ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 136

[FRL–7069–7]

Guidelines Establishing Test Procedures for the Analysis of Pollutants; Whole Effluent Toxicity Test Methods

AGENCY: Environmental Protection Agency (EPA).

ACTION: Proposed rule.

SUMMARY: Today, EPA proposes to ratify its approval of several analytic test procedures measuring ''whole effluent toxicity,'' which the Agency standardized in an earlier rulemaking. Today's proposal also would modify some of those test procedures. EPA is proposing today's notice to satisfy obligations in a settlement agreement designed to resolve litigation over that earlier rulemaking. The proposed changes are intended to improve the performance of whole effluent toxicity (WET) tests, and thus increase confidence in the reliability of the results obtained using the test procedures.

DATES: Comments on this proposal must be postmarked, delivered by hand, or electronically mailed on or before November 27, 2001. Comments provided electronically will be considered timely if they are submitted electronically by 11:59 p.m. Eastern Standard Time (EST) on November 27, 2001.

ADDRESSES: Send written or electronic comments on the proposed rule to ''Whole Effluent Toxicity (WET) Test Method Changes'' Comment Clerk (WETEU–IX); Water Docket (4101); Environmental Protection Agency; Ariel Rios Building; 1200 Pennsylvania Avenue, NW; Washington, DC—P 20460. EPA requests that commenters submit copies of any references cited in comments. Commenters also are requested to submit an original and three copies of their written comments and enclosures. Commenters that want receipt of their comments acknowledged should include a self-addressed, stamped envelope. All written comments must be postmarked or delivered by hand. No facsimiles (faxes) will be accepted. Hand deliveries should be delivered to EPA's Water Docket at 401 M Street, SW, Room EB57, Washington, D.C. 20460.

Comments may be submitted electronically to: *OW-Docket@epa.gov.* Electronic comments must be submitted as a Word Perfect 5/6/7/8 file or an ASCII file, avoiding the use of special characters and any form of encryption. Comments and data also will be accepted on disks in WordPerfect 5/6/7/8 or ASCII file format. Electronic comments on this proposed rule may be filed online at any Federal Depository Library. All electronic comments must be identified by docket number (WET–IX). Electronic comments will be transferred into a paper version for the official record. EPA will attempt to clarify electronic comments if there is an apparent error in transmission.

The record for this rulemaking has been established under docket number WET–IX. A copy of the supporting documents cited in this proposal is available for review at EPA's Water Docket, East Tower Basement (Room EB 57), 401 M Street, SW, Washington, DC 20460. For access to docket materials, call (202) 260–3027 on Monday through Friday, excluding Federal holidays, between 9:00 a.m. and 3:30 p.m. EST to schedule an appointment.

This **Federal Register** document has been placed on the Internet for public review and downloading at the following location: http://www.epa.gov/fedrgstr/. The final report of EPA's WET Interlaboratory Variability Study, Volumes 1 and 2 (USEPA, 2001a; USEPA, 2001b) and the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d), which is referenced in today's rule and provides details of proposed changes, also are available on the Internet at http://www.epa.gov/waterscience/WET.

FOR FURTHER INFORMATION CONTACT: For regulatory information regarding this proposal, contact Marion Kelly, Engineering and Analysis Division (4303), Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency, 1200 Pennsylvania Avenue, NW, Washington, DC 20460 (e-mail: kelly.marion@epa.gov) or call (202) 260–7117. For technical information regarding method changes proposed in today's rule, contact Teresa J. Norberg-King, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, Office of Research and Development, U.S. Environmental Protection Agency, 6201 Congdon Boulevard, Duluth, MN 55804 (e-mail: norberg-king.teresa@epa.gov) or call (218) 529–5163.

SUPPLEMENTARY INFORMATION:

Potentially Regulated Entities

EPA Regions, as well as State, Territories and Tribes authorized to implement the National Pollutant Discharge Elimination System (NPDES) program, issue permits that comply with the technology-based and water quality-based requirements of the Clean Water Act. In doing so, the NPDES permitting authority, including authorized States, Territories, and Tribes, make a number of discretionary choices associated with permit writing, including the selection of pollutants to be measured and, in many cases, limited in permits. If EPA has ''approved'' (i.e., promulgated through rulemaking) standardized testing procedures for a given pollutant, the NPDES permitting authority must specify one of the approved test procedures or an approved alternate test procedure for the measurements required under the permit. In addition, when a States, Territory, or authorized Tribe provides certification of Federal licenses under CWA section 401, measurements required by such certifications must be made using the approved testing procedures. Categories and entities that may be regulated include:

Category	Examples of potentially affected/regulated entities
States, Territorial, and Indian Tribal Governments	States, Territories, and Tribes authorized to administer the NPDES permitting program; States, Territories, and Tribes that certify Federal licenses.

This table is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be regulated by this action. This table lists the types of entities that EPA is now aware could potentially be regulated by this action. Other types of entities not listed in the table also could be regulated. If you have questions regarding the applicability of this action to a particular entity, consult the persons listed in the preceding **FOR FURTHER INFORMATION CONTACT** section.

Outline of Preamble

I. Statutory Authority

Today's proposal is pursuant to the authority of sections 101(a), 301, 304(h), 402, and 501(a) of the Clean Water Act (CWA), 33 U.S.C. 1251(a), 1311, 1314(h), 1342, 1361(a) (the ''Act''). Section 101(a) of the Act sets forth the ''goal of restoring and maintaining the chemical, physical, and biological integrity of the Nation's waters'' and prohibits ''the discharge of toxic pollutants in toxic amounts.'' Section 301 of the Act prohibits the discharge of any pollutant into navigable waters unless the discharge complies with a National Pollutant Discharge Elimination System (NPDES) permit, issued under section 402 of the Act. Section 304(h) of the Act requires the Administrator of the EPA to ''promulgate guidelines establishing test procedures for the analysis of pollutants that shall include the factors which must be provided in any certification pursuant to section 401 of this Act or permit applications pursuant to section 402 of this Act.'' Section 501(a) of the Act authorizes the Administrator to ''prescribe such regulations as are necessary to carry out his function under this Act.''

II. Regulatory Background

Standardized analytical procedures for monitoring and reporting required in NPDES permits (40 CFR part 122, §§ 122.21, 122.41, 122.44, and 123.25), and in the implementation of the pretreatment standards issued under section 307 of the Act (40 CFR part 403, §§ 403.10 and 402.12) appear at 40 CFR part 136. 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.'' Permits may include, for example, effluent limitations for WET using standardized testing procedures other than those published at 40 CFR part 136 that are approved for nationwide use. In such cases, use of the particular test species and test protocols would remain subject to challenge on a case-by-case basis in permit proceedings (except, for example, if an authorized State conducted rulemaking to standardize a particular testing procedure applicable within the State).

In 1995, EPA amended the ''Guidelines Establishing Test Procedures for the Analysis of Pollutants,'' 40 CFR part 136, to add a series of standardized whole effluent toxicity (WET) test methods to the list of Agency approved methods for CWA data gathering and compliance monitoring programs (60 FR 53529; October 16, 1995) (WET final rule). The WET final rule amended 40 CFR 136.3 (Tables IA and II) by adding acute toxicity methods and short-term methods for estimating chronic toxicity. These methods measure the toxicity of effluents and receiving waters to freshwater, marine, and estuarine organisms. Acute methods (USEPA, 1993b) generally use death of the test organisms during 24 to 96 hour exposure durations as the measured effect of an effluent or receiving water. The short-term methods for estimating chronic toxicity (USEPA, 1994a; USEPA, 1994b) use longer durations of exposure (up to nine days) to ascertain the adverse effects of an effluent or receiving water on survival, growth, and/or reproduction of the organisms. For this rulemaking notice, the short-term methods for estimating chronic toxicity will be referred to as chronic methods for ease of notation.

Standardized test procedures for conducting the approved acute and chronic WET tests are provided in the following three method manuals, which were incorporated by reference in the WET final rule: Methods for Measuring the Acute Toxicity of Effluents and Receiving Water to Freshwater and Marine Organisms, Fourth Edition, August 1993, EPA/600/4–90/027F (acute method manual); 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 (freshwater chronic method manual); and 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 (marine chronic method manual).

After promulgation of the WET methods, a variety of parties filed suit challenging the EPA rulemaking (*Edison Electric Institute* v. *EPA*, No. 96–1062 (D.C. Cir.); *Western Coalition of Arid States* v. *EPA*, No. 96–1124; *Lone Star Steel Co.* v. *EPA*, No. 96–1157 (D.C. Cir.)). To resolve that litigation, EPA entered into settlement agreements with the various parties. EPA proposes actions today to fulfill obligations under some of those settlement agreements.

In February 1999, EPA published a technical corrections notice that incorporated into the WET final rule an errata document to correct minor errors and omissions, provide clarification, and establish consistency among the WET final rule and method manuals (64 FR 4975; February 2, 1999). Further background on the WET test methods and these technical documents are included in the **Federal Register** notices cited above (60 FR 53529 and 64 FR 4975).

III. Explanation of Today's Action

A. Introduction

Today's proposal would make a number of revisions to the currently approved WET test methods. See section III.B. Also in today's action, EPA presents final results of an interlaboratory variability study of WET test methods and, based on these results, proposes to ratify 11 of the 12 methods evaluated in the study (see section III.C). Today's proposal requests public comment on the inclusion of additional technical changes to the approved WET test methods and on EPA's proposal to ratify 11 of 12 WET test methods.

Although today's action fulfills portions of settlement agreements resolving litigation over the 1995 WET test method rulemaking, EPA acknowledges that some stakeholders still have significant concerns related to implementation of WET control strategies through NPDES permits. By today's proposal, EPA intends to focus only on analytic testing methodologies to measure WET, not on WET implementation generally.

Since the 1995 WET final rule, EPA and authorized States have taken additional actions to improve and enhance implementation of WET control strategies. EPA, for example, has published additional guidance on the conduct of a toxicity identification evaluation (TIE) and a toxicity reduction evaluation (TRE), as well as guidance on the circumstances that trigger such evaluations (USEPA, 1999c; USEPA, 2001g).

Other questions have arisen about the significance of EPA action to standardize WET testing procedures through rulemaking. For example, some stakeholders question whether, by promulgating WET test methods, EPA has published recommended water quality criteria (pursuant to CWA section 304(a)) for ''toxicity.'' To respond and clarify, EPA's promulgation of WET test procedures are not water quality criteria recommendations under section 304(a). When States develop and implement water quality standards, including narrative water quality criteria, States should translate those criteria into measurable expressions of toxicity. The test methods themselves are not per se translators of the narrative criterion: ''no toxics in toxic amounts.'' The test methods are merely the measurement tools according to which such criteria may be translated.

Today's proposed revisions include changes to the three method manuals (USEPA, 1993b; USEPA, 1994a; USEPA, 1994b) incorporated by reference in the WET rule (60 FR 53529; October 16, 1995) and amend the ''Guidelines Establishing Test Procedures for the Analysis of Pollutants'' (40 CFR part 136) to reference the updated editions of the method manuals. Modifications to the method manuals are intended to update the methods, provide additional minor corrections and clarifications, and address specific stakeholder concerns (see Section III.B). EPA proposes to update the methods (1) by incorporating previous method addenda and errata and (2) by revising method precision statements to reflect results from recent EPA studies (USEPA, 2000d; USEPA, 2001a). In addition to corrections identified in previous method addenda and errata, EPA proposes to correct other minor technical errors and omissions. EPA also seeks comment on an additional modification to WET test methods that would require the application of upper and lower bounds on the percent minimum significant difference (PMSD) calculated in WET tests (see section V.B).

EPA also proposes method revisions in response to specific stakeholder concerns. Specifically, these revisions include: requiring ''blocking'' by known parentage in the Ceriodaphnia dubia Survival and Reproduction Test; adding procedures to control pH drift that may occur during testing; incorporating review procedures for the evaluation of concentration-response relationships; clarifying allowable nominal error rate adjustments; clarifying limitations in the generation of confidence intervals; adding guidance on dilution series selection; clarifying dilution water acceptability; and adding procedures for determining and minimizing the impact of pathogens in the Fathead Minnow Survival and Growth Test. These are summarized below in section III.B and detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d). Proposal of these revisions partially fulfills the requirements of two settlement agreements between stakeholders and EPA (*Edison Electric Institute, et al.* v. *EPA*, No. 96–1062 & consolidated case (D.C. Cir.), Settlement Agreement, July 24, 1998; *Lone Star Steel* v. *EPA*, No. 96–1157 (D.C. Cir.), Settlement Agreement, March 4, 1998).

EPA requests public comment on the proposed changes to the WET test methods and on the proposal to ratify the WET test methods (see section V). When EPA takes final action on today's proposal, the Agency intends to incorporate the modifications proposed today into the text of new editions of each of the WET method manuals.

B. Proposed Method Changes

Today, EPA proposes to revise each of the WET method manuals (USEPA, 1993b; USEPA, 1994a; USEPA, 1994b). Proposed method changes include: (1) updates to the methods, (2) minor corrections and clarifications, and (3) modifications to address specific stakeholder concerns. These method changes are described in Sections 1 through 3 below and are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d), which is included in the docket supporting today's rule and is available online at http://www.epa.gov/waterscience/WET.

1. Updates

a. Incorporation of Previous Addenda and Errata

Subsequent to promulgating the WET final rule in 1995, EPA issued several documents to correct and amend that rule and its supporting documentation. Specifically, in February 1999, EPA published a final rule that incorporated into the WET rule an errata document (USEPA, 1999a) to correct minor errors and omissions in the WET method manuals (64 FR 4975; February 2, 1999). In addition, a 1996 addenda document (USEPA, 1996a) revised the 1993 acute method manual (USEPA, 1993b). Today, EPA proposes to incorporate the changes noted in the errata and the addenda documents into the text of the appropriate method manuals by issuing revised editions of each of the three method manuals. EPA plans to issue the revised editions when it takes final action on this proposal. The incorporation of the errata and addenda into the method manual text would not further alter the methods. This action would simply assist users of the method manuals by incorporating all previous corrections into updated editions.

b. Update of Method Precision Data

Since publishing the WET method manuals, EPA has conducted two large-scale studies of WET test method precision. During 1999 and 2000, EPA conducted an interlaboratory variability study (the WET Variability Study) of 12 of the 17 WET test methods promulgated at 40 CFR part 136. This study generated data from more than 700 blind samples tested in 55 laboratories. EPA published interlaboratory precision results from the WET Variability Study in 2000 (USEPA, 2000b; USEPA, 2000c) and submitted the study results for expert

peer review in 2001 (USEPA, 2001c). Following expert peer review, EPA published a final study report (USEPA, 2001a; USEPA, 2001b).

In addition to the WET Variability Study, EPA conducted a study of intralaboratory WET test precision based on routine laboratory reference toxicant test data. EPA compiled a database of more than 1,800 reference toxicant tests conducted for 23 different methods between 1988 and 1999 in 75 laboratories. EPA used this database to quantify estimates of precision for each of the WET methods. EPA published this precision data and additional guidance on reducing method variability in a guidance document titled, Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System Program (USEPA, 2000d) (the Variability Guidance Document).

In today's action, EPA proposes to modify the WET method manuals by updating statements and inserting tables regarding the multi-laboratory (interlaboratory) and single-laboratory (intralaboratory) precision of the methods using data from the WET Variability Study and the Variability Guidance Document. Results from these two studies represent the most current and complete data available on intralaboratory and interlaboratory precision of WET test methods. The proposed changes would modify the chronic method manuals (USEPA, 1994a; USEPA, 1994b) by revising subsections on precision and accuracy for several test methods. The proposed changes also would modify Section 4 (Quality Assurance) of each of the method manuals (USEPA, 1993b; USEPA, 1994a; USEPA, 1994b) to update statements on test method variability and precision. The specifics of the proposed method manual changes related to updating precision statements are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d).

2. Minor Corrections and Clarifications

In addition to the incorporation of changes identified in the 1999 errata (USEPA, 1999a) and the acute manual addenda (USEPA, 1996a), EPA proposes to correct additional minor errors and omissions in the WET method manuals. All of the minor corrections and clarifications identified to date are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d). This list may not be exhaustive, and EPA proposes to correct additional minor errors and omissions that become apparent during the correcting or revising of sections of the WET method manuals.

3. Specific Stakeholder Concerns

Today, EPA also proposes to modify the WET method manuals to address specific stakeholder concerns. The proposed modifications are summarized in Sections a through h below and are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d), which is included in the docket supporting today's rule and is available online at http://www.epa.gov/waterscience/WET. Proposal of these revisions partially fulfills the requirements of two settlement agreements between stakeholders and EPA (*Edison Electric Institute, et al.* v. *EPA,* Settlement Agreement, July 24, 1998; *Lone Star Steel* v. *EPA,* Settlement Agreement, March 4, 1998).

a. Blocking by Known Parentage

EPA proposes to amend the Ceriodaphnia dubia Survival and Reproduction Test (section 13 of USEPA, 1994a) to require that test organisms be allocated using ''blocking by known parentage.'' Blocking by known parentage is a block randomization technique for allocating test organisms among test chambers such that offspring from a single female are distributed evenly among the test treatments (one per treatment). In this arrangement, a block consists of the set of six test chambers (one for each test treatment) containing organisms derived from a single female parent.

Currently, the promulgated method describes a blocking by known parentage procedure for use in test setup, but the method does not require the use of this procedure. Today's proposal would require the use of blocking by known parentage by using compulsory terms such as ''must'' and ''shall.'' The procedure described for test setup in the current promulgated method would be retained as an example of how blocking by known parentage may be accomplished.

In association with a blocking by known parentage requirement, today's proposal also would add guidance on the treatment of males that may occur in tests. The proposed changes would require exclusion of an entire block from reproduction analysis (i.e., calculation of the no observed effect concentration for reproduction and the 25% inhibition concentration for reproduction) when 50% or more of the surviving organisms in that block are identified as males. If less than 50% of surviving organisms in a block are identified as males, only those males would be excluded from the reproduction analysis. The proposed changes also would stipulate that a test is invalid if fewer than eight replicates remain in the control after excluding individual males and necessary blocks (i.e., those having 50% or more of surviving organisms identified as males). The specifics of all proposed method manual changes related to blocking by known parentage are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d).

Blocking by known parentage provides at least two benefits to the performance of the Ceriodaphnia dubia Survival and Reproduction Test (USEPA, 2001e). First, this technique of test organism allocation ensures that any ''brood effect'' is evenly distributed among the test treatments. Brood effects include differences in organism fecundity or sensitivity that may be attributed to the health or genetics of the parent organism. Blocking by known parentage minimizes any potential bias that may be caused by one test treatment receiving an inordinate number of underperforming (or overperforming) young from the same parent organism. In an analysis of 389 tests from EPA's reference toxicant test database (USEPA, 2000d) and 102 tests from EPA's WET Variability Study (USEPA, 2001a), 9% and 25% of tests, respectively, showed statistically significant (alpha = 0.05) block effects on the reproduction endpoint (USEPA, 2001e). This means that, for these tests, the number of offspring produced by test organisms was significantly affected by the parental source of those test organisms. The blocking by known parentage technique distributes this effect evenly across the test treatments to ensure that observed differences in reproduction between treatments are due to the effect of the treatment and not the parental source of test organisms.

A second benefit of blocking by known parentage would be that it provides a means of minimizing the impact of male production on test performance. In healthy cultures, Ceriodaphnia dubia generally reproduce parthenogenetically to produce cloned females for use in testing. Under conditions of environmental stress, however, cladocerans (such as Ceriodaphnia dubia and Daphnia magna) are known to produce males (Pennak, 1989), which can negatively affect the performance of toxicity tests designed to measure reproductive

effects (Haynes et al., 1989). When using blocking by known parentage, males produced by a given brood female are contained within a single block of the test rather than randomly scattered throughout the test. If a large number of males are produced from a given brood female, the associated block may be removed from the analysis of reproduction, thereby minimizing the effect of those males on the test. Blocking by known parentage also allows the source of males to be identified, so that potential problems with culture health can be more easily isolated.

b. pH Drift

During the conduct of static or static-renewal WET tests, the pH in test containers may fluctuate or drift from the initial pH value. This pH drift may be upward or downward depending upon test conditions and sample characteristics. For instance, the addition of food substances such as algae may cause a decrease in pH, while the loss of carbon dioxide (CO_2) from supersaturated effluent samples may cause an increase in pH. A change in pH during testing means that an effluent sample might be tested for toxicity at a different pH than the effluent sample pH at the point of discharge. Under certain circumstances, this pH drift could influence sample toxicity and be considered a test interference. For this reason, EPA is proposing to provide guidance in the chronic method manuals (USEPA, 1994a; USEPA, 1994b) on how to identify if pH drift is a test interference and how to control test pH if artifactual toxicity due to pH drift is confirmed.

For most tests, the range of pH drift is small, is well within the organisms' tolerance range, and does not interfere with the analysis of whole effluent toxicity. In EPA's WET Variability Study (USEPA, 2001a), daily pH drift in blank samples averaged only +0.1 units (with a range of −0.3 to +0.8 among 35 tests) in the Ceriodaphnia dubia Survival and Reproduction Test and −0.1 units (with a range of −1.4 to +0.7 among 25 tests) in the Fathead Minnow Larval Survival and Growth Test. For effluent samples (municipal wastewater spiked with KCl) analyzed in EPA's WET Variability Study, pH drift in the 100% sample increased slightly for the Ceriodaphnia dubia Survival and Reproduction Test, averaging +0.3 units (with a range of −0.2 to +1.1 among 28 tests). For the Fathead Minnow Larval Survival and Growth Test, daily pH drift in effluent samples averaged −0.1 units (with a range of −0.6 to +0.4 among 28 tests), the same degree of drift observed in blank samples. Ninety percent of Ceriodaphnia dubia Survival and Reproduction Tests (126 tests) experienced absolute pH drift (up or down) of less than 0.7 units, and 90% of Fathead Minnow Larval Survival and Growth Tests (105 tests) experienced absolute pH drift of less than 0.5 units.

While pH drift was relatively mild for most samples analyzed in the WET Variability Study (USEPA, 2001a), other effluent samples may routinely exhibit a greater degree of pH drift. For example, municipal wastewater from Publicly-Owned Treatment Works (POTW) is typically discharged at a pH of 7.2–7.4, but the pH may equilibrate after contact with air and stabilize at 8.0–8.5 (USEPA, 1992). In a 1998 survey of 433 POTWs, 39% of respondents indicated that upward drift of effluent sample pH had been observed during acute or chronic WET testing (DeGraeve *et al.*, 1998). Upward pH drift in POTW effluent is generally caused by dissipation of CO_2 from the sample. Biological treatment often produces an effluent that is supersaturated with CO_2. As dissolved CO_2 in the supersaturated sample equilibrates with the atmospheric CO_2 concentration, CO_2 is lost from the sample. Because dissolved CO_2 acts as a weak acid, pH increases as CO_2 is lost. In cases where pH drift is due to the effluent characteristics, the degree of drift will be greatest in the 100% effluent concentration and will decrease with decreasing test concentrations.

EPA does not consider pH drift alone to be an interference in WET testing if pH is within the organism's tolerance range (typically pH 6 to 9). Belanger and Cherry (1990) showed that Ceriodaphnia dubia survival and reproduction did not differ significantly in receiving water tests conducted at pH values ranging from 6 to 9. The degree of pH drift typically observed in effluent samples should generally only interfere with test results if the sample contains a compound with toxicity that is pH dependent and at a concentration that is near the toxicity threshold. Compounds with pH-dependent toxicity are those with chemical characteristics that allow sufficient differences in dissociation, solubility, or speciation to occur within a physiologically tolerable pH range of 6 to 9 (Schubauer-Berigan *et al.*, 1993). Examples of such compounds include ammonia, metals, hydrogen sulfide, cyanide, and ionizable organics. Ammonia, for instance, is very common in effluent samples, and its toxicity changes sharply within the typical effluent pH range of 7 to 8.5. As pH increases and the temperature is held relatively constant, the percent of total ammonia in the un-ionized form increases (USEPA, 1994a; Emerson *et al.*, 1975). Because the un-ionized form of ammonia (NH3) is significantly more toxic than the ionized form (NH_4^+), toxicity increases as pH increases. For metals, toxicity may increase or decrease with increasing pH. Lead and copper were found to be more acutely toxic at pH 6.5 than at pH 8.0 or 8.5, while nickel and zinc were more toxic at pH 8.5 than at pH 6.5 (USEPA, 1992). pH-dependent toxicity is likely to be affected by temperature, dissolved oxygen, CO_2 concentrations, and total dissolved solids (USEPA, 1992). When pH-dependent compounds are present at concentrations near the threshold for toxicity, pH drift during WET testing may produce artifactual toxicity, or toxicity that would not have been observed if the initial test pH had been maintained.

In addition to the issue of pH drift affecting toxicity in the presence of pH-dependent compounds, stakeholders have raised concerns about daily pH drift and sample renewal cycles producing toxicity even in the absence of pH-dependent compounds. The circumstance of concern would be in static-renewal tests, where the pH may change between the time test organisms are placed into the test solutions and the time at which the test solution is renewed. At renewal, the pH of test solutions may be quickly returned to the initial sample pH. For chronic tests that require daily renewal, a daily cycle of pH drift and renewal may be established. Stakeholders expressed concern that, if the difference in pH between the test solution and the renewal solution is great, these adjustments in pH at renewal may cause shock to the test organisms. Because the control treatment does not always experience the same pH drift as effluent treatments, any shock resulting from daily renewal would be experienced only in effluent treatments and artifactual toxicity could result. In a 1998 settlement agreement with these stakeholders (*Edison Electric Institute, et al.* v. *EPA*, Settlement Agreement, July 24, 1998), EPA agreed to propose changes to the WET methods that would provide methodological solutions for controlling pH drift.

Currently, the WET method manuals (USEPA, 1993b; USEPA, 1994a; USEPA, 1994b) provide guidance for effluent samples that arrive (i.e., at the testing laboratory prior to testing) with a pH outside of the 6.0 to 9.0 range. This range represents the general organism tolerance range, so pH values outside of this range may produce toxic effects due to pH alone. For samples that arrive

with a pH outside of this range, the current method manuals require adjustment of the sample to pH 7 for freshwater testing or pH 8 for marine testing. The method manuals also suggest brief aeration of samples prior to use if dissolved oxygen levels are not at or near saturation. Aeration provides the benefit of bringing other dissolved gases (e.g., CO_2) into equilibrium with the atmosphere and stabilizing pH, but use of aeration should be minimized to reduce the loss of volatile chemicals.

In 1996, EPA issued additional guidance on ammonia and pH control in chronic testing (USEPA, 1996b). This guidance recognized that the analyst has flexibility to control artifactual toxicity caused by pH drift in chronic tests provided that the analyst verifies that the source of toxicity is, in fact, artifactual. To verify that the toxicity is artifactual, EPA recommended parallel testing using one test with an adjusted pH and one test without an adjusted pH. If toxicity is removed or reduced when pH is adjusted, the source of toxicity could be artifactual and pH could be controlled in the testing of the effluent. This guidance acknowledged that pH could be controlled during testing with procedures that do not significantly alter the nature of the sample.

Today, EPA proposes to modify the chronic method manuals (USEPA, 1994a; USEPA, 1994b) to incorporate procedures for controlling pH drift in static-renewal tests when sample toxicity is confirmed to be artifactual and caused by pH drift. EPA proposes adding guidance that is consistent with the 1996 USEPA guidance on pH and ammonia control in chronic testing (USEPA, 1996b), and extending this guidance to include situations where artifactual toxicity is caused by pH drift in the absence of ammonia.

The proposed method changes would require that, prior to the use of pH control techniques, the analyst must confirm that observed toxicity is artifactual and caused by pH drift. Evidence of artifactual toxicity would be demonstrated by conducting parallel tests: one with controlled pH and one with uncontrolled pH. Several such parallel tests conducted on a given effluent may be required by the regulatory authority to verify that the toxicity observed in that effluent is artifactual and caused by pH drift (as opposed to variability in effluent samples). Following this determination, the regulatory authority may allow pH control in subsequent chronic toxicity testing of the effluent. The proposed method changes would specify the use of acid/base addition and/or a CO_2-controlled atmosphere technique for adjusting and controlling pH in chronic tests.

The CO_2-controlled atmosphere technique that is proposed for pH control in chronic tests is conducted using enclosed test chambers with CO_2 injected into the headspace above the test solution (USEPA, 1991a; USEPA, 1992; USEPA, 1996c; Mount and Mount, 1992). An enriched-CO_2 environment increases the dissolution of CO_2 into the sample, which acts as a weak acid to prevent pH increases. This technique uses the natural carbonate buffering system to control pH and requires minimal alteration of the sample. This technique is one method recommended for adjusting pH in toxicity identification evaluations (TIEs) (USEPA, 1991a; USEPA, 1992; USEPA, 1996c).

In acute testing, the proposed method changes would recommend the use of static-renewal testing or flow-through testing when artifactual toxicity due to pH drift is suspected. The use of static-renewal testing may reduce the degree of pH drift (compared to static non-renewal tests), and flow-through testing should eliminate pH drift that could occur due to static testing conditions. In flow-through testing, new sample is continually added to the test chambers, so drift from the initial sample pH should not occur. Flow-through testing also eliminates any potential for organism shock from pH drift and renewal cycles, because test renewal is continuous. Because flow-through testing provides an available option for reducing pH drift in acute tests without modifying the sample, EPA does not propose additional techniques (such as acid/base addition and/or CO_2-controlled atmosphere techniques that are proposed for chronic test methods) for pH control in acute test methods.

The specifics of all proposed method manual changes related to pH drift are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d). The proposed changes related to pH drift will affect all methods in the freshwater chronic method manual (USEPA, 1994a), except for the *Selenastrum capricornutum* Growth Test; and all methods in the marine chronic method manual (USEPA, 1994b), except for the *Arbacia punctulata Fertilization* Test and the *Champia parvula* Reproduction Test. The *Selenastrum, Arbacia,* and *Champia* tests do not require test solution renewal, so daily pH fluctuations should not be a concern. Proposed changes to the acute method manual (USEPA, 1993b) would simply recommend the use of static-renewal testing or flow-through testing when artifactual toxicity due to pH drift is suspected. EPA invites comments on how pH drift would and should be addressed in WET testing (see Section V.A).

c. Concentration-Response Relationships

The concentration-response relationship established between the concentration of a toxicant and the magnitude of the response is a fundamental principle of toxicology. This principle assumes that there is a causal relationship between the dose of a toxicant (or concentration for toxicants in solution) and a measured response. A response may be any measurable biochemical or biological parameter that is correlated with exposure to the toxicant. The classical concentration-response relationship is depicted as a sigmoidal-shaped curve with detrimental responses increasing as the concentration of the toxicant increases. Not all concentration-response relationships, however, are represented by the classical sigmoidal-shaped curve. A corollary of the concentration-response concept is that every toxicant should exhibit a concentration-response relationship, given that the appropriate response is measured and given that the concentration range evaluated is appropriate. Use of this concept can be helpful in determining whether an effluent sample causes toxicity and in identifying anomalous test results.

In July 2000, EPA published guidance on evaluating concentration-response relationships to assist in determining the validity of WET test results (USEPA, 2000a). This document explained the concentration-response concept and provided review steps for 10 different concentration-response patterns that may be encountered in WET test data. Based on the results of the review, the guidance anticipates one of three determinations: (1) that calculated effect concentrations are reliable and should be reported; (2) that calculated effect concentrations are anomalous and should be explained; or (3) that the test was inconclusive and should be repeated with a newly collected sample.

In today's action, EPA proposes to require the review of concentration-response relationships generated for all multi-concentration WET tests reported under the NPDES program. EPA proposes to modify section 10 of the two chronic method manuals (USEPA, 1994a; USEPA, 1994b) and section 12 of the acute method manual (USEPA, 1993b) to incorporate this required test review procedure. The modified sections would explain the

concentration-response concept, require the review of concentration-response relationships, and reference EPA guidance (USEPA, 2000a) describing various forms of concentration-response relationships and review procedures. Use of the concentration-response review procedures (USEPA, 2000a) would ensure that a valid concentration-response relationship is demonstrated prior to the determination of toxicity. EPA intends to maintain the review procedures described in the guidance document (USEPA, 2000a) as ''guidance'' because these procedures may be revised as new information on the review of concentration-response relationships (including additional forms of concentration-response relationships) becomes available.

To demonstrate the effectiveness of the proposed concentration-response review steps, EPA used the guidance on concentration-response relationships (USEPA, 2000a) in the review and reporting of results from EPA's WET Variability Study (USEPA, 2001a). In this study, 635 valid tests (i.e., those that met test acceptability criteria) were reviewed according to the proposed concentration-response evaluation procedures. Based on these review procedures, the calculated effect concentrations in 14 tests were determined to be anomalous, and the effect concentrations calculated in 9 tests were determined to be inconclusive. Eight of the 23 test results that were considered anomalous or inconclusive had erroneously indicated toxicity in blank samples. These results would have been reported as false positives if the concentration-response review procedures had not been used. This study indicates that the proposed concentration-response review procedures are effective in reducing the incidence of false positives in WET testing. The use of these review procedures reduced the rate of reported false positives in the WET Variability Study from 11.1% to 3.7% for the Ceriodaphnia dubia Survival and Reproduction Test; from 12.5% to 4.35% for the Fathead Minnow Larval Survival and Growth Test; from 14.3% to 0% for the Mysidopsis bahia Survival, Growth, and Fecundity Test; and from 14.3% to 0% for the Inland Silverside Larval Survival and Growth Test.

In addition to requiring the review of concentration-response relationships, EPA proposes to modify section 12 of the acute method manual (USEPA, 1993b) and section 10 of the two chronic method manuals (USEPA, 1994a; USEPA, 1994b) to consolidate other important test review components that are described elsewhere in the method manuals. These revised sections, titled ''Report Preparation and Test Review,'' would describe the review of sample collection and handling conditions, test acceptability criteria, test conditions, statistical methods, concentration-response relationships, reference toxicant testing, and test variability. The specifics of the proposed method manual changes related to concentration-response relationship evaluation and other test review components are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d).

The quality of WET Variability Study data (USEPA, 2001a; USEPA, 2001b) used to make decisions for this rulemaking is of primary importance to the Agency and to stakeholders. These data and the test review and acceptance criteria used in the WET Variability Study are detailed in a final study report contained in the record for this rulemaking (USEPA, 2001a). Some stakeholders believe that EPA improperly applied different standards in accepting or rejecting data generated in the WET Variability Study and departed from the stated objectives of the study design. EPA is proposing test review procedures consistent with the test reviews that EPA conducted on data developed in the WET Variability Study (though EPA notes that the objectives of the study differ from those associated with compliance monitoring). EPA proposes modifications to standardize the minimum elements of WET test review. While some of these test review components provide specific criteria for the acceptance or rejection of test results (e.g., the method test acceptability criteria), others (e.g., review of test conditions, reference toxicant testing, and concentration-response relationships) must be reviewed within the context of the test objective. Also, State and/or regional regulatory authorities may require additional test review components and criteria to further standardize the reporting and review of WET test data. EPA requests comment on the acceptance, interpretation, and use of the WET Variability Study data and on the proposed section of the method manuals titled, ''Report Preparation and Test Review''.

d. Nominal Error Rates

WET test results (i.e., effect concentrations) may be determined by point estimation or hypothesis testing techniques (USEPA, 1994a; USEPA, 1994b). Hypothesis testing techniques compare responses in the control treatment with responses in other treatments to test the ''null hypothesis'' that there is no statistically significant difference between the treatments (i.e., that the effluent is not toxic). To determine when a difference between treatments is large enough to be statistically significant, the statistician or analyst must select a nominal error rate. The nominal error rate, or alpha level, is an intended upper bound on the probability of incorrectly concluding that the treatments are different when, in fact, they are not (a Type I statistical error). The larger the alpha level, the greater the probability of incorrectly rejecting the null hypothesis (i.e., determining that the effluent is toxic when, in fact, it is not). For all WET tests, EPA recommends using an alpha level of 0.05, which corresponds to a 5% probability of making a Type I error.

In response to stakeholder concerns that an alpha level of 0.05 does not adequately protect against Type I errors (Moore et al., 2000; *Edison Electric Institute, et al.* v. *EPA*, Settlement Agreement, July 24, 1998), EPA published guidance on nominal error rate selection (USEPA, 2000a). This guidance clarifies that the alpha level may be reduced to 0.01 in specific circumstances. These circumstances include instances when sublethal endpoints from Ceriodaphnia dubia or fathead minnow tests are reported under NPDES permit requirements, or when WET permit limits (based on any WET method) are derived without allowing for receiving water dilution. Even under these circumstances, however, the alpha level may be reduced only in tests that meet a fixed criterion for test sensitivity because reductions in the alpha level also reduce statistical power. Specifically, the percent minimum significant difference (PMSD) calculated for the test using an alpha level of 0.01 should be less than or equal to criteria set forth in the guidance document (USEPA, 2000a). The document also provides guidance on determining the need for additional test replication to meet PMSD criteria and guidance on the decision process for reducing the nominal error rate in hypothesis testing.

In today's action, EPA proposes to modify the chronic WET method manuals (USEPA, 1994a; USEPA, 1994b) to clarify the circumstances under which the recommended alpha level may be reduced. The proposed change would modify subsection 9.4.6 (Recommended Alpha Levels) of the two chronic method manuals (USEPA, 1994a; USEPA, 1994b). This subsection would maintain the current recommendation that an alpha level of 0.05 be used for hypothesis testing. In

addition, the subsection would identify the specific circumstances where the alpha level used for hypothesis testing could appropriately be reduced from 0.05 to 0.01. The subsection would describe these circumstances and reference the published guidance (USEPA, 2000a) for information on determining adequate test sensitivity and determining the appropriateness of reductions in the alpha level. The specifics of the proposed method manual changes related to nominal error rates are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d).

e. Confidence Intervals

Point estimation techniques described in the WET method manuals are used to generate effect concentrations and associated 95% confidence intervals (USEPA, 1993b; USEPA, 1994a; USEPA, 1994b). Software used to conduct these statistical procedures occasionally do not provide the associated confidence intervals. This situation may arise when test data do not conform with specific assumptions required by the statistical methods, when point estimates are outside of the test concentration range, and when specific limitations imposed by the software are encountered. In July 2000, EPA published guidance on the specific circumstances under which confidence intervals are not generated or are not suitable (USEPA, 2000a).

In today's action, EPA proposes to modify the WET method manuals to clarify the circumstances under which confidence intervals are not generated by point estimation techniques and to reference the published guidance on this issue (USEPA, 2000a). The proposed change would modify subsection 9.3.2 (Point Estimation Techniques) of the two chronic method manuals (USEPA, 1994a; USEPA, 1994b) and subsection 11.2 (Determination of the LC50 from Definitive, Multi-Effluent-Concentration Acute Toxicity Tests) of the acute method manual (USEPA, 1993b). The specifics of the proposed method manual changes related to confidence intervals are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d).

f. Dilution Series

In multi-concentration (definitive) WET tests, organism effects are measured in a range of effluent concentrations. The dilution series selected for the test defines the concentrations of effluent tested. The WET methods recommend preparing test concentrations using a dilution factor of greater than or equal to 0.5 and provide an example dilution series of 100%, 50%, 25%, 12.5%, and 6.25% effluent. While this particular dilution series is commonly used in WET testing, test concentrations for each test should be selected independently based on the objective of the study, the expected range of toxicity, the receiving water concentration (or instream waste concentration), and any available historical testing information on the effluent. The dilution series should be selected to optimize the precision of calculated effect concentrations and assist in establishing concentration-response relationships. In July 2000, EPA published guidance on selecting appropriate dilution series for WET testing (USEPA, 2000a).

In today's action, EPA proposes to modify the WET method manuals to reference the published guidance on selecting dilution series (USEPA, 2000a) and to clarify that dilution series should be selected independently for each test based on the objective of the study, the expected range of toxicity, the receiving water concentration (or instream waste concentration), and any available historical testing information on the effluent. The proposed change would modify subsection 8.10 (Multi-concentration [Definitive] Effluent Toxicity Tests) of the two chronic method manuals (USEPA, 1994a; USEPA, 1994b) and subsection 9.3 (Multi-concentration [Definitive] Effluent Toxicity Tests) of the acute method manual (USEPA, 1993b). The specifics of the proposed method manual changes related to dilution series selection are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d).

g. Dilution Waters

Test concentrations in definitive WET tests are prepared by diluting the effluent sample with an appropriate dilution water. The WET methods allow the use of natural receiving waters or synthetically prepared waters for dilution. Because the choice of dilution water can affect WET test results (Cooney et al., 1992; Belanger et al., 1989; DeLisle and Roberts, 1988), selecting an appropriate dilution water is important. To assist in this process, EPA published guidance on dilution water selection (USEPA, 2000a) that clarifies what EPA considers to be an acceptable dilution water. An acceptable dilution water is one that is appropriate for the objectives of the test; supports adequate performance of the test organisms with respect to survival, growth, reproduction, or other responses that may be measured in the test (i.e., consistently meets test acceptability criteria for control responses); is consistent in quality; and does not contain contaminants that could produce toxicity. The guidance also provides recommendations on how to select an appropriate dilution water based on the objectives of the test, the condition and quality of ambient receiving water, in-stream dilution potential, and recommendations or requirements from local regulatory authorities. Lastly, the guidance explains the use of dual controls when dilution water differs from organism culture water.

In today's action, EPA proposes to modify the WET method manuals by clarifying the definition of acceptable dilution waters and referencing the published guidance (USEPA, 2000a) for more information on selecting appropriate dilution waters. The proposed change would modify subsection 7.1 (Types of Dilution Water) of each of the method manuals (USEPA, 1993b; USEPA, 1994a; USEPA, 1994b). The specifics of the proposed method manual changes related to dilution waters are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d).

h. Pathogen Interference

WET testing is designed to measure the aggregate toxicity of an aqueous test sample. The presence of pathogens and/or parasites in the test sample, however, may confound this measurement of toxicity by causing sporadic mortality among test organisms. Today, EPA proposes to modify the Fathead Minnow (Pimephales promelas) Larval Survival and Growth Test to provide guidance on the adverse effects of pathogens and/or parasites on test performance (*i.e.*, pathogen and/or parasite test interference). EPA proposes procedures to control pathogen and/or parasite effects without compromising the capacity of the test to measure the toxicity of the test sample. The proposed method modifications are summarized below and detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d).

Pathogens that interfere with the test may come from the receiving water used for test dilutions, from the effluent, or from the receiving water that is used as intake water. Most receiving waters contain all the common fish pathogens, but these fish pathogens do not cause a problem in the stream. At times, however, the test conditions during

WET tests (e.g., 24 hour durations between sample renewals, beakers used for seven days without change, or uneaten brine shrimp) may promote bacterial growth. Some opportunistic bacteria take advantage of these conditions and flourish or ''bloom.'' The bacteria that bloom may be harmless or they may be fish pathogens. Blooms may even differ between replicates. In some cases, the presence of uncontrolled pathogen and/or parasite effects in the WET test may suggest the selection of a different test species.

Stakeholders have identified particular concerns with the adverse effect of pathogens on the performance of the Fathead Minnow Larval Survival and Growth Test. A typical indication that pathogen interference has occurred in a WET test is when test organisms exhibit ''sporadic mortality.'' This sporadic mortality phenomenon is characterized by an unexpected concentration-response relationship (i.e., effects that do not increase with increasing effluent concentration) and fathead minnow survival that varies greatly among replicates and among effluent dilutions. The observed sporadic mortality among replicates tends to occur in receiving water controls and in lower effluent concentrations (or occasionally in the full-strength effluent samples) on day three or day four of the Fathead Minnow Larval Survival and Growth Test. EPA does not have evidence of such sporadic mortality occurring in concurrently conducted chronic tests using the cladoceran, Ceriodaphnia dubia, or concurrent acute tests with the fathead minnow, C. dubia, or other acute test species.

When sporadic mortality is observed, often a fungal growth occurs directly on the fish, especially in the gill area. This growth interferes with measuring toxicity in the WET test. Biological test interference due to this type of fungal growth may occur during the toxicity test when effluents and water samples tested are derived from the receiving water (i.e., their source is a receiving water intake) or when the receiving water is used as the diluent. The fungal growth has been attributed to Saprolegnia sp. (Downey et al., 2000) which may be a secondary infection following infection from a known fish pathogen. Microbiological evaluations on receiving waters, the fish, and their food indicated the ubiquitous nature of pathogenic organisms (e.g., Flexibacter spp., Aeromonas hydrophila). Eradicating these types of organisms from the test through the decontamination of the fish and their food has not been practical (Geis et al., 2000a).

Data from the WET test must be reviewed carefully to ascertain if pathogens are suspected. The key indicators that pathogen interference has occurred are the presence of an unexpected concentration-response relationship (i.e., effects that do not increase with increasing effluent concentration), and organism survival that varies greatly among replicates and among effluent dilutions. The analyst should evaluate the test data to determine a cause for any unexpected concentration-response pattern and subsequently to determine the validity of calculated results (USEPA, 2000a). Normal, reversed, or bimodal concentration-response relationships are not considered indicators of test interference by pathogenic bacteria (USEPA, 2000a). The analyst also should evaluate the responses at each test concentration for unusually high mortality and/or for unevenness of mortalities among replicates. If the within-treatment coefficient of variation (CVs) for survival in an effluent treatment is greater than 40% and relatively low for control replicates in standard synthetic water, pathogen interference should be considered. Following data evaluations, additional testing would be required to ascertain that sporadic mortality observed in the WET test is due to interference by pathogenic bacteria. Parallel tests should be conducted using reconstituted water and receiving water as diluents with the effluent.

Before modifying any test procedures that will allow the analyst to account for pathogen interference, all available options within the flexibility of the method should be exhausted. Samples should be filtered through a 2–4 mm mesh opening (as described in Subsection 8.8.2 of the freshwater chronic method manual (USEPA, 1994a)) to remove indigenous organisms. Tests should be conducted using separate glassware, pipettes, and siphons for each concentration to minimize cross contaminating replicates of all treatments. The analyst also must keep laboratory equipment clean and dry when not in use. Use of reconstituted laboratory waters instead of receiving waters may eliminate the interference, and the use of reconstituted water would be preferable to invalid tests. However, for those instances when receiving water is required as the diluent or when the effluent and the subsequent dilutions exhibit the interference, EPA recommends modifying the test design to prevent the spread of the pathogen among the test chambers during the test.

Once pathogenic test interference has been confirmed by additional testing, the proposed modifications to the Fathead Minnow Larval Survival and Growth Test would recommend use of an altered test design to minimize the effects of the pathogenic interference. The use of fewer fish per test chamber and new test chambers daily has been the most effective technique for controlling the effects of pathogenic bacteria in the Fathead Minnow Larval Survival and Growth Test. Use of small plastic 30-ml cups containing two fish per cup showed the greatest improvement to the test method, removing the pathogenic effect 91% of the time (Geis et al., 2000a). For instance, use of 20 ml of test solution in a 1 ounce plastic cup and two fish per beaker significantly reduced the sporadic mortality not attributed to the effluent toxicity. The total number of fish tested is not reduced (i.e., 40 per treatment), and the fish are combined at the end of the test into the typical number of replicates so that data analysis following the test method manuals is unchanged.

When parallel testing has confirmed pathogen interference and the modifications to the test design for the number of fish per chamber does not reduce the pathogen interference, the regulatory authority may allow modifications of the effluent samples to remove or inactivate the pathogens. The analyst should apply TIE filtration steps (USEPA, 1991a; USEPA, 1992) in combination with various sterilization techniques listed below to ascertain and control adverse influences on tests caused by pathogens in the intake or receiving waters used for dilution. For some samples, one or more techniques such as irradiation with ultraviolet light, pasteurization, filtration (0.2 µ m pore size), and addition of antibiotics has been shown to improve survival and reduce variability among replicates effectively (SETAC, 1999). EPA cautions that some treatment methods that might control pathogens in the test, (e.g., ultraviolet light treatment or the addition of antibiotics (Downey et al., 2000)) may also improperly reduce or increase the toxicity of the sample. Filtration also may remove some toxicity in the sample as shown in toxicity identification evaluations (USEPA, 1991a; 1992; 1993a). The use of ultrafiltration on an effluent sample containing particulate matter to which process-induced metals have adsorbed may improperly remove a significant source of process-related toxicity. Also, chlorination and dechlorination may be

a treatment option where pathogenic bacteria are suspected as the sole source of toxicity in the ambient intake waters. However, when the analyst prepares samples using techniques of chlorination and/or dechlorination, potential exists for oxidation and reduction of other compounds (USEPA, 1991a; 1992). All toxicity tests conducted on modified samples (e.g., sterilized) must include an additional blank preparation (control) consisting of similarly treated reconsituted laboratory water (USEPA, 1991a; 1992).

Procedures to control the adverse influences of pathogens must not be used to reduce process-related sources of toxicity. With effluents and ambient waters, the pathogen(s) may mask the presence of a chemical that is, by itself, toxic. It is also possible that the pathogen infection is induced by some predisposing factor in the receiving water and would not occur without that factor. The need to evaluate both intake water and effluent samples to determine the cause of the pathogen or the source of pathogens is essential before applying any pathogen/parasite control technology and cannot be overemphasized. The analyst must evaluate whether the intake water is contributing the interference observed in the toxicity test of the final effluent.

The method modifications proposed today provide techniques to assess and control the effects of pathogens in the Fathead Minnow Larval Survival and Growth Test. Today's proposal does not address, however, the determination as to the conditions under which this control is appropriate for purposes of NPDES permit compliance. By today's proposal, EPA does not concede that the discharge of toxic biological agents to waters of the US is appropriate or authorized but merely that pathogens in test samples may confound measurement of whole effluent toxicity.

C. Ratification or Withdrawal of Methods

In a 1998 settlement agreement with Edison Electric Institute et al. (*Edison Electric Institute, et al.* v. *EPA*, No. 96–1062 & consolidated case (D.C. Cir.), Settlement Agreement, July 24, 1998), EPA agreed to conduct an interlaboratory variability study of 12 of the 17 approved WET test methods (the WET Variability Study). The 12 methods evaluated in the study (Table 1) represent a combination of acute and chronic test methods; freshwater and marine test methods; and invertebrate, fish, and algal species. EPA conducted the WET Variability Study in 1999 through 2000, and published preliminary results from the study in October 2000 (USEPA, 2000b; USEPA, 2000c). In 2001, EPA submitted the preliminary results of the study for expert peer review (USEPA, 2001c). The peer review comments and EPA's response to those comments are included in the record established for this rulemaking (see Addresses section of this rule). Based on peer review comments, EPA revised the preliminary study report to produce a final study report. In conjunction with today's action, EPA is publishing a final study report (USEPA, 2001a; USEPA, 2001b) that presents the final results of EPA's WET Variability Study. These results are discussed in section III.C.1 below.

The settlement agreement (*Edison Electric Institute, et al.* v. *EPA*, Settlement Agreement, July 24, 1998) also required that EPA propose to ratify or withdraw each of the 12 WET test methods evaluated in the WET Variability Study. Based on the results of the WET Variability Study, consideration of peer review comments, and an overall evaluation of the WET program, EPA proposes to ratify 11 of the methods evaluated in the WET Variability Study. EPA proposes to ratify nine of these methods, in an amended form, as described in Section III.B of this rule. EPA proposes to ratify two other methods (the Selenastrum capricornutum Growth Test and the Mysidopsis bahia Survival, Growth and Fecundity Test) with additional modifications (i.e., in addition those described in Section III.B of this rule) to improve the performance of the methods. EPA proposes to withdraw and propose a new Holmesimysis costata Acute Test method. The Holmesimysis costata Acute Test method was promulgated and tested in the WET Variability Study using acute test procedures designed for the Mysidopsis bahia Acute Test (except at a temperature of 12°C, instead of 20°C or 25°C; and a salinity of 32–34‰, instead of 5–30‰). Results of the WET Variability Study revealed that acute test procedures designed for Mysidopsis bahia were insufficient for successful test conduct using Holmesimysis costata. For this reason, EPA proposes to withdraw Holmesimysis costata as an acceptable species for use in the Mysidopsis bahia Acute Test method and to propose it as an acute toxicity test method designed specifically for Holmesimysis costata. Sections 2–7 below discuss the proposed ratification and/or withdrawal of each method evaluated in the WET Variability Study.

TABLE 1.—WHOLE EFFLUENT TOXICITY TEST METHODS INCLUDED IN EPA'S WET VARIABILITY STUDY

Test method	Common test method name	Test method No. [a]
Cladoceran, Ceriodaphnia dubia, Acute Test	Ceriodaphnia— dubia Acute Test	
Cladoceran, Ceriodaphnia dubia, Survival and Reproduction Test.	Ceriodaphnia dubia Survival and Reproduction Test	1002.0
Fathead Minnow, Pimephales promelas, Acute Test	Fathead Minnow Acute Test	
Fathead Minnow, Pimephales promelas, Larval Survival and Growth Test.	Fathead Minnow Larval Survival and Growth Test	1000.0
Green Alga, Selenastrum capricornutum, Growth Test	Selenastrum capricornutum Growth Test	1003.0
Mysid, Mysidopsis bahia, Survival, Growth, and Fecundity Test	Mysidopsis bahia Survival, Growth, and Fecundity Test	1007.0
Sheepshead Minnow, Cyprinodon variegatus, Acute Test	Sheepshead Minnow Acute Test	
Sheepshead Minnow, Cyprinodon variegatus, Larval Survival and Growth Test.	Sheepshead Minnow Larval Survival and Growth Test	1004.0
Inland Silverside, Menidia beryllina, Acute Test	Inland Silverside Acute Test	
Inland Silverside, Menidia beryllina, Larval Survival and Growth Test.	Inland Silverside Larval Survival and Growth Test	1006.0
Red Macroalga, Champia parvula, Reproduction Test [b]	Champia parvula Reproduction Test	1009.0
Mysid, Holmesimysis costata, Acute Test [b c]	Holmesimysis costata Acute Test	

[a] Test method numbers were not designated for acute test methods in USEPA, 1993b.
[b] Due to insufficient laboratory support, interlaboratory data were not obtained for this method.
[c] The EPA-approved acute test with Holmesimysis costata was performed using the test conditions for the Mysidopsis bahia Acute Test method (except at a temperature of 12°C, instead of 20°C or 25°C; and a salinity of 32–34‰, instead of 5–30‰).

In ratifying WET test methods, EPA reaffirms the conclusion expressed in the 1995 WET final rule (60 FR 53529; October 16, 1995), that these methods are applicable for use in NPDES permits. In the 1995 WET final rule, this conclusion was based on the well-established use of the methods, the existence of extensive guidance on quality assurance and routine quality control activities, and validation data from a number of studies conducted by EPA, State programs, and universities. Since promulgation of the methods, this basis for approval has been strengthened by more widespread use of the methods, additional guidance on quality assurance and quality control issues (USEPA, 2000a; USEPA, 2000d), and the WET Variability Study to confirm method performance data from original validation studies (USEPA, 2001a; USEPA, 2001b).

1. WET Variability Study

EPA designed the WET Variability Study to characterize interlaboratory variability, the rate of successful test completion, and the rate of ''false positive'' incidence (i.e., the measurement of toxicity in non-toxic blank samples) for the 12 test methods listed in Table 1. For two of these methods (the Champia parvula Reproduction Test and the Holmesimysis costata Acute Test), EPA was unable to obtain interlaboratory data due to laboratory unavailability (i.e., EPA was unable to contract with a minimum of six laboratories qualified and willing to conduct these test methods within the time frame of the study). Intralaboratory data were obtained for the Champia parvula Reproduction Test, but no valid intralaboratory or interlaboratory data were obtained for the Holmesimysis costata acute test. For each of the remaining 10 methods, 7 to 35 laboratories participated in multi-laboratory testing of 3 or 4 ''blind'' test samples. Laboratories received some combination of the following test sample types: reagent water (or ''blank''); reference toxicant; municipal or industrial effluent; and receiving water. Participant laboratories were required to analyze each blind test sample according to the promulgated WET test method manuals and specific instructions in participant laboratory standard operating procedures developed for the study (appendix B, USEPA, 2001b). In total, the study generated interlaboratory precision data from testing more than 700 blind samples among 55 participant laboratories. EPA had not previously conducted a study of this magnitude with these objectives in this time frame.

The results of the WET Variability Study (Table 2) supported the conclusions of the 1995 WET final rule and confirmed the acceptability of the WET test methods for use in NPDES permits, except as noted below in sections 2 through 7. The analysis of successful test completion rates revealed that most WET test methods could be consistently and reliably performed by qualified testing laboratories. For the purposes of the study, EPA defined successful test completion rates to be the percentage of initiated and properly terminated tests that met the test acceptability criteria as specified in the WET method manuals. Successful test completion rates were above 90% for 8 of the 10 methods evaluated during interlaboratory testing. Only the Ceriodaphnia dubia Survival and Reproduction Test method (see section 2 below) and the Selenastrum capricornutum Growth Test method (see section 5 below) produced successful test completion rates less than 90%.

The analysis of false positive rates revealed that the WET test methodologies, including applicable guidance on reviewing WET test results (USEPA, 2000a), effectively control the incidence of falsely identifying toxicity in non-toxic ''blank'' samples. False positive rates were defined as the percentage of valid tests conducted on blank samples that indicated toxicity by producing LC50 (median lethal concentration), NOEC (no observed effect concentration), or IC25 (25% inhibition concentration) values of less than 100% sample. False positive results were reported for three test methods, and the rates of false positives were below the theoretical false positive rate of 5% (based on the recommended 0.05 alpha level for hypothesis testing) for all but the Selenastrum capricornutum Growth Test conducted without EDTA.

The analysis of interlaboratory precision data revealed that the WET test methods are sufficiently precise for use in NPDES permits. Interlaboratory coefficients of variation (CVs) calculated in the WET Variability Study ranged from 10.5% to 58.5% (Table 2). This observed range of interlaboratory variability is consistent with the range of variability reported for chemical methods approved at 40 CFR part 136 (USEPA, 1991b). For chemical methods measuring metals at the low end of the detection range, interlaboratory CVs range from 18% to 129%, with a median CV of 45%. Interlaboratory CVs for chemical methods for organic analyses range from greater than 12% to 91%, and interlaboratory CVs for nonmetal inorganic analyses range from 4.6% to 70%.

TABLE 2.—SUMMARY OF TEST RESULTS FROM EPA'S WET VARIABILITY STUDY

Test method	Successful test completion rate (%)	False positive rate[a] (%)	Interlaboratory precision (% CV)[b]
Ceriodaphnia dubia Acute Test	95.2	0.00	29.0
Ceriodaphnia dubia Survival and Reproduction Test	82.0	3.70	35.0
Fathead Minnow Acute Test	100	0.00	20.0
Fathead Minnow Larval Survival and Growth Test	98.0	4.35	20.9
Selenastrum capricornutum Growth Test (with EDTA)[c]	63.6	0.00	34.3
Selenastrum capricornutum Growth Test (without EDTA)[c]	65.9	33.3	58.5
Mysidopsis bahia Survival, Growth, and Fecundity Test	[d]97.7	0.00	41.3
Sheepshead Minnow Acute Test	100	0.00	26.0
Sheepshead Minnow Larval Survival and Growth Test	100	0.00	10.5
Inland Silverside Acute Test	94.4	0.00	38.5
Inland Silverside Larval Survival and Growth Test	100	0.00	43.8
Champia parvula Reproduction Test[e]	ND	ND	[f]ND
Holmesimysis costata Acute[e]	ND	ND	ND

[a] False positive rates reported for each method represent the higher of false positive rates observed for hypothesis testing or point estimate endpoints.

[b] Coefficients of variation (CVs) reported for each method represent the CV of LC50 values for acute test methods and IC25 values for chronic test methods. CVs reported are based on total interlaboratory variability (including within-laboratory and between-laboratory components of variability) and averaged across sample types.

[c] The Selenastrum capricornutum Growth Test method was conducted with and without ethylenediaminetetraacetic acid (EDTA) as a component of the nutrients added to test and control treatments. Due to improved test performance with the addition of EDTA, EPA is proposing to recommend the addition of EDTA in the Selenastrum capricornutum Growth Test.

[d] Successful test completion for the optional fecundity endpoint was 50%.

[e] ND = not determined. Due to insufficient laboratory support, interlaboratory data were not obtained for the Champia parvula Reproduction Test method and the Holmesimysis costata Acute Test method.

[f] While interlaboratory test data were not obtained for the Champia parvula Reproduction Test method, intralaboratory data was obtained from the referee laboratory. Intralaboratory CVs were 27.6%, 49.7%, and 50.0% for reference toxicant, receiving water, and effluent sample types, respectively.

2. Ceriodaphnia dubia Acute Test, Ceriodaphnia dubia Survival and Reproduction Test, Fathead Minnow Acute Test, Fathead Minnow Larval Survival and Growth Test, Sheepshead Minnow Acute Test, Sheepshead Minnow Larval Survival and Growth Test, and Inland Silverside Acute Test

Today, EPA proposes to ratify its previous rulemaking standardizing the following WET test methods: Ceriodaphnia dubia Acute Test, Ceriodaphnia dubia Survival and Reproduction Test, Fathead Minnow Acute Test, Fathead Minnow Larval Survival and Growth Test, Sheepshead Minnow Acute Test, Sheepshead Minnow Larval Survival and Growth Test, and the Inland Silverside Acute Test. At the time of method promulgation, interlaboratory precision data were available for each of these test methods. Based on these precision data, EPA concluded that toxicity tests are no more variable than chemical analytical methods in 40 CFR part 136, and that toxicity tests provide reliable indicators of whole effluent toxicity. At that time, EPA also anticipated that laboratory performance would improve with use of the methods over time. Results from the WET Variability Study not only confirmed the level of precision previously cited for these methods, but indicated that the methods currently exhibit even lower variability than estimated at the time of method promulgation (60 FR 53529; October 16, 1995). Such data also confirm EPA's assumptions regarding the likely improvement in laboratory performance over time. The average of interlaboratory CVs reported (in the WET method manuals and/or the Technical Support Document for Water Quality-based Toxics Control (USEPA, 1991b)) for each method at the time of promulgation ranged from 34% to 44.2% (Table 3). Interlaboratory CVs reported for these methods in the WET Variability Study ranged from 10.5% to 38.5%. For each method, interlaboratory variability measured in the WET Variability Study was lower than that cited at the time of promulgation (Table 3). Interlaboratory CVs measured in the WET Variability Study were 4% to 34% lower than average values cited in the method manuals for the same methods. On average, interlaboratory variability measured in the WET Variability Study was 15% lower than originally reported at the time of method promulgation. These results strongly confirm EPA's conclusions that these methods provide sufficient precision for use in NPDES permits.

TABLE 3.—COMPARISON OF INTERLABORATORY METHOD PRECISION AT THE TIME OF METHOD PROMULGATION AND MEASURED IN EPA'S WET VARIABILITY STUDY

Method	Interlaboratory precision estimates (%CV) at time of method promulgation [a]	Updated interlaboratory precision estimates (%CV) [b]	Improved precision?
Ceriodaphnia dubia Acute Test	44.2	29.0	Yes
Ceriodaphnia dubia Survival and Reproduction Test	42	35.0	Yes
Fathead Minnow Acute Test	35	20.0	Yes
Fathead Minnow Larval Survival and Growth Test	34	20.9	Yes
Selenastrum capricornutum Growth Test	[c] NR	[d] 34.3	NA [e]
Mysidopsis bahia Survival, Growth, and Fecundity Test	[c] NR	41.3	NA [e]
Sheepshead Minnow Acute Test	42	26.0	Yes
Sheepshead Minnow Larval Survival and Growth Test	44.2	10.5	Yes
Inland Silverside Acute Test	42.2	38.5	Yes
Inland Silverside Larval Survival and Growth Test	[c] NR	43.8	NA [e]
Champia parvula Reproduction Test	[c] NR	[c] NR	NA [f]
Holmesimysis costata Acute Test	[c] NR	[c] NR	NA [f]

[a] Precision estimates represent an average of all interlaboratory CVs reported for a given method in the WET method manuals (USEPA, 1993b; USEPA, 1994a; USEPA, 1994b) and/or the Technical Support Document for Water Quality-based Toxics Control (USEPA, 1991b). The number of significant figures displayed differs because these data are obtained from various sources, which reported results to either two or three significant figures.

[b] Precision estimates were obtained from EPA's WET Variability Study conducted in 1999–2000 (USEPA, 2001a).

[c] NR = None reported.

[d] Precision estimates for the Selenastrum capricornutum Growth Test method are based on conduct of the test with Ethylenediaminetetraacetic acid (EDTA) as a component of the nutrients added to test and control treatments.

[e] NA = not applicable. Improved precision could not be determined because estimates of interlaboratory precision were not reported at the time of method promulgation.

[f] NA = not applicable. Improved precision could not be determined because estimates of interlaboratory precision were not reported at the time of method promulgation or determined in the WET Variability Study.

Other test performance characteristics measured in the WET Variability Study also confirmed EPA's conclusions that these methods are applicable for use in NDPES permits. False positive rates for these methods were below the theoretical false positive rate of 5% (based on the recommended 0.05 alpha level for hypothesis testing), indicating that the methods do not routinely indicate toxicity in non-toxic samples. Successful test completion rates for these methods were also at acceptable levels (82.0% to 100%), with 6 of these 7 methods exhibiting successful test completion rates above 90%. While the 82.0% successful test completion rate for the Ceriodaphnia dubia Survival and Reproduction Test method was lower than for most other methods evaluated in the WET Variability Study, this rate is consistent with successful test completion rate information available for this method at the time of promulgation. The 82.0% successful test completion rate observed in the WET Variability Study is consistent with the 80% rate reported for this method in a 1989 interlaboratory study (USEPA, 1991b) and represents tremendous improvement from a 1987 interlaboratory study that reported a successful test completion rate of 56% (DeGraeve et al., 1992).

The overall successful test completion rate observed for the Ceriodaphnia dubia Survival and Reproduction Test method in the WET Variability Study was also suppressed by poor performance in a subset of laboratories. Only 10 of the 34 participant laboratories performed invalid tests, but 8 of these laboratories performed invalid tests on 50% or more of the samples tested. The low rate of successful test completion in these 8 laboratories may have been influenced by the study's strict testing schedule, which required each test to be conducted on a given day and all tests to be conducted within a 15-day time period. When invalid tests conducted in a given laboratory were due to marginal or poor health of the test organism cultures, then it was logical that the laboratory would fail a high percentage of tests during this study because culture health was unlikely to fully recover within 15 days. EPA believes that successful test completion rates for this method improve when testing laboratories are allowed flexibility in the timing of sample collection and can avoid initiating tests during periods of marginal to poor culture health.

3. Inland Silverside Larval Survival and Growth Test

EPA proposes to ratify the Inland Silverside Larval Survival and Growth Test method. Similarly to the methods listed in section 2 above, the Inland Silverside Larval Survival and Growth Test method exhibited acceptable successful test completion rates and false positive rates (Table 2). No false positives were observed for the method in the WET Variability Study, and the successful test completion rate was 100%. Unlike the methods listed in section 2 above, however, EPA cannot compare interlaboratory precision data cited at the time of method promulgation and data reported from the WET Variability Study because EPA did not rely on interlaboratory precision data for this method at the time of promulgation (Table 3). Instead, EPA relied on intralaboratory data for the method. The Agency's previous experience with method variability evaluations supported EPA's assumption that, though WET tests typically have lower CVs (higher precision) in intralaboratory studies than in interlaboratory studies, acceptable ranges of precision demonstrated in intralaboratory studies tend to subsequently be confirmed by interlaboratory studies.

In the WET Variability Study, an interlaboratory CV of 43.8% was reported for the Inland Silverside Larval Survival and Growth Test method. While interlaboratory variability for this method is higher than for other methods reported in the study, it is within the range of interlaboratory CVs (34% to 44.2%) cited for other WET methods at the time of promulgation (Table 3). It is also within the range of interlaboratory CVs reported for chemical methods approved at 40 CFR part 136 (USEPA, 1991b). Therefore, EPA reaffirms the conclusions that this method is no more variable than chemical analytical methods approved at 40 CFR part 136 and that this method is applicable for use in NPDES permits (60 FR 53529; October 16, 1995).

4. Champia parvula Reproduction Test

In the WET Variability Study, insufficient participant laboratory support was available to conduct interlaboratory testing of the Champia parvula Reproduction Test method within the time frame of the study. In addition to the referee laboratory, only one laboratory submitted the necessary quality control information to prequalify for participation in the interlaboratory study of this method. Due to insufficient laboratory support and failure to meet the study's data quality objective of a minimum of six laboratories, EPA canceled interlaboratory testing of the Champia parvula Reproduction Test method. Though interlaboratory testing was canceled, the referee laboratory conducted single-laboratory testing of the Champia parvula Reproduction Test method. In the 1995 WET rule, EPA addressed the issue of limited laboratory availability for conduct of the Champia parvula Reproduction Test method. EPA predicted that as the requirements for use of this organism in the NPDES permit program increased, the resulting increase in market demand would result in an increase in the number of laboratories capable of performing this test. However, the number of permits requiring the Champia parvula Reproduction Test method has remained low (DeGraeve et al., 1998), so few laboratories have invested in developing Champia parvula cultures or standard operating procedures for conduct of the method.

EPA believes that the limited use of the Champia parvula Reproduction Test method does not reduce the value of the test method. The Champia parvula Reproduction Test represents the only approved test method for a marine plant species. Maintaining an approved test method for this functional group (marine/plant/chronic test) is important for proper implementation of the WET program. The Technical Support Document for Water Quality-Based Toxics Control (USEPA, 1991b) recommends the use of at least three marine species representing three different phyla (e.g., a fish, an invertebrate, and a plant) for testing the toxicity of effluents discharged to estuarine and marine environments.

The limited use of the Champia parvula Reproduction Test method also does not affect the performance of the test method in laboratories that are qualified to conduct the test. While the WET Variability Study did not provide interlaboratory precision data for the Champia parvula Reproduction Test method, referee laboratory data confirmed the estimates of intralaboratory precision cited at the time of method promulgation (USEPA, 1994b). Intralaboratory CVs cited in the method manual for Champia parvula Reproduction Tests conducted using copper sulfate and sodium dodecyl sulfate averaged 63%. In preliminary testing for the WET Variability Study, the referee laboratory achieved an intralaboratory CV of 27.6% for 3 reference toxicant tests using copper sulfate, and an intralaboratory CV of 49.7% for 4 tests of spiked receiving water. Only one pair of replicate

effluent samples was tested using the Champia parvula Reproduction Test method. Tests of these duplicate effluent samples yielded a CV of 50.0%. All other testing of the effluent sample type was conducted on samples from different sampling dates, so additional precision measurements were not obtained for this sample type. In addition to intralaboratory test data from the WET Variability Study, EPA's Variability Guidance Document (USEPA, 2000d) reported an intralaboratory CV of 59% for the Champia parvula Reproduction Test based on 23 reference toxicant tests conducted in 2 laboratories. Intralaboratory data from both the WET Variability Study and the Variability Guidance Document support the intralaboratory precision data previously cited in the method manual (USEPA, 1994b) for the Champia parvula Reproduction Test method. Based on the confirmation of intralaboratory precision data cited at the time of method promulgation, EPA proposes to ratify the Champia parvula Reproduction Test method.

5. Mysidopsis bahia Survival, Growth, and Fecundity Test

The Mysidopsis bahia Survival, Growth, and Fecundity Test uses three test endpoints to evaluate toxicity: survival, growth, and fecundity (or reproduction). The survival and growth endpoints are required endpoints and specific test acceptability criteria for these endpoints must be met (80% survival and mean weight of 0.20 mg in the control treatment) to produce a valid test. The fecundity endpoint is optional and may be used if the test acceptability criterion for fecundity (egg production by 50% or more of control females) is met. Failure to meet the test acceptability criterion for fecundity does not invalidate a test but means that the fecundity endpoint may not be used in calculating test results. In the WET Variability Study, 97.7% of tests met the required test acceptability criteria for survival and growth, but only 50% of tests met the test acceptability criterion for fecundity. While failure to generate fecundity data does not invalidate a test, it may affect the sensitivity of the measurement. Researchers have shown that the fecundity endpoint is often the most sensitive endpoint and that the test most effectively estimates the chronic toxicity of effluents when all three endpoints are used (Lussier et al., 1999).

EPA proposes to ratify the Mysidopsis bahia Survival, Growth, and Fecundity Test method with an additional modification to improve the performance of the method. EPA proposes to add guidance to improve the success of obtaining fecundity data. The specifics of the proposed method manual changes to implement this modification are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d). The additional guidance would recommend optimizing temperature, feeding, and organism densities during the seven-day pre-test holding period and during the testing period. These factors are critical to the success of the fecundity endpoint, because they control the rate of mysid development and maturation. While these factors are typically controlled during the testing period, equal attention should be paid to these factors during the pre-test holding period to ensure maximum mysid development. Lussier et al. (1999) found that by increasing holding temperature and test temperature from 26°C ± 1°C to 26°C–27°C and maintaining holding densities at ≤10 organisms/L, the percentage of tests meeting the test acceptability criteria for fecundity increased from 60% to 97%.

With the exception of the low successful test completion rate for the fecundity endpoint, other test method performance measures evaluated in the WET Variability Study for the Mysidopsis bahia Survival, Growth, and Fecundity Test were acceptable. No false positives were observed for the method, the successful test completion rate was 97.7% for the survival and growth endpoints, and interlaboratory variability (%CV) was 41.3% for the growth IC25 endpoint (Table 2). No interlaboratory precision data were reported for the Mysidopsis bahia Survival, Growth, and Fecundity Test method at the time of method promulgation; therefore interlaboratory precision data from the WET Variability Study could not be compared to previously cited values for this method (Table 3). While interlaboratory variability for this method is higher than for most other methods reported in the study, it is within the range of interlaboratory CVs (34% to 44.2%) cited for other WET methods at the time of promulgation (Table 3). It is also within the range of interlaboratory CVs reported for chemical methods approved at 40 CFR part 136 (USEPA, 1991b). Therefore, EPA reaffirms the conclusions that this method is applicable for use in NPDES permits (60 FR 53529; October 16, 1995).

6. Selenastrum capricornutum Growth Test

In the WET Variability Study, the Selenastrum capricornutum Growth Test method was conducted with and without the addition of ethylenediaminetetraacetic acid (EDTA). In the approved Selenastrum capricornutum Growth Test method, EDTA is an optional component of the nutrient mixture that is added to test and control treatments. While algal growth is enhanced by the addition of EDTA, the method recommends excluding EDTA from the nutrient mixture when testing samples that may contain metals. EDTA is a chelating agent that effectively binds metals, thereby potentially reducing the toxic effect of metals present in the analyzed sample. Because the presence of metals in WET samples is often unknown at the time of testing, laboratories often conduct the Selenastrum capricornutum Growth Test method without the addition of EDTA.

Results from the WET Variability Study revealed that Selenastrum capricornutum Growth Test method performance was substantially better when EDTA was added to the nutrient mixture than when it was excluded. No false positives were observed when EDTA was used, but 2 of the 6 blank samples (33.3%) analyzed without EDTA produced false positive results (USEPA, 2001a). Interlaboratory variability of the Selenastrum capricornutum Growth Test method was also much lower with EDTA (34.3%) than without EDTA (58.5%). When conducted with EDTA, the Selenastrum capricornutum Growth Test method exhibited interlaboratory precision similar to other chronic methods evaluated in the WET Variability Study. No interlaboratory precision data were reported for the Selenastrum capricornutum Growth Test method at the time of method promulgation, so interlaboratory precision data from the WET Variability Study could not be compared to previously cited values for this method. When compared to interlaboratory precision cited for other WET test methods at the time of promulgation, the Selenastrum capricornutum Growth Test method (conducted with EDTA) was well within the range (Table 3).

The successful test completion rate of the Selenastrum capricornutum Growth Test method was low for tests conducted with and without EDTA (63.6% and 65.9%, respectively), however, the low successful test completion rates were in part due to laboratory inexperience in using both the with and without-EDTA techniques. Two laboratories that cultured organisms without EDTA and generally conducted tests without EDTA showed poor successful test completion rates

(failing eight of eight tests) when EDTA was used. These laboratories failed all eight tests conducted with EDTA and passed all but one test (seven) without EDTA. When these two laboratories were removed from the analysis, the successful test completion rate for tests conducted with EDTA increased to 77.8%.

Based on WET Variability Study results, EPA proposes to ratify the Selenastrum capricornutum Growth Test method with a modification to recommend the addition of EDTA to the nutrient mixture added to control and test treatments. The specifics of the proposed method manual changes to implement this modification are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d). This method modification will improve overall test method performance by reducing false positives and increasing interlaboratory precision. EPA also believes that recommending the use of EDTA will improve successful test completion rates for the method as laboratories consistently culture and test with EDTA. In addition to improving test method performance, the method modification to recommend the use of EDTA is consistent with other established Selenastrum capricornutum toxicity testing protocols. Both ASTM (1992) and Environment Canada (1992) methods for toxicity testing using Selenastrum capricornutum recommend the use of EDTA.

EPA recognizes that the proposed modification to the Selenastrum capricornutum Growth Test method may cause the method to underestimate the toxicity of metals. EPA believes, however, that this modification is necessary to ensure adequate performance of the Selenastrum capricornutum Growth Test method. EPA also believes that under appropriate implementation of the WET program, this modification will not significantly reduce environmental protection. The Technical Support Document for Water Quality-based Toxics Control (USEPA, 1991b) recommends that permitting decisions be based on testing using a minimum of three species representing three different phyla (e.g., a fish, invertebrate, and plant). This recommendation is based on the recognition that species differ in their sensitivity to toxicants. By using a battery of species to test the toxicity of an effluent, permitting decisions can be made to protect the most sensitive species tested. Using this approach, the addition of EDTA in the Selenastrum capricornutum Growth Test method would affect environmental protection only when Selenastrum capricornutum is determined to be the most sensitive species and when the effluent contains metals whose toxicity is reduced by the addition of EDTA. This situation should be infrequent, and result in only minor decreases in test sensitivity. Geis et al. (2000b) showed that Ceriodaphnia dubia was more sensitive than Selenastrum capricornutum to three of five metals tested (copper, nickel, and cadmium), and Selenastrum capricornutum was only slightly more sensitive than Ceriodaphnia dubia to zinc and lead.

7. Holmesimysis costata Acute Test

Holmesimysis costata is a Pacific coast mysid species that was elevated from the supplemental species list in the previous acute method manual and added to the list of approved acute toxicity test species at the time of the WET final rule (60 FR 53529; October 16, 1995). This species was added in response to comments that the recommended test species in the acute method manual did not include any invertebrate species indigenous to Pacific coastal waters. One commenter also submitted data showing that Holmesimysis costata was at least as sensitive to toxicants as the recommended acute toxicity test species. Based on these comments, the acute method manual was modified to add a footnote listing Holmesimysis costata as an acceptable species for use with the Mysidopsis bahia Acute Test procedures. The footnote to the table of test conditions for the Mysidopsis bahia Acute Test states that ''Holmesimysis costata can be used with the test conditions in this table, 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 it is the required test organism in discharge permits.'' Because the acute method manual was incorporated by reference in the final rule, the incorporation of this footnote established Holmesimysis costata as an approved acute toxicity test species. The WET final rule (60 FR 53529; October 16, 1995) clarified this by stating that ''EPA accepts the use of * * * Holmesimysis 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‰).''

EPA decided to evaluate the Holmesimysis costata Acute Test method in the WET Variability Study according to the protocol as the method was promulgated, i.e., using the test conditions for Mysidopsis bahia (except at a temperature of 12°C, instead of 20°C or 25°C, and a salinity of 32‰ to 34‰, instead of 5‰ to 30‰). Sufficient participant laboratory support, however, was not available to conduct interlaboratory testing of the Holmesimysis costata Acute Test method within the time frame of the study. In addition to the referee laboratory, only two laboratories submitted the necessary quality control information to prequalify for participation in the interlaboratory study of this method. This method is required only in NPDES permits issued in California, so few laboratories currently conduct this test routinely. Due to insufficient laboratory support and failure to meet the study's data quality objective of a minimum of six laboratories, EPA canceled interlaboratory testing of the Holmesimysis costata Acute Test method. Though interlaboratory testing was canceled, the referee laboratory did attempt to conduct single-laboratory testing of the Holmesimysis costata Acute Test.

During the WET Variability Study, the referee laboratory initiated five Holmesimysis costata acute tests. The referee laboratory did not initiate additional tests due to difficulties in obtaining test organisms. Juvenile Holmesimysis costata used for testing are generally obtained from field-collected gravid females. The referee laboratory was unable to collect sufficient numbers of gravid females during most of the time frame for the WET Variability Study (September 1999 through April 2000). Of the five tests that were initiated, none successfully met test acceptability criteria and required test conditions. Three tests failed to meet test acceptability criteria for control survival, and two tests failed to meet requirements for the age of test organisms (all within 24 hours). These test failures demonstrated the inadequacy of Mysidopsis bahia Acute Test procedures for use in conducting acute tests with Holmesimysis costata. EPA has since concluded that modified test procedures are needed for successful conduct of the Holmesimysis costata Acute Test. These modifications include more detailed organism collection and holding procedures, specific dilution water requirements, revised temperature requirements, and less restrictive test organism age requirements.

Today, EPA proposes to withdraw Holmesimysis costata as an acceptable species for use in the Mysidopsis bahia Acute Test method and proposes a separate Holmesimysis costata Acute Test method. This proposal would add

to the acute method manual a table of test conditions specific to Holmesimysis costata and information in Appendix A.3 on the morphology, taxonomy, collection, holding, culturing, feeding, and testing of Holmesimysis costata. The specifics of the proposed Holmesimysis costata Acute Test method and the method manual changes necessary to implement the addition of this method are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d).

The proposed Holmesimysis costata Acute Test method is based on method development data from the California Water Resources Control Board's Marine Bioassay Project (State Water Resources Control Board, 1990) and from peer-reviewed literature (Martin et al., 1989; Hunt et al., 1997). These data show that given the appropriate test procedures and test conditions, the Holmesimysis costata Acute Test can produce reliable and sensitive toxicity results with adequate precision. Single-laboratory testing of zinc with the Holmesimysis costata Acute Test method yielded intralaboratory precision (CVs) of 19% and 23% in 48-h and 96-h acute tests, respectively. Multi-laboratory testing of zinc with the Holmesimysis costata Acute Test method yielded interlaboratory precision (CVs) of 24% and 1% in 2 separate trials.

In addition to the proposed Holmesimysis costata Acute Test method, EPA requests comment on the applicability of similar methods published by voluntary consensus standard bodies. A mysid toxicity test method with specific test procedures for Holmesimysis costata is published in Standard Methods for the Examination of Water and Wastewater (APHA et al., 1998), and a West Coast mysid toxicity test method is published by the American Society for Testing and Materials (ASTM, 1993). EPA does not believe that these methods from voluntary consensus standard bodies provide the detailed requirements necessary for routine use in compliance monitoring, so EPA is proposing a new Holmesimysis costata Acute Test method for inclusion in EPA's acute method manual (USEPA, 1993b). EPA invites comment, however, on whether to approve the other organizations' testing procedures, including comment on their use for compliance monitoring.

IV. Regulatory Requirements

A. Executive Order 12866—Regulatory Planning and Review

Under Executive Order 12866 (58 FR 51735 (October 4, 1993)), the Agency must determine whether a regulatory action is ''significant'' and therefore subject to Office of Management and Budget (OMB) review and the requirements of the Executive Order. The Executive Order defines ''significant regulatory action'' as one that is likely to result in a rule that may: (1) Have an annual effect on the economy of $100 million or more or adversely affect in a material way the economy, a sector of the economy, productivity, competition, jobs, the environment, public health or safety, or State, local, or tribal governments or communities; (2) create a serious inconsistency or otherwise interfere with an action taken or planned by another agency; (3) materially alter the budgetary impact of entitlements, grants, user fees, or loan programs or the rights and obligations of recipients thereof; or (4) raise novel legal or policy issues arising out of legal mandates, the President's priorities, or the principles set forth in the Executive Order.''

Pursuant to the terms of Executive Order 12866, it has been determined that this rule is not a ''significant regulatory action.'' Therefore, this action is not subject to OMB review.

B. Unfunded Mandates Reform Act

Title II of the Unfunded Mandates Reform Act of 1995 (UMRA), Public Law 104–4, establishes requirements for Federal agencies to assess the effects of their regulatory actions on State, local, and tribal governments and the private sector. Under section 202 of the UMRA, EPA generally must prepare a written statement, including a cost-benefit analysis, for proposed and final rules with ''Federal mandates'' that may result in expenditures to State, local, and tribal governments, in the aggregate, or to the private sector, of $100 million or more in any one year. Before promulgating an EPA rule for which a written statement is needed, section 205 of the UMRA generally requires EPA to identify and consider a reasonable number of regulatory alternatives and adopt the least costly, most cost-effective or least burdensome alternative that achieves the objectives of the rule. The provisions of section 205 do not apply when they are inconsistent with applicable law. Moreover, section 205 allows EPA to adopt an alternative other than the least costly, most cost-effective or least burdensome alternative if the Administrator publishes with the final rule an explanation of why that alternative was not adopted. Before EPA establishes any regulatory requirements that may significantly or uniquely affect small governments, including tribal governments, it must have developed, under section 203 of the UMRA, a small government agency plan. The plan must provide for the notification of potentially affected small governments, enabling officials of affected small governments to have meaningful and timely input in the development of EPA regulatory proposals with significant Federal intergovernmental mandates, and informing, educating, and advising small governments on compliance with the regulatory requirements.

EPA has determined that today's proposed rule does not contain a Federal mandate that may result in expenditures of $100 million or more for State, local, and tribal governments, in the aggregate, or the private sector in any one year. Today's rule proposes revisions to WET test methods that are currently approved for use in NPDES permits and certification of Federal licenses under the CWA. The revisions are minor and the cost to implement them is minimal. Thus, today's rule is not subject to the requirements of sections 202 and 205 of the UMRA.

EPA has determined that this rule contains no regulatory requirements that might significantly or uniquely affect small governments. It would not significantly affect them because any incremental costs incurred are minimal, and it would not uniquely affect them because it would affect entities of all sizes required to test for whole effluent toxicity by a regulatory authority the same. Further, whole effluent toxicity monitoring by small entities is generally expected to be less frequent than such monitoring by larger entities. Therefore, today's rule is not subject to the requirements of section 203 of UMRA.

C. Regulatory Flexibility Act (RFA), as Amended by the Small Business Regulatory Enforcement Fairness Act of 1996 (SBREFA), 5 U.S.C. 601 et seq.

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

For purposes of assessing the impacts of today's rule on small entities, small entity is defined as: (1) a small business as defined by the U.S. Small Business Administration definitions at 13 CFR 121.201; (2) a small governmental jurisdiction that is a government of a city, county, town, school district or special district with a population of less

than 50,000; and (3) a small organization that is any not-for-profit enterprise which is independently owned and operated and is not dominant in its field.

After considering the economic impacts of today's proposed rule on small entities, I certify that this action will not have a significant economic impact on a substantial number of small entities. Today's rule proposes revisions to WET test methods that are currently approved for use in NPDES permits and certification of Federal licenses under the CWA. The revisions are minor and the cost to implement them is minimal. The proposed revisions are intended to improve the performance of WET tests, and thus increase confidence in the reliability of the results obtained using the test methods. EPA estimates that any incremental costs associated with the proposed revisions would be alleviated by a potential reduction in retesting that may result from improved test performance and increased confidence in the reliability of testing results. We continue to be interested in the potential impacts of the proposed rule on small entities and welcome comments on issues related to such impacts.

D. Paperwork Reduction Act

This action does not impose an information collection burden under the provisions of the *Paperwork Reduction Act,* 44 U.S.C. 3501 *et seq.* It does not contain any information, collection, reporting, or record keeping requirements.

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

An Agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. The OMB control numbers for EPA's regulations are listed in 40 CFR part 9 and 48 CFR chapter 15.

E. National Technology Transfer and Advancement Act

Section 12(d) of the National Technology Transfer and Advancement Act of 1995 (NTTAA), Public Law 104–113, section 12(d) (15 U.S.C. 272 note) directs EPA to use voluntary consensus standards in its regulatory activities unless to do so would be inconsistent with applicable law or otherwise impractical. Voluntary consensus standards are technical standards (*e.g.*, materials specifications, test methods, sampling procedures, and business practices) that are developed or adopted by voluntary consensus standards bodies. The NTTAA directs EPA to provide Congress, through OMB, explanations when the Agency decides not to use available and applicable voluntary consensus standards.

Today's action would revise existing EPA WET test methods and add a new Holmesimysis costata Acute Test method. For the methods that EPA is proposing to revise, the Agency did not conduct a search to identify potentially applicable voluntary consensus standards, because the revisions EPA proposes today would merely incorporate more specificity and detail into already approved EPA test methods. EPA invites comment, however, on the extent to which voluntary consensus standard organizations' methods would be consistent with the EPA methods for which revisions are proposed today. For the new Holmesimysis costata Acute Test method, the Agency reviewed applicable voluntary consensus standards and identified two mysid methods (ASTM, 1993; APHA et al., 1998) that provide specific test procedures for use with Holmesimysis costata. While EPA requests comment on the applicability of these voluntary consensus standards, the Agency does not believe that these methods would provide the additional detailed requirements EPA proposes today. For this reason, EPA proposes a new EPA Holmesimysis costata Acute Test method. EPA welcomes comments on this aspect of the proposed rulemaking and, specifically, invites the public to identify potentially-applicable voluntary consensus standards and to explain why such standards should be used in this regulation.

F. Executive Order 13045—Protection of Children From Environmental Health Risks and Safety Risks

Executive Order 13045 (62 FR 19885, April 23, 1997) applies to any rule that: (1) Is determined to be ''economically significant'' as defined under Executive Order 12866, and (2) concerns an environmental health or safety risk that EPA has reason to believe may have a disproportionate effect on children. If the regulatory action meets both criteria, the Agency must evaluate the environmental health or safety effects of the planned rule on children, and explain why the planned regulation is preferable to other potentially effective and reasonably feasible alternatives considered by the Agency. This rule is not subject to the Executive Order because it is not economically significant as defined in Executive Order 12866, nor does it concern an environmental health or safety risk that EPA has reason to believe may have a disproportionate effect on children.

G. Executive Order 13175—Consultation and Coordination With Indian Tribal Governments

Executive Order 13175, entitled ''Consultation and Coordination with Indian Tribal Governments'' (65 FR 67249; November 6, 2000), requires EPA to develop an accountable process to ensure ''meaningful and timely input by tribal officials in the development of regulatory policies that have tribal implications.'' ''Policies that have tribal implications'' is defined in the Executive Order to include regulations that have ''substantial direct effects on one or more Indian tribes, on the relationship between the Federal government and the Indian tribes, or on the distribution of power and responsibilities between the Federal government and Indian tribes.''

This proposed rule does not have tribal implications. It will not have substantial direct effects on tribal governments, on the relationship between the Federal government and Indian tribes, or on the distribution of power and responsibilities between the Federal government and Indian tribes, as defined in Executive Order 13175. Today's proposed rule would revise WET test methods that are currently approved for use in NPDES permits and certification of Federal licenses under the CWA. The revisions are minor and the cost to implement them is minimal. Thus, Executive Order 13175 does not apply to this rule. In the spirit of Executive Order 13175, and consistent with EPA policy to promote communications between EPA and tribal governments, EPA specifically solicits comment on this proposed rule from tribal officials.

H. Executive Order 13132—Federalism

Executive Order 13132, entitled ''Federalism'' (64 FR 43255; August 10, 1999), requires EPA to develop an

accountable process to ensure ''meaningful and timely input by State and local officials in the development of regulatory policies that have federalism implications.'' ''Policies that have federalism implications'' is defined in the Executive Order to include regulations that have ''substantial direct effects on the States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government.''

This proposed rule does not have federalism implications. It will not have substantial direct effects on the States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government, as specified in Executive Order 13132. Today's rule proposes revisions to WET test methods that are currently approved for use in NPDES permits and certification of Federal licenses under the CWA. The revisions are minor and the cost to implement them is minimal. Thus, Executive Order 13132 does not apply to this rule. In the sprit of Executive Order 13132, and consistent with EPA policy to promote communications between EPA and State and local governments, EPA specifically solicits comment on this proposed rule from State and local officials.

I. Executive Order 13211—Energy Effects

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

J. Plain Language Directive

Executive Order 12866 requires each agency to write all rules in plain language. We invite your comments on how to make this proposed rule easier to understand. For example, have we organized the material to suit your needs? Are the requirements in the rule clearly stated? Does the rule contain technical language or jargon that isn't clear? Would a different format (grouping and order of sections, use of headings, paragraphing) make the rule easier to understand? Would more (but shorter) sections be better? Could we improve clarity by adding tables, lists, or diagrams? What else could we do to make the rule easier to understand?

V. Request for Comments and Available Data

EPA requests public comments on this proposed rule. EPA invites comment on the technical merit, applicability, and implementation of the specific WET test method changes included in this proposal. EPA also invites comments on the ratification of the methods listed. EPA encourages commenters to provide copies of supporting data and/or references cited in comments.

EPA recognizes that stakeholders continue to have concerns over a variety of issues related to implementation of whole effluent toxicity controls through NPDES permits. Today's notice, however, invites comments only on the conduct of WET test methods and not on the implementation of WET control strategies through NPDES permits. EPA is interested in comments on the extent to which some aspect(s) of the technical components of the method revisions proposed today may affect implementation of WET control strategies. For example, today's notice solicits comments related to the proposed application of percent minimum significant difference (PMSD) approaches to evaluate the precision of WET test results (see Section B below). Application of the PMSD approach is intended to control the within-test variability in WET methods. Nationwide, however, NPDES agencies have implemented other concepts, such as limits on CVs to control for within-test variability rather than the PMSD concepts about which EPA solicits comment today. It is not EPA's objective to create conflict with the current implementation of WET control strategies that do not presently apply the PMSD concepts, but instead to enhance ongoing implementation efforts by providing an additional review step for WET test results to promote WET test precision. To the extent that application of the PMSD concepts could result in conflicts with the current and ongoing WET implementation, EPA invites comments on how to ameliorate any such adverse effects on WET implementation.

A. pH Drift

In particular, EPA requests comments and available data to support or refute test method changes related to pH drift (see Section III.B.3.b). EPA requests that commenters provide any data that show the value of proposed pH control measures in situations where ammonia or other pH-dependent toxicants are not present. EPA specifically requests chronic toxicity data from parallel controlled-pH and uncontrolled-pH tests on well-treated municipal or industrial effluents. Such data should include raw toxicity test data sheets, ammonia measurements on tested samples, and daily initial and final pH measurements on each test treatment. EPA also requests data from multiple tests conducted on a given effluent over time to demonstrate a trend of artifactual toxicity due to pH drift in that effluent. Data sets should include full strength effluent, as well as a range of effluent concentrations and a dilution water control. Electronic as well as hard copy formats of raw test data and statistical analysis are encouraged. Though EPA continues to search for and may yet develop data supporting the need for procedures to control pH drift in the absence of ammonia or other pH-dependent toxicants, if sufficient data are not available at the time of final action on today's proposal, EPA may not incorporate changes to the methods beyond the 1996 guidance in the final rule.

B. Percent Minimum Significant Difference

The percent minimum significant difference (PMSD) is a measure of within-test variability and test sensitivity. The PMSD for a given WET test can be defined as the smallest percentage difference between the control and a treatment (an effluent dilution) that could be declared as statistically significant. As test variability increases, the ability of a test to detect small toxic effects diminishes and the test becomes a less sensitive measure of toxicity. Appendix C of the WET method manuals (USEPA, 1994a; USEPA, 1994b) describes the calculation of the minimum significant difference (MSD). The PMSD is simply the MSD expressed as a percentage of the control response (i.e., PMSD = MSD/control mean * 100).

In June 2000, EPA published guidance on WET test variability that recommended placing upper and lower bounds on the PMSD to control variability and ensure a specified range of test sensitivity (USEPA, 2000d). This guidance derived lower and upper bounds as the 10th and 90th percentiles, respectively, of PMSDs from a large number of reference toxicant tests. Based on this guidance, tests for which the PMSD exceeds an upper bound would be conducted again (with a newly collected sample), if the test leads to a decision that there is no significant toxicity at the concentration identified in the permit as a limit (''Instream Waste Concentration'' (IWC) or ''Receiving Water Concentration''). This

guidance also applies lower PMSD bounds for the purpose of determining the no observed effect concentration (NOEC). The purpose of the lower PMSD bound is to avoid declaring as ''significant'' toxic effects that are smaller than those that can generally and routinely be detected by the method as currently conducted by qualified laboratories. Application of a lower bound does not imply that EPA has knowledge that, or considers that, percent differences smaller than the lower bound represent non-toxic effects. The lower bound PMSD is used here not as a threshold for toxicity but as a measure of method precision.

Today, EPA seeks comment on proposing to require the application of the upper and lower PMSD bounds for sublethal endpoints in the (1) Ceriodaphnia dubia Survival and Reproduction Test; (2) Fathead Minnow Larval Survival and Growth Test; (3) Mysidopsis bahia Survival, Growth, and Fecundity Test; and (4) Inland Silverside Larval Survival and Growth Test. The proposed requirement would apply to the determination of the NOEC and LOEC (lowest observed effect concentration) for sublethal endpoints in multi-concentration tests. In the proposed application, the upper and lower PMSD bounds would be used to determine when a treatment differs significantly from the control treatment. Any test treatment with a percentage difference from the control (i.e., [mean control response—mean treatment response]/ mean control response * 100) that is greater than the upper PMSD bound would be considered as significantly different. Any test treatment with a percentage difference from the control that is less than the lower PMSD bound would not be considered as significantly different. The specifics of method manual changes proposed to institute the required application of PMSD bounds are detailed in the document titled, Proposed Changes to Whole Effluent Toxicity Method Manuals (USEPA, 2001d). The PMSD procedures about which EPA invites comment today would not preclude application of the current recommended guidance (USEPA 2000d) on PMSD bounds because today's proposed procedures are less restrictive than the guidance recommendation. EPA will consider using additional sources of data for developing lower and upper bounds for PMSD, including, but not limited to, data from EPA's WET Variability Study (USEPA, 2001a).

EPA considered the appropriateness of requiring PMSD bounds for the growth endpoints of the Sheepshead Minnow Larval Survival and Growth Test and the Selenastrum capricornutum Growth Test. At this time, EPA does not believe that requiring PMSD bounds for these test methods would be appropriate because: (a) These methods appear to achieve smaller PMSDs than the other chronic methods (USEPA 2000d), and (b) the PMSD bounds for these methods (USEPA 2000d) would be based upon fewer laboratories and tests (albeit a substantial number) than the PMSD bounds for the methods for which EPA invites comment today. EPA also considered the appropriateness of PMSD bounds for the survival endpoints of test methods for chronic toxicity, and test methods for acute toxicity. At this time, EPA does not believe that imposing PMSD bounds for the survival endpoints would be necessary because precision for survival endpoints appears to be, in most cases, better than precision for sublethal endpoints (USEPA 2000d). EPA seeks comment on the appropriateness of imposing PMSD bounds for four test methods and for sublethal endpoints.

EPA considered other measures of test precision, including the standard deviations and coefficients of variation for treatments and control, MSD, and the mean square for error from the analysis of variance of treatment effects (USEPA 1994a, 1994b). EPA considers the PMSD to be the measure that would be most easily understood and that could be directly applied to determination of NOEC and LOEC values. The PMSD quantifies the smallest percentage difference between the control and a treatment (effluent dilution) that could be declared as statistically significant. It thus includes exactly that variability affecting determination of the NOEC and LOEC. The CV for the control or any one treatment, or for selected treatments, represents only a portion of the variability affecting the NOEC, LOEC, and point estimates. Some State or Regional WET programs have requirements on the CV for the control and the treatment representing the IWC concentration. Such requirements can provide finer control over the variability influencing a comparison, especially a direct comparison between the control and the IWC treatment. The PMSD upper bound provides control over the average variability and would be used here specifically for multi-concentration tests in which the NOEC or LOEC are determined by using the MSD. EPA seeks comment on (1) the need for increased within-test precision, (2) the merits and drawbacks of applying PMSD bounds as described above, and (3) additional or alternative applications of PMSD bounds to control test precision. Alternative applications of PMSD bounds could include quality control requirements for laboratories to track PMSD values over time (e.g., control charts for PMSD performance in reference toxicant and/or effluent tests); a requirement to demonstrate recent, ongoing precision (PMSD less than an upper bound) in multiple tests before starting an effluent test; and/or use of PMSD bounds as a component of test review. EPA also requests that commenters submit data (hard copy and electronic format) to support their comments or recommendations regarding the application of PMSDs.

C. Other Method Modifications

In addition to the method modifications proposed today, EPA seeks comment and recommendations on other method modifications that would improve the performance of the WET test methods. Specifically, EPA requests comment and recommendations on (1) increasing the test acceptability criteria for mean control reproduction (number of young per surviving female) in the Ceriodaphnia dubia Survival and Reproduction Test; (2) increasing the test acceptability criteria for mean control weight (mean weight per original) in the Fathead Minnow Larval Survival and Growth Test; (3) increasing the number of replicate chambers per concentration from a minimum of three to a minimum of four in the Fathead Minnow Larval Survival and Growth Test Method, Sheepshead Minnow Larval Survival and Growth Test Method, the Inland Silverside Larval Survival and Growth Test Method, and the Sea Urchin Fertilization Test Method; and (4) increasing the minimum number of replicates in the Ceriodaphnia dubia Survival and Reproduction Test Method. Modifications to the minimum number of replicates would be made to improve the precision of the test methods. EPA intends to evaluate these and other options for improving WET test method performance using existing data (from the WET Variability Study and the Variability Guidance Document) and data submitted to EPA in response to this request. EPA requests comments and recommendations on any additional quality control measures that would increase test precision or the overall quality of data generated. Comments should be supported by data (hard copy and electronic format) and other technical information whenever possible. Comments that contain

suggestions that are not supported by submitted data will be considered, but will be given less weight than those supported by data. EPA also requests that commenters submit information on estimated increases in testing costs that may be associated with any recommended method modification.

Lastly, EPA requests comment on the document titled, Study Report and Recommended Standard Operating Procedure (SOP) for Shipping Large Volume Samples at Less Than 4°C (USEPA, 2001f), which is included in the record for this rulemaking (see Addresses section of this rule for more information on obtaining copies of referenced materials). This report presents data to support a recommended SOP for meeting sample temperature requirements (less than 4°C) during shipping of WET samples.

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List of Subjects in 40 CFR Part 136

Environmental protection, Reporting and recordkeeping requirements, Water pollution control.

Dated: September 24, 2001.

Christine Todd Whitman,

Administrator.

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

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

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

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

2. Section 136.3 is amended:

a. In Table IA paragraph (a) by revising entries 6 to 9.

b. In paragraph (a) by revising footnotes 7–9 to Table IA.

c. In paragraph (b) by revising references (34), (38), and (39). d. In paragraph (b) by removing and reserving reference (42).

§ 136.3 Identification of test procedures.

(a) * * *

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS

Parameter and units	Method [1]	EPA	Standard methods 18th, 19th, 20th Ed.	ASTM	AOAC	USGS	Other
*	*	*	*	*	*	*	
Aquatic Toxicity:							
6. Toxicity, acute, fresh water organisms, LC50, percent effluent..	Daphnia, Ceriodaphnia, Fathead Minnow, Rainbow Trout, Brook Trout, or Bannerfin Shiner mortality.	Sec. 9 [7]					
7. Toxicity, acute, estuarine and marine organisms, LC50, percent effluent..	Mysidopsis bahia, Holmesimysis costata, 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.	1000.0 [8]					
	Fathead minnow embryo-larval survival and teratogenicity.	1001.0 [8]					
	Ceriodaphnia survival and reproduction.	1002.0 [8]					
	Selenastrum growth	1003.0 [8]					
9. Toxicity, chronic, estuarine and marine organisms, NOEC or IC25, percent effluent..	Sheepshead minnow larval survival and growth.	1004.0 [9]					
	Sheepshead minnow embryo-larval survival and teratogenicity.	1005.0 [9]					
	Menidia beryllina larval survival and growth.	1006.0 [9]					
	Mysidopsis bahia survival, growth, a 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.
[7] USEPA. [Date: To be completed at final rule]. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. Fifth Edition. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio. [EPA number: To be completed at final rule].
[8] USEPA. [Date: To be completed at final rule]. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio. [EPA number: To be completed at final rule].
[9] USEPA [Date: to be completed at final rule]. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Third Edition. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio. [EPA number: To be completed at final rule]. These methods do not apply to marine waters of the Pacific Ocean.

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(b) * * *

References, Sources, Costs, and Table Citations:

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(34) USEPA. [Date: To be completed at final rule]. Methods for Measuring the Acute Toxicity of Effluents and Receiving Water to Freshwater and Marine Organisms. Fifth Edition. [Date: To be completed at final rule]. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio [EPA number: To be completed at final rule]. Available from: National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Publ. No. [Publication number: To be completed at final rule]. Cost: $[Cost: To be completed at final rule]. Table IA, Note 7.

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(38) USEPA. [Date: To be completed at final rule]. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms. Fourth Edition. [Date: To be completed at final rule]. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio. [EPA number: To be completed at final rule]. Available from: National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Publ. No. [Publication number: To be completed at final rule]. Cost: $[Cost: To be completed at final rule]. Table IA, Note 8.

(39) USEPA. [Date: To be completed at final rule]. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Marine and Estuarine Organisms. Third Edition. [Date: To be completed at final rule]. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio. [EPA number: To be completed at final rule]. Available from: National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Publ. No. [Publication number: To be completed at final rule]. Cost: $[Cost: To be completed at final rule]. Table IA, Note 9.

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(42) [Reserved]

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