of Table II, the Office of Hazardous Materials, Transportation Bureau, Department of Transportation, has determined that the Hazardous Materials Regulations do not apply to the following materials: Hydrochloric Acid (HCl) in water solutions at concentrations of 0.04% by weight or less (pH about 1.96 or greater); Nitric Acid (HNO₃) in water solutions of 0.15% by weight or less (pH about 1.62 or greater); Sulfuric Acid (H₂SO₄) in water solutions of 0.35% or less (pH about 1.15 or greater); and Sodium Hydroxide (NaOH) in water solutions at concentrations of 0.080% by weight or less (pH about 12.30 or less).

⁴ Samples should be analyzed as soon as possible after collection. The times listed in the table are the maximum times that samples may be held before analyses and still be considered valid. Samples used for toxicity tests are to be used for test initiation or for renewal of test solutions within 36 h of collection as grab samples, or within 36 hours of the collection of the last sample of the composite. Samples for bacteria or chemical analysis may be held for longer periods than specified in this table only if the permittee or monitoring laboratory has data on file to show that the specific types of samples under study, the analytes are stable for the longer time, and has received a variance from the Regional Administrator under Para. 136.3(e). Some samples may not be stable for the maximum time period given in the table. A permittee or monitoring laboratory is obligated to hold the samples for a shorter time if knowledge exists to show that this is necessary to maintain sample stability. See Para. 136.3(e) for details. The term "analyze immediately" usually means within 15 minutes or less of sample collection.

⁵ Should only be used in the presence of residual chlorine.

¹⁶ Sufficient ice should be placed with the samples in the shipping container to ensure that ice is still present when the samples arrive at the laboratory. However, even if ice is present when the samples arrive, it is necessary to immediately measure the temperature of the samples and confirm that the 4C temperature maximum has not been exceeded. In the isolated cases where it can be documented that this holding temperature can not be met, the permittee can be given the option of on-site testing or can request a variance. The request for a variance should include supportive data which show that the toxicity of the effluent samples is not reduced because of the increased holding temperature.

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FEDERAL COMMUNICATIONS COMMISSION

47 CFR Parts 32 and 36

[DA 95-2036]

Reporting Requirements on Video Dialtone Costs and Jurisdictional Separations for Local Exchange Carriers Offering Video Dialtone Services

AGENCY: Federal Communications
Commission.

ACTION: Final rule.

SUMMARY: On September 29, 1995, the Bureau issued a Memorandum Opinion and Order (MO&O) that adopted reports for local exchange carriers offering video dialtone service. The reports will enable the Commission, State regulatory agencies, local exchange carriers ("LEC"), and other interested parties to analyze LECs' video dialtone investment, revenue, and costs. Specifically, the data will allow the Commission to monitor the implementation of video dialtone service, to assist the Commission in ensuring that local telephone service ratepayers do not absorb any of the costs of a LEC's video dialtone operations, to track the impact of video dialtone on jurisdictional separations and local telephone rates, and to aid the Commission in its tariff review process.

EFFECTIVE DATE: September 29, 1995.

FOR FURTHER INFORMATION CONTACT: Timothy Peterson, Common Carrier Bureau, Accounting and Audits Division, (202) 418-0810.

SUPPLEMENTARY INFORMATION: In the MO&O the Bureau addressed the issues raised by the parties in response to its June 23, 1995, Order Inviting Comments that solicited comment on the proposed content and format of the video dialtone reporting requirements.

Comments were filed by local exchange carriers, the cable industry and representatives of the states. Generally, the local exchange carriers

believed that the reporting requirements were overly burdensome. The cable industry and representatives of the states believed that the reporting requirements should be expanded to include additional data. In response to the comments of the parties, the Bureau revised its original proposal to eliminate certain data that it determined were not essential to meet the Commission objectives.

FCC Report 43-09A was adopted by the Common Carrier Bureau in the Memorandum Opinion and Order released September 29, 1995 to establish reporting requirements on video dialtone costs for local exchange carriers offering video dialtone service. The report is prescribed for every local exchange carrier that has obtained Section 214 authorization from the Commission to provide video dialtone trials or commercial services.

Affected carriers shall file by June 30, September 30, and December 31 of each year the report for the previous quarter. The initial report will be filed on the last day of the calendar quarter after the end of the calendar quarter in which a carrier received Section 214 authorization. The report shall be filed on a study area basis.

FCC Report 43-09A provides a quarterly report of wholly dedicated and shared video dialtone investment, expense, and revenue captured in a carrier's subsidiary accounting records. The report line items generally follow those provided in existing FCC Report 43-01, ARMIS Quarterly Report, with minor exceptions. The report columns identify data for each line item by dedicated video dialtone costs and revenues, shared costs and revenues, and video dialtone's portion of shared costs and revenues.

FCC Report 43-09B was adopted by the Common Carrier Bureau to establish reporting requirements on video dialtone costs and jurisdictional separations for local exchange carriers offering video dialtone service. The report is prescribed for every local exchange carrier that has obtained Section 214 authorization from the Commission to provide video dialtone trials or commercial services.

Affected carriers shall file by March 31 of each year the report for the fourth calendar quarter. The report shall be filed on a study area basis.

FCC Report 43-09B provides a fourth quarter report of video dialtone investment, expense, and revenue disaggregated by regulated and nonregulated classification and by jurisdictional categories. The reports summarize the impact of video dialtone on the interstate and intrastate jurisdictions and local telephone rates. The report line items generally follow those provided in existing FCC Report 43-01, ARMIS Quarterly Report, with minor exceptions. The report columns identify data for each line item by total costs and revenues, dedicated video dialtone costs and revenues, shared costs and revenues, video dialtone's portion of shared costs and revenues, total video dialtone costs and revenues, video dialtone's percentage of total costs and revenues, nonregulated and nonregulated video dialtone costs and revenues, and video dialtone costs and revenues subject to separations and those allocated to the intrastate and interstate jurisdictions. These reporting requirements have been approved by OMB under OMB control number 3060-0680.

Complete text of the Memorandum Opinion and Order is available for inspection and copying in the Accounting and Audits Division public reference room, 2000 L Street NW., Suite 812, Washington DC.

Copies are also available from International Transcription Service, Inc., at 2100 M Street NW., Suite 140, Washington, DC 20037, or call (202) 857-3800.

List of Subjects

47 CFR Part 32

Uniform System of Accounts.

47 CFR Part 36

Jurisdictional separations procedures, Telephone.

William F. Caton,

Acting Secretary, Federal Communications
Commission.

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