MEMORANDUM

Office of Water Permit Programs
Division of Water Program Coordination
Department of Environmental Quality

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SUBJECT: Guidance Memo No. 00-2012
Toxics Management Program Implementation Guidance

TO: Regional Directors

FROM: Larry G. Lawson

DATE: August 24, 2000

COPIES: Martin Ferguson, Regional Office Permit Managers, Regional Office Water Permit Managers, Regional Office Compliance and Enforcement Managers, TMP Staff, David Paylor, Mary Jo Leugers

The purpose of this guidance is to replace/update Guidance memo No. 93-029, "Toxics Management Program Implementation Guidance", all Appendices, and subsequent addenda.

The guidance was last updated August 1994. The TMR has been repealed, WET testing methods and application requirements for POTWs have been published in the FR. This guidance replaces all previous guidance on the subjects covered herein.

The Toxics Management Program guidance contained herein is based on the best technical and procedural information that now exists. However, the entire Toxics program is still evolving and being developed at both the state and national level. We will keep track of these developments and as warranted, we will either amend this guidance or issue new guidance as appropriate.

NOTE TO USERS: This document is provided as guidance and as such, sets forth standard operating procedures for the agency. However, it does not mandate any particular method nor does it prohibit any particular method for the analysis of data, establishment of a wasteload allocation, or establishment of a permit limit. If alternative proposals are made, such proposals should be reviewed and accepted or denied based on their technical adequacy and compliance with appropriate laws and regulations.

Deborah L. DeBiasi, at 804/698-4028 or dldebiasi@deq.state.va.us is the contact person if you or your permit managers have any questions.

IMPLEMENTATION GUIDANCE

for the

TOXICS MANAGEMENT PROGRAM

Revised August 24, 2000

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Toxics Management Program Guidance

Toxics Management Program Guidance
Revision Date: August 24, 2000

I. Purpose

In order to ensure Virginia's surface waters meet their beneficial uses, it may be necessary to utilize whole effluent toxicity tests, as complex effluents may contain numerous toxicants leading to possible additive, synergistic, or antagonistic effects to the organisms of the receiving waters. This approach employs acute and chronic toxicity tests to measure the aggregate toxicity of pollutants present in wastewater. Standard vertebrate and invertebrate surrogate species are both tested since different species often exhibit different sensitivities to various toxicants contained in the effluents. The value of whole effluent tests is further recognized as the combined effects of chemicals in complex effluents are addressed. It should be noted that these tests do not provide information regarding human health. The purpose of this guidance is to recommend how and when to use aquatic toxicity testing to assess the reasonable potential for toxicity of a discharge to surface waters.

II. Authority

The authority to utilize aquatic toxicity testing to determine the reasonable potential for toxicity of a discharge is part of the VPDES Permit Regulation, 9VAC 25-31-220 D.1.a.-d.

*1. **Achieve water quality standards established under the Law and Section 303 of the CWA, including state narrative criteria for water quality.***

*a. **Limitations must control all pollutants or pollutant parameters (either conventional, nonconventional, or toxic pollutants) which the Board determines are or may be discharged at a level which will cause, have the reasonable potential to cause, or contribute to an excursion above any Virginia water quality standard, including Virginia narrative criteria for water quality.***

*b. **When determining whether a discharge causes, has the reasonable potential to cause, or contributes to an in-stream excursion above a narrative or numeric criteria within a Virginia water quality standard, the Board shall use procedures which account for existing controls on point and non-point sources of pollution, the variability of the pollutant or pollutant parameter in the effluent, the sensitivity of the species to toxicity testing (when evaluating whole effluent toxicity), and where appropriate, the dilution of the effluent in the receiving water.***

*c. **When the Board determines, using the procedures in paragraph D 1 b.***

of this section, that a discharge causes, has the reasonable potential to cause, or contributes to an in-stream excursion above the allowable ambient concentration of a Virginia numeric criteria within a Virginia water quality standard for an individual pollutant, the permit must contain effluent limits for that pollutant.

d. Except as provided in this subparagraph, when the Board determines, using the procedures in paragraph D.1.b. of this section, toxicity testing data, or other information, that a discharge causes, has the reasonable potential to cause, or contributes to an in-stream excursion above a narrative criterion within an applicable Virginia water quality standard, the permit must contain effluent limits for whole effluent toxicity. Limits of whole effluent toxicity are not necessary where the Board demonstrates in the fact sheet or statement of basis of the VPDES permit, using the procedures in paragraph D.1.b. of this section, that chemical-specific limits for the effluent are sufficient to attain and maintain applicable numeric and narrative Virginia water quality standards.

III. Definitions, Acronyms and Abbreviations

ACR Acute-to-chronic ratio is the ratio of the acute toxicity of an effluent or a toxicant to its chronic toxicity. It is used as a factor for estimating chronic toxicity on the basis of acute toxicity data, or for estimating acute toxicity on the basis of chronic toxicity data.

Acute Toxicity An effect that usually occurs shortly after the administration of either a single dose or multiple doses of a pollutant. Lethality to an organism is the usual measure of acute toxicity. Where death is not easily detected, immobilization is considered equivalent to death.

Acute Toxicity Test A test to determine the concentration of effluent or ambient waters that causes an adverse effect (usually death) on a group of test organisms during a short-term exposure (e.g., 24, 48, or 96 hours). Acute toxicity is measured using statistical procedures (e.g., point estimate techniques or a t-test).

AML Average monthly limit

Biological Monitoring or Biomonitoring The repeated measurement of physiological responses of organisms and/or their systems to environmentally induced conditions. These may include:

1. Aquatic life, including accumulation of pollutants in tissue, in state waters due to the discharge of pollutants by techniques and procedures, including sampling of organisms representative of appropriate levels of the food chain appropriate to the volume and the physical, chemical, and biological characteristics of the effluent,

and at appropriate frequencies and locations.

2. The use of acute and chronic tests which directly measure effluent toxicity to aquatic organisms. These toxicity tests can be used to identify toxic discharges and may help establish effluent limits for permits.

CCC The EPA national water quality criteria recommendation for the highest instream concentration of a toxicant or an effluent to which organisms can be exposed indefinitely without causing unacceptable effects.

CFR Code of Federal Regulations

CFS Cubic feet per minute, a velocity measure. 1 CFS = 0.6463 MGD (million gallons per day)

Chronic Toxicity An effect that is irreversible or progressive or occurs because the rate of injury is greater than the rate of repair during prolonged exposure to a pollutant. This includes low level, long-term effects such as reduction in growth, reproduction, or fecundity.

Chronic Toxicity Test A short-term test in which sublethal effects (e.g., reduced growth or reproduction) are usually measured in addition to lethality. Chronic toxicity is defined as $TU_c = 100/NOEC$ or $TU_c = 100/IC_{25}$.

Clean Water Act, CWA or "Act" Refers to 33 USC 1251 et. Seq.

CMC The EPA national water quality criteria recommendation for the highest instream concentration of a toxicant or an effluent to which organisms can be exposed for a brief period of time without causing an acute effect.

Contaminated Non-process Wastewater Any water which, during manufacturing or processing, comes into incidental contact with any raw material, intermediate product, finished product, by-product, or waste product by means of rainfall runoff, accidental spills, leaks caused by failure of process equipment or discharges from safety showers and related personal safety equipment.

Contiguous Zone The entire zone established by the United States under Article 24 of the Convention on the Territorial Sea and the Contiguous Zone.

Continuous Discharge A discharge which occurs without interruption throughout the operating hours of the facility, except for infrequent shutdowns for maintenance, process changes, or other similar activities. (If chronic bioassays are required, the discharge must be 5 days in duration.)

Criteria Continuous Concentration CCC is the EPA national water quality criteria

recommendation for the highest instream concentration of a toxicant or an effluent to which organisms can be exposed indefinitely without causing unacceptable (chronic) effect. Numerically, this equates to 1.0 TU_c.

Criteria Maximum Concentration CMC is the EPA national water quality criteria recommendation for the highest instream concentration of a toxicant or an effluent to which organisms can be exposed for a brief period of time without causing an acute effect. Numerically, this equates to 0.3 TU_a.

CV Coefficient of Variation is a standard statistical measure of the relative variation of a distribution or set of data, defined as the standard deviation divided by the mean. It is also called the relative standard deviation (RSD). The CV can be used as a measure of precision within and among laboratories, or among replicates for each treatment concentration.

Department, DEQ The Department of Environmental Quality

Discharge When used without qualification means the discharge of a pollutant.

DMR, Discharge Monitoring Report The form supplied by the Department, or an equivalent form developed by the permittee and approved by the Board, for the reporting of self-monitoring results by permittees.

Effluent Limitation Any restriction imposed by the Board on quantities, discharge rates, and concentrations of pollutants that are discharged from point sources into surface waters, the waters of the contiguous zone, or the ocean.

Ephemeral Stream Any drainage way, ditch, hollow, or swale that contains only (1) flowing water during or immediately following periods of rainfall or (2) water supplied by the discharger.

Flows: 7Q10 The critical receiving stream flow used to calculate chronic aquatic life water quality standards. It is the low flow which, on a statistical basis, would occur for a 7 consecutive day period once every 10 years.

1Q10 The critical receiving stream flow used to calculate acute aquatic life water quality standards. It is the lowest stream flow which, on a statistical basis, would occur over a 1-day period once every 10 years.

30Q5 The critical receiving stream flow which is used to calculate the non-carcinogenic human health water quality standards. It is the lowest stream flow which, on a statistical basis, would occur for a 30-day consecutive period once every 5 years.

FR Federal Register

Hypothesis Testing A statistical technique (e.g., Dunnetts test) that determines if a tested concentration is statistically different from the control. Endpoints determined from hypothesis testing are the NOEC and the LOEC, and can only be represented by the test dilutions selected.

IC Inhibition Concentration – Usually seen as IC_{25} – The estimated concentration that would cause a 25% reduction in effect from the control organisms

Industrial Facility Establishments with activity in which they are engaged as an economic unit, generally at a single location where business is conducted, services or industrial operations performed, or in which raw materials are changed into useful products.

Instream Waste Concentration (IWC) The concentration of an effluent, expressed as a percentage, which occurs in the receiving waterbody after mixing. To calculate the IWC, divide the effluent flow by the 7Q10 (chronic IWC, or IWC_c) or 1Q10 (acute IWC, or IWC_a) added to the effluent flow. Also known as RWC, or receiving water concentration.

Intermittent Stream A stream that contains flowing water for extended periods during a year, but does not carry flow at all times.

LC_{50} The concentration of a toxic pollutant or effluent expressed as percent volume that is lethal to 50% of the test organisms within the prescribed period of time. It is the Lethal Concentration to 50% of the organisms, expressed simply.

LOEC Lowest Observed Effect Concentration – The lowest concentration of an effluent or toxicant that results in statistically adverse effects on the test organisms (i.e., where the values for the observed endpoint are statistically different from the control. It is seen as a secondary end point for chronic tests

LTA Long Term Average (LTA_a = acute, LTA_c = Chronic)

Major Facility Municipal facility with design capacity equal to or greater than 1.0 MGD; or an industrial facility that either scores 80 or more points on the NPDES Permit Rating Worksheet or that may be agreed to by EPA and DEQ. Permits for major facilities must go to EPA for review and concurrence prior to issuance.

MDL Maximum daily limit

MGD Million gallons per day. 1 MGD = 1.5473 CFS (cubic feet per second)

Minimum Significant Difference (MSD) The magnitude of difference from the control where the null hypothesis is rejected in a statistical test comparing a treatment with a control. MSD is based on the number of replicates, control performance, and power of the test.

Minor Facility A facility that does not fall within the "Major Facility" category.

Mixing The process by which an effluent is incorporated into the receiving waterbody.

Mixing Zone An area where an effluent discharge undergoes initial dilution and is extended to cover the secondary mixing in the ambient waterbody. A mixing zone is an allocated impact zone where water quality criteria can be exceeded as long as acutely toxic conditions are prevented.

MSD Minimum significant difference

Municipal Facility A treatment works, other than an industrial facility, whose primary function is to receive and treat wastewater from domestic sources or from indirect domestic, commercial or industrial sources.

NOAEC No Observed Adverse Effect Concentration – An acute test endpoint. – The highest concentration at which survival is not significantly different from the controls., and below which there is no statistically significant adverse effect.

NOEC No Observed Effect Concentration – A chronic test endpoint - The highest concentration of toxicant to which organisms are exposed in which the values for the observed responses are not statistically different from the controls, and below which there is no statistically significant adverse effect.

Non-Contact Cooling water Water which is used to reduce temperature which does not come into direct contact with any raw material, intermediate product, waste product (other than heat), by-product or finished product.

NPDES National Pollutant Discharge Elimination System

Point Estimate Techniques Statistical analyses such as Probit, Interpolation Method, and Spearman-Karber are used to determine the effluent concentration at which adverse effects (e.g., growth or survival) occurred. For example, the concentration at which a 25 percent reduction in growth occurred. The test endpoints derived by point estimation are LC_{50} and IC_{25}.

Privately Owned Treatment Works (PVOTW) Any device or system that is 1) used to treat indirect domestic, commercial, and/or industrial wastes, whose operator is not the owner of the treatment works; and 2) not a POTW.

Publicly Owned Treatment Works (POTW) Any device or system used in the treatment of municipal sewage or industrial wastes of a liquid nature which is owned by a state or municipality. Sewers, pipes, or other conveyances are

included in this definition only if they convey wastewater to a POTW providing treatment.

Reasonable potential Where an effluent is projected or calculated to cause an excursion above a water quality standard based on a number of factors including, as a minimum, the four factors listed in 40 CFR 122.44(d)(1)(ii).

Reference Toxicant Test A toxicity test performed with a quantified chemical in accordance with the procedures required for effluent tests. It checks the sensitivity of the organisms being used and the suitability of the test methodology. Reference toxicant data are part of a routine QA/QC program to evaluate the performance of laboratory personnel, and the robustness and sensitivity of the test organisms.

SETAC Society of Environmental Toxicology and Chemistry

Significant Industrial User (SIU) This includes, except as provided in paragraph 3. of this definition:

1. All industrial users subject to Categorical Pretreatment Standards under 9 VAC 25-31-780 and incorporated by reference in 9 VAC 25-31-30; and

2. Any other industrial user that:

   - discharges an average of 25,000 gallons per day or more of process wastewater to the POTW (excluding sanitary, noncontact cooling and boiler blowdown wastewater);

   - contributes a process wastestream which makes up 5 percent or more of the average dry weather hydraulic or organic capacity of the POTW treatment plant;

   - or is designated as such by the Control Authority (DEQ), as defined in 9 VAC 25-31-840A, on the basis that the industrial user has a reasonable potential for adversely affecting the POTW's operation or for violating any pretreatment standard or requirement.

3. Upon a finding that an industrial user meeting the criteria in paragraph 2. of this definition has no reasonable potential for adversely affecting the POTW's operation or for violating any pretreatment standard or requirement, the control authority may at any time, on its own initiative or in response to a petition received from an industrial user or POTW, and in accordance with Part VII (9 VAC 25-31-730 et seq.) of this regulation, determine that such industrial user is not a significant industrial user.

Stormwater Flows which are from conveyances or systems of conveyances

used for collecting and conveying precipitation runoff and which are not contaminated by contact with, or do not come into contact with any raw material, intermediate products, finished products, byproduct, or waste products located on the site of such operations.

Test Acceptability Criteria (TAC) In order that toxicity test results be considered acceptable, the effluent and the reference toxicant must meet specific criteria as defined in the test method (e.g., for the chronic *Ceriodaphnia dubia* survival and reproduction test, the criteria are as follows: the test must achieve at least 80 percent survival and an average of 15 young per surviving female in the controls).

TMDL Total Maximum Daily Load is the sum of the individual wasteload allocations and load allocations. A margin of safety is included with the two types of allocations so that any additional loading, regardless of source, would not produce a violation of water quality standards.

Toxicity The inherent potential or capacity of a material to cause adverse effects in a living organism, including acute or chronic effects to aquatic life, bioaccumulation of pollutants in the tissues of aquatic organisms at levels which result in potential harm to the organism or pose a risk to organisms in the food chain, or detrimental effects on human health or other adverse environmental effects.

TSD EPA's Technical Support Document for Water Quality-based Toxics Control (March 1991, EPA505/2-90-001)

TU Toxic Unit (TU_a = acute toxic unit, TU_c = chronic toxic unit)

VPDES Permit Virginia Pollutant Discharge Elimination System permit - A document issued by the Board, pursuant to the regulation 9 VAC 25-31-10 et Seq, authorizing, under prescribed conditions the potential or actual discharge of pollutants from a point source to surface waters and the use or disposal of sewage sludge. Under the approved state program, a VPDES permit is equivalent to an NPDES permit.

WET Whole Effluent Toxicity – The aggregate toxic effect of an effluent measured directly by an aquatic toxicity test (CFR 122.2)

WLA Wasteload Allocation is the portion of a receiving water's total maximum daily load that is allocated to one of its existing or future point sources of pollution.

IV. Applicability Criteria for a Facility to Perform Aquatic Toxicity Tests

All permit applications should be carefully reviewed to see if there is "reasonable potential for toxicity" from the discharger. The following criteria have been developed for industries and domestic dischargers to see if they should be included in the Toxics Management Program:

1. Industrial Facilities

A. Any industry that falls into one of the Standard Industrial Classification (SIC) codes identified in Appendix A.

B. Any industry with an IWC (Instream Waste Concentration) greater than or equal to 33%.

C. Any other discharge that is deemed to have the potential for toxicity or instream impact based on an evaluation of manufacturing processes, indirect discharges, treatment processes, effluent or receiving stream data, or other relevant information.

The following is a partial list of possible candidates for Criterion C. These wastewater sources have a history of containing components that are toxic to aquatic organisms. For this reason, effluents containing these sources should be scrutinized for toxicity.

1) Bulk Oil Storage Facilities (including hydrostatic testing of storage tanks).

2) Water Treatment Plants

3) Tunnels (Assess cleaning operations for toxicity)

4) Certain Coal Mining Operations (e.g., Vertical Ventilation Hole wastewater, and those which contain emulsion oils). Coal pile runoff should be monitored with toxicity testing included the BMPs.

5) Water Conditioning Facilities (e.g., Carbon/Resin re-generation)

6) Facilities that may not actually discharge process water but due to the nature of the facility, the storm water may be contaminated from different aspects of the operation. A prime example includes a foundry operation where dust from the smokestacks settles on the

property and is contained in holding ponds.

7) Heating/Cooling Compressor wastewater

8) Boiler blowdown/Steam Condensate

9) Wastewater treated through an oil/water separator

10) Effluents with significant concentrations of degreasers

11) Noncontact cooling water discharges with an IWC less than 1% but which are treated with chemical additives.

Discharges that **may be excluded** from the toxics program include:

a. Discharges of storm water in which the storm water does not come into contact with raw materials intermediates, by-products, finished products, or waste materials; and

b. Discharges of noncontact cooling waters with instream waste concentrations of less than 1% and which are not treated with chemical additives.

c. Pump-outs of non-contaminated ground water (and pump-outs of petroleum contaminated ground water which receive appropriate treatment and where BTEX limits are applied).

d. Hydrostatic tests at petroleum pipeline pump stations (excluding bulk oil storage facilities) if the permit is drafted in accordance with OWPS guidance.

e. Corrective Action Plan (CAP) permits which involve discharges to surface waters.

2. Municipal/Domestic Facilities[1]

A. All POTW's and any other facility permitted as a major municipal facility, with design flow rates ≥1.0 MGD

B. All POTW's:

1. The criteria and data submission requirements for municipal/domestic facilities were published in the Federal Register, Vol. 64, No. 149, Page 42465, Wednesday, August 4, 1999. Virginia DEQ is in the process of review with EPA to approve a revision to the VPDES Permit Regulation (9 VAC 25-31-10 et seq.) that would include these applicability criteria. The current citation in the draft is 9 VAC 25-31-100 J.5.a through j.

- **with approved pretreatment programs or**
- **required to develop a pretreatment program**

C. Other POTW's, as required by the Board, based on consideration of the following factors:

1.) The variability of the pollutants or pollutant parameters in the POTW effluent (based on chemical-specific information, the type of treatment plant, and types of industrial contributors);

2.) The ratio of effluent flow to the receiving stream flow;

3.) Existing controls on point or non-point sources, including total maximum daily load (TMDL) calculations for the receiving stream segment and the relative contribution of the POTW;

4.) Receiving stream characteristics, including possible or known water quality impairment, and whether the POTW discharges to a coastal water, or a water designated as an outstanding natural resource water; or

5.) Other considerations (including, but not limited to, the history of toxic impacts and compliance problems at the POTW) that the Board determines could cause or contribute to adverse water quality impacts.

V. Test types and when to apply them

There are two types of aquatic toxicity tests that are used to determine the Whole Effluent Toxicity of a discharge: acute tests and chronic tests. Acute tests are used to determine survival, whereas chronic tests are used to determine not only survival, but also reproduction, growth, and with mysids, fecundity.

ACUTE TOXICITY TESTS: All facilities that meet the applicability criteria of Section IV. to be placed in the Toxics Management Program will need to be assessed for acute toxicity. The LC_{50} test and the NOAEC test are used for this determination.

LC_{50} This test statistically estimates the concentration of the sample that is lethal to 50% of the organisms tested. It can be run as a 48-hour static test, or a 96-hour static renewal test. A minimum of 5 concentrations of the sample is set up in a geometrically derived dilution series (usually a 0.5 dilution series), along with

controls. The dilution series may have to add concentrations at the lower end of the series in order to achieve a calculable LC_{50} at test conclusion. Chemical parameters are measured and recorded daily. The test requirements and guidelines can be found in Appendix B. The LC_{50} is divided into 100 to get TU_a. The TU_a values can be used with the WLA_a if only acute toxicity is of concern. For use in the WLA program when both acute and chronic data are available, the LC_{50} must be converted to TU_a, and then to TU_c by the following formula: $100/LC_{50}$ X ACR (Acute to Chronic Ratio). If WLA determines that an acute limit or monitoring endpoint is needed, the TU_c from WLA.EXE should be converted back to TU_a by dividing it by the ACR and then dividing it into 100.

For example: LC_{50} = 40% ACR = 5

100/40 = 2.5 TU_a
2.5 X 5 = 12.5 TU_c

If WLA determines needing an acute limit based on 10 TU_c,
then 100/(10/5) = LC_{50} 50%

If the resulting LC_{50} includes a decimal point, it should be rounded 'up' to the nearest whole number.

NOAEC This test is recommended when the acute IWC is greater than 33%. It will determine the highest effluent concentration that is not significantly different from the control (No Observed Adverse Effect Concentration) and is expressed as NOAEC = 100% or NOAEC = the highest percent concentration where there was no significant difference when compared to the controls. (Note: This is interpreted as the highest percent concentration where there is no significant difference when compared to the controls, and below which there is no statistically significant adverse effect.) The test can be run as a single dilution (with replicates), usually 100% effluent and controls, or as a multi-dilution test, for 48 hours in duration. The multi-dilution test is set up and monitored the same as an LC_{50} test with the results calculated more like a chronic test. The LC_{50} can also be calculated from the mutidilutional test for use in WLA.EXE or to calculate a CV. The NOAEC result can be converted to TU_a by 100/NOAEC. Test requirements and guidelines can be found in Appendix B.

The rationale to use the NOAEC test for IWC_a's greater than 33% instead of the LC_{50} test is because of the 0.3 CMC which is to be met at end-of-pipe. The factor of 0.3 in the CMC is used to adjust the typical LC_{50} point estimate (50% mortality) from an acute toxicity test to an LC_1 (virtually no mortality). The conversion of 0.3 TU_a to its equivalent LC_{50} value is shown below:

$100/0.3\ TU_a = LC_{50}\ 333.333\%$

The endpoint of 333.333% effluent is impossible to test. The highest dilution of effluent that can be tested is 100%, which if using the LC_{50} test, could allow for up to 50% of the organisms to die and still meet that endpoint. This is not protective of the acute criterion of "no discharge of toxic chemicals in toxic amounts". The LC_1 test is not practical in that no mortality is allowed to the test organisms; yet, up to 10% mortality is allowed for by the control organisms for an acceptable test. The TSD (page 35) states that the 0.3 factor was found to include 91 percent of observed LC_1 to LC_{50} ratios in 496 acute effluent tests. As a result, whenever there is a dilution ratio of less than approximately three parts receiving water to one part effluent (3:1), the resulting WLA will be lower than the minimum level of acute toxicity that the LC_{50} test can measure. The NOAEC test is more appropriate, in that it statistically determines whether the 100% effluent is significantly different than the controls.

The following organisms are used in the acute tests required of Virginia permittees:

FRESHWATER			
Organism Name*	**Common Name**	**Organism Type**	**Test Type and Duration**
Ceriodaphnia dubia	Water flea, daphnid	Invertebrate	48 Hr Static Acute - LC_{50} 48 Hr Static Acute - NOAEC
Oncorhynchus mykiss	Rainbow trout	Vertebrate	48 Hr Static Acute - LC_{50} 96 Hr Static Renewal Acute - LC_{50} 48 Hr Static Acute - NOAEC
Pimephales promelas	Fathead minnow	Vertebrate	48 Hr Static Acute - LC_{50} 96 Hr Static Renewal Acute - LC_{50} 48 Hr Static Acute - NOAEC
SALTWATER			
Mysidopsis bahia	Opossum shrimp	Invertebrate	48 Hr Static Acute - LC_{50} 96 Hr Static Renewal Acute - LC_{50} 48 Hr Static Acute - NOAEC
Cyprinodon variegatus	Sheepshead minnow	Vertebrate	48 Hr Static Acute - LC_{50} 96 Hr Static Renewal Acute - LC_{50} 48 Hr Static Acute - NOAEC

* Note that the name of the organism should be italicized, or have the genus and species underlined separately. (Example: *Ceriodaphnia dubia* or Ceriodaphnia dubia)

If the need for acute monitoring has been determined for a discharger that makes up 33% or less of the instream waste concentration, both a vertebrate and an invertebrate species should be used with 48-hour static acute LC_{50} tests. For a discharger who has an IWC of greater than 33%, the NOAEC tests should be used with both a vertebrate and an invertebrate species. The multi-dilution NOAEC test should be used if monitoring is required; either the single dilution (100% effluent and the controls) or multi-dilution NOAEC test can be used for a limitation.

CHRONIC TOXICITY TESTS: A facility should monitor for chronic toxicity if the chronic instream waste concentration (IWC_c) is greater than or equal to 1%, and the discharge is continuous. IWC_c's less than 1% should present little to no effects for chronic toxicity. A "continuous discharge" is defined as a discharge that occurs without interruption throughout the operating hours of the facility, except for infrequent shutdowns for maintenance, process changes, or other similar activities. A

chronic test is performed with a minimum of 5 effluent dilutions and the controls for a duration of 6-8 days. The statistics compare each dilution to the controls to see if there is a significant difference. Chronic tests determine survival, as well as growth, reproduction or fecundity.

Chronic toxicity testing is not required for outfalls which are either intermittent in nature, **(for the purposes of this guidance, intermittent is defined as having a continuous discharge for less than five consecutive days)**, or those continuous discharges with an IWC of less than 1%. This exemption from chronic testing is due to the short duration of the discharge that reduces exposure time of the toxicants to the organisms in the receiving stream. Consequently, with reduced exposure time to toxicants there is less chance that the instream biota are being chronically affected. For discharges that comprise less than 1% of the receiving stream, the effluent receives enough dilution such that chronic toxicity should not occur instream.

NOEC No Observed Effect Concentration - The highest concentration of toxicant to which organisms are exposed in which the values for the observed responses are not statistically different from the controls, and below which there is no statistically significant adverse effect.

IC_{25} The linear interpolation method is used to calculate a point estimate, called the inhibition concentration (IC), of a toxicant that causes a given percent reduction (e.g., 25%, 50%) in effect as compared to the controls.

The following tests and organisms are used for chronic toxicity determinations for Virginia permittees:

Freshwater Tests

Invertebrate - Chronic Static Renewal 3-Brood Survival and Reproduction Test with *Ceriodaphnia dubia*
Vertebrate - Chronic Static Renewal 7-Day Survival and Growth Test with *Pimephales promelas*

Saltwater Tests

Invertebrate - Chronic Static Renewal 7-Day Survival, Growth and Fecundity Test with *Mysidopsis bahia*
Vertebrate - Chronic Static Renewal 7-Day Survival and Growth Test with *Cyprinodon variegatus*

It is recommended that if chronic testing is to be done on an effluent where the IWC_c is $\geq$1%, that both the invertebrate and vertebrate are tested. For municipalities, this is required by

the published rule[2]. For a facility which discharges into trout waters where the *Oncorhynchus mykiss* static acute test is warranted, the chronic test with *Pimephales promelas* should be used. There is no acceptable chronic test method for *O. mykiss.*

The NOEC (No Observed Effect Concentration) is determined by statistics, following the flow chart in the EPA guidance manual referenced in Appendix B. **Additionally, the testing laboratory should report the LC_{50} at 48 hours and the IC_{25} at the conclusion of the test.** While the IC_{25} endpoint is not used for compliance purposes, it may be used for calculation of an effluent specific CV (coefficient of variation) or ACR, which will effect the NOEC or TU_c permit endpoint.

To date, we have encouraged the use of a dilution series for chronic testing which included the monitoring endpoint as a mid-range dilution. If we were to continue with that approach, tests that resulted in an NOEC of the endpoint (or slightly less) would cause the WLA.EXE program to indicate a limit is needed. It would be advantageous to the permittee to use a dilution series that incorporates the TU_c value (converted to an NOEC) representing the mean of the data entered into WLA.EXE that is just below (by about 0.000001 TU_c) the "triggering" point. Tests resulting in that dilution or one of the two NOEC's above it would be less likely to cause the program to require a limit. This "mean value" appears in the box for permit limits in the top right hand side of the WETLIM10.xls spreadsheet in purple, with a recommended dilution series calculated for you in Table 4. on page 3 of the spreadsheet.

EXAMPLE:

Chronic MDL	5.850298736	TU_c	=	NOEC 18%	(Rounded from 17.09%)
Mean of the data	2.40414909	TU_c	=	NOEC 41.6%	

Dilution series for monitoring would be:	100%	64.5%	41.6%	26.8%	17.2%	Controls.
Dilution series written as TU_c:	1.00	1.55	2.40	3.73	5.78	Controls

For compliance with a WET limit, the dilutions selected for use in the chronic test should include the limit. The dilution series can be determined by using the chart or calculation in Appendix C, or Table 4. on WETLIM10.xls. Using the Example above:

Chronic MDL	5.850298736	TU_c	=	NOEC 18%	=	5.55 TU_c

Dilution series for compliance testing:	100%	42.4%	18%	7.6%	3.2%	Controls
Dilution series written as TU_c:	1.00	2.36	5.56	13.09	30.86	Controls

The TU_c is determined (by WETLIM10) which is then converted to an NOEC of 17.085292. This is an unrealistic endpoint for a laboratory to achieve, so the NOEC needs to be rounded to something that is feasible. The NOEC is rounded 'up' to a whole number (18), and

2 The published rule requires data to be submitted with the application of a municipal facility. A minimum of two species is required to have been tested for a minimum of four test periods. It would be advantageous for the permittee to provide additional testing for evaluation.

then the corresponding TU_c is recalculated to 5.55 TU_c. If the NOEC were to be rounded "down" to 17%, the resulting TU_c would back-calculate to 5.8823529 TU_c. Tests resulting in this endpoint would exceed the MDL, and would be non-compliant despite a calculated difference of only 0.0293656. Therefore, the WETLIM10.xls spreadsheet compensates by adding in a factor of 0.5 prior to rounding the NOEC 'up'.

VI. Programs and Spreadsheets for Endpoint/Limit Determinations

Once a facility is placed into the Toxics Management Program based on the applicability criteria, we need to determine the test endpoints to be met so an assessment of "reasonable potential" can be made. This approach uses existing effluent data (where available) to project an estimated maximum pollutant concentration in the effluent. The projected maximum concentration is used as an input to a water quality model to determine whether the effluent has the reasonable potential to cause or contribute to an excursion of ambient water quality criteria. If there is "reasonable potential" the permit writer must derive a permit limit for the parameter of concern for that facility.

Previously, we used end-of-pipe for the acute test endpoint, which was to test to an endpoint of $LC_{50} \geq 100\%$. No dilution was allowed to assess for acute toxicity. The chronic test used the IWC_c as the monitoring endpoint, determined by taking the facility flow, and dividing it by the facility flow added to the 7Q10 of the receiving stream. The criterion was for 75% of the tests (acute or chronic) to meet the endpoint, which (by regulation) indicated that there was "no reasonable potential" for toxicity. If reasonable potential was determined by more than 25% of the tests failing to meet the endpoint, then a facility was required to perform a Toxicity Reduction Evaluation (TRE). A TRE plan was required to be submitted performed and completed during an established compliance period. At the conclusion of the TRE, the facility had to meet a limit calculated by following the guidance for the Toxics Management Program, or by using previous versions of the WET limit spreadsheet. The calculation allowed for effluent variability, laboratory variability, and test variability. Because of the allowance of variability, the calculated limit was rarely the same as the monitoring endpoint; in most cases, it was less stringent. However, the limit was to be met all of the time.

Monitoring endpoints should be developed by the same rationale as limitations, with the same allowances for variability inherent to limits. Using the following "tools", the guidance will recommend how best to achieve that:

MIX.EXE	This program is used to determine the percentage of the receiving stream flow that can be used for complete mix with the effluent.
WETLIM10.xls	This is an Excel spreadsheet which will calculate the WLA_a and WLA_c, MDLs, and AML. It also will calculate a site specific Coefficient of Variation (CV) and Acute to Chronic Ratio (ACR) with sufficient usable data. The percentage of 1Q10 and 7Q10 flows for complete mix are needed from MIX.EXE.

WLA.EXE — This program needs to have at least 1 data point, the acute WLA ($WLA_{a,c}$) and the chronic WLA (WLA_c) to calculate whether a limit is needed or not, based on the 97th percentile probability.

VII. VPDES Permitting

1. New Discharger – Applicability and Test Requirements

The application should contain the information you need to see if the facility should be assessed for having "reasonable potential for toxicity" and be included in the Toxics Management Program:

(IV.1.A.) If the facility is an industry, check the SIC code(s) against the list in Appendix A. The first column of SIC codes are primary industries which should be considered for the TMP. The second column contains SIC codes of facilities who are in the TMP database (list is current with the date of the document). Queries can be made of the TMP database to see how facilities with the same (or similar) SIC code have done with their monitoring.

(IV.1.B.) If the facility is an industry, use the 30-day maximum flow and the 1Q10 to calculate the IWC. If the IWC_a is greater than or equal to 33%, there may be potential for toxicity due to lack of dilution.

(IV.1.C.) Any other discharge that is deemed to have the potential for toxicity or instream impact should be monitored for toxicity. Chemical monitoring can be compared to aquatic toxicity data in the EPA database AQUIRE (WEB address is http://www.epa.gov/ecotox), or with the water quality standards. Consider the site inspection write-up to see if the discharger is environmentally responsible in terms of preventing pollutants from entering the waterways.

Contaminated stormwater discharges should be addressed through BMP's and the Stormwater Pollution Prevention Plan. Acute toxicity testing should be included and used to show the benefits of actions taken.

(IV.2.A.) If the facility is a municipality with a design flow greater than or equal to 1.0 MGD (major), it should have toxics monitoring in the permit.

(IV.2.B.) If the municipal plant has a pretreatment program or is required to develop a pretreatment program, it should perform toxics monitoring. Note the industrial contributors (SIU's, Primary Industries, etc.) to the plant and what percentage of the influent

flow they comprise. It is also useful to note the hours of operation for the industries, so as to gauge optimal times for sampling.

(IV.2.C.) If a municipality does not fall into either of the two criteria above, it may be considered to meet this criterion. There are several factors listed (i.e., variability of effluent, IWC, controls on contributors, TMDL for receiving stream, history of impacts and compliance problems) that would merit a special condition for aquatic toxicity testing to determine reasonable potential.

The next step is to use the WETLIM10.xls spreadsheet (or the calculations can be performed manually by using the formulae in Appendix D) to determine the test endpoints for compliance, and the acute and chronic WLA's. Fill in the facility flow (for municipal facilities, use the design flow; for industrial facilities, use the 30 day max flow), the 1Q10 and 7Q10. If MIX.EXE has been run, enter the percentage of the flows than can be used next to the flows on the spreadsheet. Facilities that have performed a mixing zone study or have a diffuser should have acute and chronic mixing ratios.[3,4] Enter that information on the spreadsheet. The acute and chronic WLA's, and the acute and chronic endpoints/limits will appear in the box (will show up outlined in green on screen) at the top of page 1.

All facilities that meet any of the TMP applicability criteria will need to be assessed for acute toxicity. If the IWC_a is less than or equal to 33%, the 48-hour static LC_{50} test is appropriate. The multi-dilution 48-hour NOAEC test should be used if the IWC_a is greater than 33%. Chronic testing should be performed if the IWC_c is greater than or equal to 1%.

The request has been made, on occasion, to allow for acute data to be reported from the chronic test data when a permittee has a requirement for both types of tests. Up till now, we have not allowed for that option. The acute tests are performed at a different temperature than the chronic tests ($20\pm1^{\circ}$ vs. $25\pm1^{\circ}$ or $26\pm1^{\circ}$), the test solutions are handled differently (static vs. static renewal), the number of tested organisms, feeding versus not feeding, and the age of the organisms are different. In order to allow for an acute endpoint determination from the chronic test data, it is important that the minimum requirements of the acute test are met. The significance of these differences was evaluated, and the following concessions have been made:

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3. For discharges into tidal estuaries, estuarine embayments, or the open ocean, specific data on waste dispersion or dilution will be applied when available and where appropriate. Where waste dispersion/dilution data are not available, a chronic dilution ratio of 50:1 (IWC = 2%) should be applied for comparisons with chronic toxicity data. Results from any permittee conducted dilution study should be submitted to, and approved by OWPP prior to implementation.
4. For all discharges into lakes, marshes or swamps, the chronic IWC (or dilution ratio) should be 100% (1:1) unless data exist that defines the actual mixing zone.

In the event both acute and chronic toxicity testing are warranted, the permittee may request to perform only chronic testing for the vertebrate species (*Pimephales promelas* or *Cyprinodon variegatus)* to ease their financial burden. This is acceptable, as long as the NOAEC or LC_{50} (whichever endpoint is in the permit) at 48 hours is calculated and the percent survival at each dilution is determined from the chronic test data and submitted with the test report. The organisms used in these chronic tests are younger and more sensitive to toxicity than those at the ages specified for the acute tests; therefore, the acute test results calculated from the chronic tests may be more representative of potential toxicity.

This allowance could not be made for the chronic tests using the invertebrates because minimum test requirements were not met. The chronic test using *Ceriodaphnia dubia* only has 10 replicates, which is insufficient to evaluate statistically for acute toxicity. The chronic test with *Mysidopsis bahia* uses 7 day old organisms instead of the 1-5 day old organisms (all within 24 hours in age of each other) used in the acute test.

If a decision is made to allow the acute data to be calculated from the chronic data, it must be put into the permit to require the chronic test with endpoints calculated for NOEC, IC_{25}, and the LC_{50} at 48 hours. The testing laboratory should provide a bench sheet with the replicate survival used for the acute statistics.

Data can be requested and submitted during the application process for a new minor facility that does not fully meet the applicability criteria, but may have reasonable potential for toxicity. Both species (vertebrate and invertebrate) should be required for acute and/or chronic tests. WETLIM10.xls can calculate the test endpoints and WLA's; the data can then be evaluated by WLA.EXE to see if more monitoring or a limit is needed.

2. Data to be supplied with the application for Municipalities

EPA published a final rule in the Federal Register, Volume 64, No. 149, Page 42465, on Wednesday, August 4, 1999. The rule outlined the specific WET test requirements to be met for municipalities when submitting an application for a permit (40 CFR 122.21(j),1999). The entire document can be found at:

http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=1999_register&docid=99-18866-filed

The forms used to supply the information are found at:
http://www.epa.gov/owm/npdes.htm#forms

The WET testing methods are cited in the Federal Register, October 16, 1995, Volume

60, Number 199, Pages 53529-53544, and at the site below:

http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=1995_register&docid=fr16oc95-10

Technical corrections to the document above can be found in the Federal Register, February 2, 1999, Volume 64, number 21, Pages 4975-4978, and at the site below:

http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=1999_register&docid=fr02fe99-7

EPA has included the following facility types (TWTDS, or "treatment works treating domestic sewage") as regulated entities under this rule:

Category	Examples of regulated entities
Local Government	Publicly Owned Treatment Works, owners and operators of treatment works treating domestic sewage
Private	Privately owned treatment works or other treatment works treating domestic sewage
State Government	Treatment works owned or operated by States and Tribes
Federal Government	Federally owned treatment works

The text of this section has been modified from the FR version to reflect the language in the VPDES Permit Regulation. The numbering is the same as in 9 VAC 25-31-100 J.5.a through j. so that referencing sections can be consistent.

5. *Effluent monitoring for whole effluent toxicity*

   a. *All applicants must provide an identification of any whole effluent toxicity tests conducted during the four and one-half years prior to the date of the application on any of the applicant's discharges or on any receiving water near the discharge.*

   b. *As provided in paragraphs J.5.c. through i. of this section, the following applicants must submit to the Department the results of valid whole effluent toxicity tests for acute or chronic toxicity for samples taken from each outfall through which effluent is discharged to surface waters, except for combined sewer overflows:*

      (1) All POTW's with design flow rates greater than or equal to one million gallons per day.

      (2) All POTW's with approved pretreatment programs or required to develop a pretreatment program

      (3) Other POTW's, as required by the Board, based on consideration of the following factors:

         (a) The variability of the pollutants or pollutant parameters in the POTW effluent (based on chemical–specific information, the type of treatment plant, and types of industrial contributors);

         (b) The ratio of effluent flow to the receiving stream flow

(c) Existing controls on point or non-point sources, including total maximum daily load (TMDL) calculations for the receiving stream segment and the relative contribution of the POTW;

(d) Receiving stream characteristics, including possible or known water quality impairment, and whether the POTW discharges to a coastal water, or a water designated as an outstanding natural resource water; or

(e) Other considerations (including, but not limited to, the history of toxic impacts and compliance problems at the POTW) that the Board determines could cause or contribute to adverse water quality impacts.

c. Where the POTW has two or more outfalls with substantially identical effluent discharging to the same receiving stream segment, the Board may allow applicants to submit whole effluent toxicity data for only one outfall on a case-by-case basis. The Board may also allow applicants to composite samples from one or more outfalls that discharge into the same mixing zone.

d. Each applicant required to perform whole effluent toxicity testing pursuant to paragraph J.5.b. (Applicability) of this section must provide:

(1) Results of a minimum of four quarterly tests for a year, from the year preceding the permit application; or

(2) Results from four tests performed at least annually in the four and one half year period prior to the application, provided the results show no appreciable toxicity using a safety factor determined by the Board.

e. Applicants must conduct tests with multiple species (no less than two species; e.g., fish, invertebrate, plant), and test for acute and chronic toxicity, depending on the range of receiving water dilution. All applicants conduct acute testing and applicants must conduct chronic testing if the dilution of the effluent is less than 100:1 at the edge of the mixing zone.

f. Each applicant required to perform whole effluent toxicity testing pursuant to paragraph J.5.b. (Applicability) of this section must provide the number of chronic or acute whole effluent toxicity tests that have been conducted since the last permit reissuance.

g. Applicants must provide the results using the form provided by the Department, or test summaries if available and comprehensive, for each whole effluent toxicity test conducted pursuant to paragraph J.5.b. (Applicability) of this section for which such information has not been reported previously to the Department.

h. Whole effluent toxicity testing conducted pursuant to paragraph J.5.b. of this section must be conducted using methods approved under 40 CFR part 136 (1999) (Biomonitoring), as directed by the Board.

i. For whole effluent toxicity data submitted to the Department within four and one-half years prior to the date of the application, applicants must provide the dates on which the data were submitted and a summary of the results.

j. Each POTW required to perform whole effluent toxicity testing pursuant to paragraph J.5.b. (Applicability) of this section must provide any information on the cause of toxicity and written details of any toxicity reduction evaluation conducted, if any whole effluent toxicity test conducted within the past four and one-half years revealed toxicity.

In summary, every new POTW (J.5.d.(1)) that meets the applicability criteria and every POTW preparing for permit reissuance (J.5.d.(2)) will have to perform (or have already performed during the previous permit term) and submit a minimum of four (4) valid tests using a vertebrate and four (4) tests using an invertebrate prior to submitting an application for a VPDES permit.

If the permit writer has the flow information (design flow, 7Q10 and 1Q10) and the information necessary to run MIX.EXE, it should be used in the WETLIM10 spreadsheet to derive endpoints to use for the tests (particularly the chronic test). The test types and organisms to use should be specified for the permittee, as well as recommended time frames in which to conduct the tests. At a minimum, provide the permittee with Appendix B of this guidance to aid with biological test requirements.

In the past, DEQ has allowed the permittee who had successfully completed their quarterly toxicity testing requirements to perform annual toxicity testing using the species most sensitive to their effluent. Hence, many permittees may have only 4 or 5 annual tests from a permit cycle for a single species and test type, or, those who alternated between the vertebrate and invertebrate will only have 2 or 3 tests with each species. This puts the permittee into the position of having to do 4 more tests with the less sensitive species, submitting an incomplete application, or for DEQ to help them comply with the application requirements. Therefore, it is recommended that:

A. For the permittee who is performing annual testing with only one species (vertebrate or invertebrate) per test type (acute or acute/chronic), or the permittee who is alternating the use of the vertebrate and invertebrate for each test type, a waiver will have to be submitted to the regional office no less than 240 days from permit expiration. If granted, it is then submitted to the EPA Regional Administrator **no less than 210 days (7 months) prior to permit expiration**. The request must include the State's justification for granting the waiver. If the waiver is submitted in the proper time frame and EPA either approves the waiver or does not act on the waiver within 181 days prior to permit expiration, the permit application (based on the subject of the waiver) is considered "complete". If EPA disapproves the waiver, the permit application based on the waiver is not "complete". Note that a Regional Administrators' disapproval of the Board's proposed waiver does not constitute final Agency action, but does provide notice to the Board and permit applicant(s) that EPA may object to any Board-issued permit issued in the absence of the required information.

B. Reissued permits for POTW's which have been in annual test frequency should require testing (acute, or acute and chronic) using **both** the vertebrate and invertebrate instead of one organism per test type. Exceptions to this recommendation may be considered on a case by case basis if either of the following conditions is met:

1) The average percent survival in 100% effluent for <u>all</u> the acceptable acute tests during a permit term with a particular species is $\geq$ 90%, or

2) The average percent survival in 100% effluent for all of the acceptable chronic tests during a permit term with a particular species is $\geq$ 80% and the secondary endpoint for reproduction, growth, or fecundity is an NOEC=100%.

There should be no possibility for toxicity from tests with the evaluated species, so annual testing with the other tested species should be sufficient. **Even if both species are able to meet the criteria of B.1) or B.2) above, it is necessary for a POTW to perform annual compliance testing with at least one species.**

Once the test reports have been submitted, they should be reviewed by the permit writer for acceptability (Refer to Appendix B for test guidelines). **The permittee must be notified as soon as possible if any of the tests are deemed "unacceptable" so that retests can be performed. The application will be**

considered <u>incomplete</u> until the requirements of this Section 2. are met.

The data should then be entered into WETLIM10 to determine an ACR, the acute $WLA_{a,c}$ and chronic WLA_c, and to convert the data into TU_c's. If only acute data are available, enter the WLA_a into WLA.EXE and the data in TU_a 's. Enter the required information into WLA.EXE to see if monitoring should continue or a limitation is needed. Each species should be evaluated separately. Acute and chronic data may be used together, if available, since both have been converted to TU_c's. Species must be evaluated separately.

The permittee should also be made aware that more than the minimum of 4 tests per test type and species may be performed, and that it may be in their benefit to do so. Monitoring endpoints and limitations will be more accurate if an effluent specific ACR and CV can be developed instead of having to use the default values.

The following form can be used to indicate the test type and organism to use for the permittee along with a copy of Appendix B of this guidance.

FORM FOR WET REQUIREMENTS TO BE SUBMITTED FOR PERMIT ISSUANCE

I. Fresh Water Acute Test Types

☐ 48 Hour Static Acute LC_{50} Test with *Ceriodaphnia dubia* (Invertebrate)
☐ 48 Hour Static Definitive NOAEC Test with *Ceriodaphnia dubia* (Invertebrate)

☐ 48 Hour Static Acute LC_{50} Test with *Oncorhyncus mykiss* (Cold Water Vertebrate)
☐ 48 Hour Static Definitive NOAEC Test with *Oncorhyncus mykiss* (Cold Water Vertebrate)
☐ 96 Hour Static Renewal Acute LC_{50} Test with *Oncorhyncus mykiss* (Cold Water Vertebrate)

☐ 48 Hour Static Acute LC_{50} Test with *Pimephales promelas* (Vertebrate)
☐ 48 Hour Static Definitive NOAEC Test with *Pimephales promelas* (Vertebrate)
☐ 96 Hour Static Renewal Acute LC_{50} Test with *Pimephales promelas* (Vertebrate)

II. Fresh Water Chronic Test Types

☐ Chronic Static Renewal 3-Brood Survival and Reproduction Test with *Ceriodaphnia dubia* (Invertebrate)
☐ Chronic Static Renewal 7-Day Survival and Growth Test with *Pimephales promelas (Vertebrate)*

Saltwater Tests

III. Salt Water Acute Test Types

☐ 48 Hour Static Acute LC_{50} Test with *Mysidopsis bahia* (Invertebrate)
☐ 48 Hour Static Definitive NOAEC Test with *Mysidopsis bahia* (Invertebrate)

☐ 48 Hour Static Acute LC_{50} Test with *Cyprinodon variegatus* (Vertebrate)
☐ 48 Hour Static Definitive NOAEC Test with *Cyprinodon variegatus* (Vertebrate)
☐ 96 Hour Static Renewal Acute LC_{50} Test with *Cyprinodon variegatus* (Vertebrate)

IV. Salt Water Chronic Test Types

☐ Chronic Static Renewal 7-Day Survival, Growth and Fecundity Test with *Mysidopsis bahia*

☐ Chronic Static Renewal 7-Day Survival and Growth Test with *Cyprinodon variegatus*

"Less than" (<) NOEC results are not acceptable for chronic tests. A retest must be performed with lower dilutions. For additional test requirements, refer to APPENDIX B of this guidance, which references the EPA document below:

Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fourth Edition, EPA/600/4-90/027F, August 1993.

Short-term Methods to Estimate the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Third Edition, EPA/600/4-91/002, July 1994.

Short-term Methods to Estimate the Chronic Toxicity of Effluents and Receiving Waters to Estuarine and Marine Organisms, Second Edition, EPA/600/4-91/003, July 1994.

3. Other monitoring situations for municipalities

Municipal facilities often have to upgrade, expand, or retrofit so as to better handle the loading of their contributors. There are also situations where new industrial discharges will be added or existing industrial contributors may cease to discharge. In these situations, the characterization of the effluent will change and will need to be reassessed for toxicity.

As we have done in the past, quarterly monitoring should be put into the permit to be initiated after the changes to the plant have occurred. In general, there should be at least a six month period before testing should start after the plant has received a CTO for the plant modifications or an expansion has occurred so that operation of the facility will be stabilized. Only data from tests performed after the changes to the plant have occurred should be evaluated by WLA.EXE for the next permit cycle, as those data are most representative of the effluent. For that reason, it is not necessary for a facility to perform annual testing prior to those changes, if quarterly testing has been scheduled in the permit. The test schedule should take into account that the permittee will have to have a minimum of four tests for each species and test type to submit with the application for the next permit.

If a municipal facility experiences a change in the contributing industrial users, they should perform quarterly testing from that point, so that they will have a minimum of the four tests for each species and test type to submit with the application for the next permit.

4. Major industrial facilities

Industrial facilities may have a discharge that is not subject to much variability, or one that changes frequently. The application and inspection should offer information that will help establish test frequency. If the effluent is highly variable due to changes in process, chemical usage, etc. then biological testing should be performed on a monthly basis, using both organisms, for a minimum of 1 year. **(Note that for some industries, the test period may be extended based on the professional judgement of the permit writer.)** At that time, calculate the ACR, determine the $WLA_{a,c}$ and WLA_C, and evaluate the data using WLA.EXE. The effluent specific CV (coefficient of variation) will be calculated using real data, instead of the default value of 0.6 when more than 10 data points per species tested are entered. It can then be determined if a limit will be needed for the reissued permit. The permittee will have up to 4 years (or the duration of the current permit) to become compliant with the WET limit that will be placed in the next permit. The frequency of testing for a WET limit should be, at a minimum, quarterly; however, if effluent variability is still of concern, then more frequent testing may be warranted. The permit writer should specify the time periods in which testing should occur, and the dates for test submittal.

At a minimum, the monitoring frequency should be set at 4 times per year until testing has been performed during each month when evaluated cumulatively. For example, the first year of testing could be performed in January, April, July, and October; the second year would test in February, May, August, November; the third year would complete the evaluation by testing in March, June, September, and December. The data should be evaluated by WLA.EXE at the conclusion of the test period to see if a limit is needed for the next permit. If no limit is needed, annual monitoring with both organisms should be considered.[5]

If the facility undergoes any changes that would alter the effluent characterization, then a new evaluation (monthly or 4 times per year) will need to be performed.

5. Minor industrial facilities

There are some of the same concerns with minor industrial facilities as there are with major industrial facilities, so we need to collect sufficient data to maintain water quality. An additional consideration should be made if the effluent is discharged as a batch, or on irregular intervals. It is recommended that test frequency be set at 4 times per year until a minimum of 12 sets (vertebrate and invertebrate) of data are collected. The permit writer should set the test times, at specific intervals, during certain months, or with the onset of a discharge. The data should be evaluated at the conclusion of the testing period to see if a limit is needed for the next permit.

If, when the data are evaluated, it is determined that no limit is necessary, the permit write should use professional judgement to see if:

A. additional biological monitoring will be required in a reduced frequency, or

B. no further monitoring is necessary until permit application time, when acute, or acute and chronic tests should be performed with both the vertebrate and invertebrate, to be submitted with the application, or

C. no further monitoring is necessary.

Assuming there have been no process changes at the facility, the new data can be evaluated with the data previously submitted. If there have been process changes, retrofitting or expansion, then a new evaluation (4 times per year for 3

5 Refer to 2.B. in the section on municipal permitting for considerations for requiring only one species for testing. While EPA has not mandated using both organisms for industrial dischargers, they are concerned with effluent variability that can be discovered more readily by the use of multiple species.

years, or 12 sets of data over a 4 year period) will need to be performed.

If the facility has an intermittent or batch discharge, there should be an asterisk and notation on the DMR allowing them to report "No Discharge" instead of simply leaving the form blank.

6. Reporting monitoring results on the DMR

Monitoring results can now be listed and required on the DMR to allow for easier tracking by compliance auditors. Each test type should be listed separately, since there are separate codes for each one. It would be optimal for the TMP to contain a schedule with dates, so that those dates can be picked up in necessary fields of the CEDS database. The permittee should still be required to submit the complete test report, along with a data summary, bench sheets with raw data, and the statistical evaluation for review.

The permittee should review their data carefully prior to submission to DEQ to ensure that it will be acceptable. Unacceptable data should be retested so that acceptable data can be reported on the DMR. Data reported on the DMR for monitoring or a WET limit that are found to be unacceptable by the reviewer will subject the permittee to an issue of non-compliance.

It should also be recommended to the permittee that testing should be performed early in the testing time frame, so that if a retest is necessary, the data can be completed and reported in a timely fashion on the DMR. If they wait until the end of the testing period and then find out the test was not acceptable, then they will run into problems trying to get a retest and the data report in time for submission with the DMR.

7. Permit issuance prior to facility having a discharge

Occasionally a facility will be issued a permit prior to having a discharge commence. Obviously, no data can be submitted with the application for prescreening. The special condition should schedule toxicity testing to begin within 6 months of initiation of the discharge, or receipt of a CTO. That should give the permittee sufficient time to get their operation in order.

8. Toxicity Reduction Evaluations

In the past, a permittee who failed to pass the acceptability criteria would have to submit a TRE plan outlining exactly what was to be done to reduce toxicity. The plan and subsequent reports would be staff reviewed and comments would be made regarding the effectiveness of the plan. At the conclusion of the TRE, the permittee would perform confirmation testing to show that the TRE had

reduced or eliminated the toxicity.

The current recommendation is for DEQ to not be involved in the TRE process unless it is to provide the permittee with resources to perform the task, or we have been asked for assistance. **It is up to the permittee to reduce or eliminate toxicity to the point of compliance with the upcoming WET limit.** This recommendation is consistent with the way we handle chemical limitations. If a limit is required by WLA.EXE, the permit writer should determine a reasonable period of time to be a compliance period (not to exceed 4 years), and then notify the permittee of the calculated limit and effective date. The compliance period is when the TRE should be performed.

WET limits are to be handled in the same manner as chemical limits, in terms of exceedances.

9. Existing WET limits on Stormwater discharges

There are several facilities in the state that have TMP monitoring for contaminated stormwater, and some which have WET limits. The current philosophy of DEQ is not to put limitations on a stormwater discharge; so, the concern is what to do with the facilities that have limits.

A. If the facility has an effective WET limit, it has to stay in the permit to be in accordance with antibacksliding.

B. If a facility has a WET limit that is not yet effective, it can be removed from the permit. Monitoring should be placed in the permit to determine the effects and anticipated reduction in toxicity due to the BMP's done by the facility.

10. Existing permits with TMP's

Permits with TMP's should have the data assessed by the criteria stated in the permit. The criteria state that if more than 25% of the data do not meet the LC_{50} or NOEC endpoint listed in the permit, the permittee will be considered to have reasonable potential and must do a TRE and have a WET limit established.

APPENDIX A

SIC/NAICS CODES

APPENDIX A

Primary Industry SIC Codes	SIC Codes Currently in TMP Program	N.A.I.C.S.	Categories *abbreviation "pt" means "part of"
1011		21221	Iron Ore Mining
1021		212234	Copper Ore and Nickel Ore Mining (pt)
1031		212231	Lead Ore and Zinc Ore Mining
1041		212221	Gold Ore Mining
1044		212222	Silver Ore Mining
1061		212234	Copper Ore and Nickel Ore Mining (pt)
1094		212291	Uranium-Radium-Vanadium Mining
1099		212299	Other Metal Ore Mining (pt)
	1422	212312	Crushed and Broken Limestone Mining and Quarrying
	1479	212393	Other Chemical and Fertilizer Mineral Mining
	1499	212319	Other Crushed and Broken Stone Mining or Quarrying (pt)
	2011	311611	Animal (except poultry) Slaughtering (pt)
	2015	311615	Poultry Processing
	2016-Old Code Now 2015		Poultry Slaughtering and Processing
	2021	311512	Creamery Butter Manufacturing
	2033	311421	Fruit and Vegetable Canning (pt)
	2077	311613	Rendering and Meat By-product Processing
	2082	31212	Breweries
	2092	311711	Fresh and Frozen Seafood Processing
	2141	312221	Narrow Fabric and Other Smware Mills: Cotton. Wool, Silk, & Manmade Fiber
	2211	31321	Broadwoven Fabric Mills (pt)
	2231	313311	Broadwoven Fabric Finishing Mills - Wool Finishing
	2241	313221	Narrow Fabric Mills (pt)
	2253	315191	Outerwear Knitting Mills
	2261	313311	Broadwoven Fabric Finishing Mills (pt)
	2262	313311	Broadwoven Fabric Finishing Mills (pt)
	2272-Old Code Now 2273		
	2273	31411	Carpet and Rug Mills, Tufted
	2283 - Old Code Now 2284		Carpet and Rug Mills, Yarn

Primary Industry SIC Codes	SIC Codes Currently in TMP Program	N.A.I.C.S.	Categories *abbreviation "pt" means "part of"
2431	2431	321911	Wood Window and Door Manufacturing
2434		33711	Wood Kitchen Cabinet and Counter Top Manufacturing (pt)
2435		321211	Hardwood Veneer and Plywood Manufacturing
2436		321212	Softwood Veneer and Plywood Manufacturing
2491	2491	321114	Wood Preservation
	2492 - Old Code Now 2493		
2493	2493	321219	Reconstituted Wood Product Manufacturing
2499		339999	All Other Miscellaneous Manufacturing of Wood Products
	2511	337122	Wood Household Furniture (except Upholstered) Manufacturing (pt)
	2514	337124	Metal Household Furniture Manufacturing
2611	2611	32211	Pulp Mills
2621	2621	322121	Paper (except Newsprint) Mills (pt)
2631	2631	32213	Paperboard Mills
2652		322213	Setup Paperboard Box Manufacturing
2653		322211	Corrugated and Solid Fiber Boxes
2655		322214	Fiber Can, tube, Drum, and Similar Products Manufacturing
2656		322215	Non-folding Sanitary Food container Manufacturing (pt)
2657		322212	Folding Paperboard Box Manufacturing
2671		322221	Coated and Laminated Packaging Paper and Plastics Film Mfg
2672		322222	Coated and Laminated Paper Manufacturing (pt)
2673		322223	Plastics, Foil , and Coated Paper Bag Manufacturing
2674		322224	Uncoated Paper and Multiwall Bag Manufacturing
2675		32231	Die-Cut Paper and Paperboard Office Supplies Manufacturing (pt)
2676		322291	Sanitary Paper Products
2677		322232	Envelope Manufacturing
2678		322233	Stationery, Tablet, and Related Product Manufacturing
2679	2679	322215	Non-folding Sanitary Food Container Manufacturing
2711		51111	Newspaper Publishers
2721		51112	Periodical Publishers
2731		51113	Book Publishers
2732		323117	Book Printing
2741		51114	Database Publishing
2752		323114	Quick Printing
2754		323111	Commercial Gravure Printing (pt)

Primary Industry SIC Codes	SIC Codes Currently in TMP Program	N.A.I.C.S.	Categories *abbreviation "pt" means "part of"
2759		323113	Commercial Screen Printing
2761		323116	Manifold Business Form Printing
2771		323110	Commercial Lithographic Printing (pt)
2782		323110	Commercial Lithographic Printing (pt)
2796		323122	Prepress Services (pt)
2812		325181	Alkalis and Chlorine Manufacturing
2813	2813	32512	Industrial Gases Manufacturing
2816	2816	325131	Inorganic Dye and Pigment Manufacturing
2819	2819	325998	All Other Miscellaneous Chemical Product Manufacturing (pt)
	2821	325211	Plastics Material and Resin Manufacturing
2822		325212	Synthetic Rubber Manufacturing
	2824	325222	Noncellulosic Organic Fiber Manufacturing
2833	2833	325411	Medicinal and Botanical Manufacturing
2834		325412	Pharmaceutical Preparation Manufacturing (pt)
2835		325412	Pharmaceutical Preparation Manufacturing (pt)
2836		325414	Biological Product (except Diagnostic) Manufacturing
2841		325611	Soap and Other Detergent Manufacturing
	2844	32562	Toilet Preparation Manufacturing
2851	2851	32551	Paint and Coating Manufacturing (pt)
2861	2861	325191	Gum and Wood Chemical Manufacturing
2865		32511	Petrochemical Manufacturing
2869	2869	32511	Petrochemical Manufacturing
	2874	325312	Phosphate Fertilizer Manufacturing
	2875	325314	Fertilizer (Mixing Only) Manufacturing
2879		32532	Pesticide and Other Agricultural Chemical Manufacturing
2891		32552	Adhesive Manufacturing
2892	2892	32592	Explosive Manufacturing
2893		32591	Printing Ink Manufacturing
2895		325182	Carbon Black Manufacturing
2899	2899	32551	Paint and Coating Manufacturing (pt)
2911	2911	32411	Petroleum Refineries
2951	2951	324121	Asphalt Paving Mixtures and Block Manufacturing
2952		324122	Asphalt Shingle and Coating Materials Manufacturing
	2992	324191	Petroleum Lubricating Oil and Grease Manufacturing

Primary Industry SIC Codes	SIC Codes Currently in TMP Program	N.A.I.C.S.	Categories *abbreviation "pt" means "part of"
	2999	324199	All Other Petroleum and Coal Products Manufacturing (pt)
3011	3011	326211	Tire Manufacturing (except Retreading)
3021		315211	Rubber and Plastics Footwear Manufacturing
3052		32622	Rubber and Plastics Hoses and Belting Manufacturing
3053		339991	Gasket, Packing, and Sealing Device Manufacturing
3069	3069	31332	Fabric Coating Mills (pt)
	3079-Old Code		
	3081	326113	Unsupported Plastics Film and Sheet (except Packaging) Manufacturing
	3229	327212	Other Pressed and Blown Glass and Glassware Manufacturing
	3241	32731	Cement Manufacturing
	3274	32741	Lime Manufacturing
	3292	33634	Motor Vehicle Brake System Manufacturing (pt)
	3295	327992	Ground or Treated Mineral and Earth Manufacturing
3312	3312	324199	All Other Petroleum and Coal Products Manufacturing (pt)
3313		331112	Electometalurgical Ferroalloy Product Manufacturing
3315		331222	Steel Wire Drawing
3316		331221	Cold-rolled Steel Shape Manufacturing
3317		33121	Iron and Steel Pipes and Tubes Manufacturing from Purchased Steel
3321	3321	331511	Iron Foundries (pt)
3322		331511	Iron Foundries (pt)
3324		331512	Steel Investment Foundries
3325	3325	331513	Steel Foundries (except Investment)
3331		331411	Primary Smelting and Refining of Copper
3334		331312	Primary Aluminum Production
3339		331419	Primary Smelting and Refining of Nonferrous Metals (except Copper and Aluminum)
3341		331314	Secondary Smelting and Alloying of Aluminum (pt)
3351		331421	Copper Rolling, Drawing, and Extruding
3353	3353	331315	Aluminum Sheet, Plate, and Foil Manufacturing
3354		331316	Aluminum Extruded Product Manufacturing
3355		331319	Other Aluminum Rolling and Drawing, (pt)
3356		331491	Nonferrous Metal (except Copper and Aluminum) Rolling, Drawing, and Extruding (pt)
3357		331319	Other Aluminum Rolling and Drawing, (pt)
	3362 - Old Code		

Primary Industry SIC Codes	SIC Codes Currently in TMP Program	N.A.I.C.S.	Categories *abbreviation "pt" means "part of"
3363		331521	Aluminum Die-Castings
3364		331522	Nonferrous (except Aluminum) Die-Castings
3365		331524	Aluminum Foundries
3366		331525	Copper Foundries
3369		331528	Other Nonferrous Foundries
3398		332811	Metal Heat Treating
3399		331111	Iron and Steel Mills (pt) -Ferrous Powder, Paste, Flakes, etc.
3411		332431	Metal Can Manufacturing
3412		332439	Other Metal Container Manufacturing
3421	3421	332211	Cutlery and Flatware (except Precious) Manufacturing
3423		332212	Hand and Edge Tool Manufacturing
3425		332213	Saw Blade and Handsaw Manufacturing
3429		332439	Other Metal Container Manufacturing
3431		332998	Enameled Iron and Metal Sanitary Ware Manufacturing
3432		332913	Plumbing Fixture Fitting and Trim Manufacturing
3433		333414	Heating Equipment Manufacturing (except Electric and Warm Air Furnaces) (pt)
3441		332312	Fabricated Structural Metal Manufacturing
3442		332321	Metal Window and Door Manufacturing
3443	3443	332313	Plate Work Manufacturing
3444		332322	Sheet Metal Work Manufacturing
3446		332323	Ornamental and Architectural Metal Work Manufacturing
3448		332311	Prefabricated Metal Building and Component Manufacturing
3449		332114	Custom Roll Forming
3451		332721	Precision Turned Product Manufacturing
3452	3452	332722	Bolt, Nut, Screw, Rivet, and Washer Manufacturing
3462		332111	Iron and Steel Forging
3463		332112	Nonferrous Forging
3465		33637	Motor Vehicle Metal Stamping
3466		332115	Crown and Closure Manufacturing
3469		339911	Jewelry (except Costume) Manufacturing (pt)
	3471	332813	Electroplating, Plating, Polishing, Anodizing, and Coloring (pt)
3482		332992	Small Arms Ammunition Manufacturing
3483		332993	Ammunition (except Small Arms) Manufacturing
3484		332994	Small Arms Manufacturing

Primary Industry SIC Codes	SIC Codes Currently in TMP Program	N.A.I.C.S.	Categories *abbreviation "pt" means "part of"
3489		332995	Other Ordnance and Accessories Manufacturing
3493		332611	Steel Spring (except Wire) Manufacturing
3494		332999	All Other Miscellaneous Fabricated Metal Product Manufacturing (pt)
3496		332618	Other Fabricated Wire Product Manufacturing
3497		322225	Laminated Aluminum Foil Manufacturing for Flexible Packaging Uses
3498		332996	Fabricated Pipe and Pipe Fitting Manufacturing
3499	3499	337215	Showcase, Partition, Shelving, and Locker Manufacturing (pt)
3511		333611	Farm Machinery and Equipment Manufacture
3519		336399	All Other Motor Vehicle Parts Manufacturing (pt)
3523		333111	Farm Machinery and Equipment Manufacturing
3524		333112	Lawn and Garden Tractor and Home Lawn and Garden Equipment Manufacturing
3531		333923	Overhead Traveling Crane, Hoist and Monorail System Mfg
3532		333131	Mining Machinery and Equipment Manufacturing
3533		333132	Oil and Gas Field Machinery and Equipment Manufacturing
3534		333921	Elevator and Moving Stairway Manufacturing
3535		333922	Conveyor and Conveying Equipment Manufacturing
3536		333923	Overhead Traveling Crane, Hoist and Monorail System Mfg (pt)
3537		333924	Industrial Truck, Tractor, Trailer, and Stacker Machinery Mfg
3541		333512	Machine Tool (Metal Cutting Types) Manufacturing
3542		333513	Machine Tool (Metal Forming Types) Manufacturing
3544		333511	Industrial Mold Manufacturing
3545		333515	Cutting Tool and Machine Tool Accessory Manufacturing
3456		333991	Power-Driven Hand Tool Manufacturing (pt)
3547		333516	Rolling Mill Machinery and Equipment Manufacturing
3548		333992	Welding and Soldering Equipment Manufacturing
3549		333518	Other Metalworking Machinery Manufacturing
3552		333292	Textile Machinery Manufacturing
3553		33321	Sawmill and Woodworking Machinery Manufacturing
3554		333291	Paper Industry Machinery Manufacturing
3555		333293	Printing Machinery and Equipment Manufacturing
3556		333294	Food Product Machinery Manufacturing
3559		33322	Rubber and Plastics Industry Machinery Manufacturing
3561		333911	Pump and Pumping Equipment Manufacturing (pt)
3562		332991	Ball and Roller Bearing Manufacturing

Primary Industry SIC Codes	SIC Codes Currently in TMP Program	N.A.I.C.S.	Categories *abbreviation "pt" means "part of"
3563		333912	Air and Gas Compressor Manufacturing
3564		333411	Air Purification Equipment Manufacturing
3565		333993	Packaging Machinery Manufacturing
3566		333612	Speed Changer, Industrial High-Speed Drive, and Gear Mfg
3567		333994	Industrial Process Furnace and Oven Manufacturing
3568		333613	Mechanical Power Transmission Equipment Manufacturing
3569		333999	All Other General Purpose Machinery Manufacturing (pt)
3571		334111	Electronic Computer Manufacturing
3572		334112	Computer Storage Device Manufacturing
3577		334119	Other Computer Peripheral Equipment Manufacturing (pt)
3578		334119	Other Computer Peripheral Equipment Manufacturing (pt)
3579		339942	Lead Pencil and Art Good Manufacturing
3581		333311	Automatic Vending Machine Manufacturing
3582		333312	Commercial Laundry, Dry-cleaning, and Pressing Machine Mfg
3585	3585	336391	Motor Vehicle Air Conditioning Manufacturing
3886		333913	Measuring and Dispensing Pump Manufacturing
3592		336311	Carburetor, Piston, Piston Ring and Valve Manufacturing
3599		336399	All Other Motor Vehicle Part Manufacturing (pt)
3612		335311	Power, Distribution, and Specialty Transformer Manufacturing
3613	3613	335313	Switchgear and Switchboard Apparatus Manufacturing
3621		335312	Motor and Generator Manufacturing (pt)
3624		335991	Carbon and Graphite Product Manufacturing
3625		335314	Relay and Industrial Control Manufacturing
3629		335999	All Other Miscellaneous Electrical Equipment and Component Mfg (pt)
3631		335221	Household Cooking Appliance Manufacturing
3632		335222	Household Refrigerator and Home Freezer Manufacturing
3633		335224	Household Laundry Equipment Manufacturing
3634		335211	Electric Housewares and Household Fan Manufacturing
3635		335212	Household Vacuum Cleaner Manufacturing
3639		335212	Household Vacuum Cleaner Manufacturing
		333298	All Other Industrial Machinery Manufacturing
3641		33511	Electric Lamp Bulb and Part Manufacturing
3643		335931	Current-Carrying Wiring Device Manufacturing
3644		335932	Noncurrent-Carrying Wiring Device Manufacturing

Primary Industry SIC Codes	SIC Codes Currently in TMP Program	N.A.I.C.S.	Categories *abbreviation "pt" means "part of"
3645		335121	Residential Electric Lighting Fixture Manufacturing (pt)
3646		335122	Commercial Industrial, and Institutional Electric Lighting Fixture Mfg
3647		336321	Vehicular Lighting Equipment Manufacturing
3648		335129	Other Light Equipment Manufacturing
3651		33431	Audio and Video Equipment Manufacturing
3652		334612	Prerecorded Compact Disc (except Software), Tape, and Record Reproducing (pt)
3661		33421	Telephone Apparatus Manufacturing
3663		33422	Radio and Television Broadcasting and Wireless Communications Equipment Mfg (pt)
3671		334411	Electron Tube Manufacturing
3674		334413	Semiconductor and Related Device Manufacturing
3675		334414	Electronic Capacitor Manufacturing
3676		334415	Electronic Resistor Manufacturing
3677		334416	Electronic Coil, Transformer, and Other Inductor Manufacturing (pt)
3678		334417	Electronic Connector Manufacturing
3679		33422	Radio and Television Broadcasting and Wireless Communications Equipment Mfg (pt)
3691		335911	Storage Battery Manufacturing
3692		335912	Dry and Wet Primary Battery Manufacturing
3694		336322	Other Motor Vehicle Electrical and Electronic Equipment Mfg (pt)
3699		333319	Other Commercial and Service Industry Machinery Manufacturing (pt) - electronic teaching machines, flight simulators
3711		336111	Automobile Manufacturing
3713		336211	Motor Vehicle Body Manufacturing
3714	3714	336211	Motor Vehicle Body Manufacturing
3715		336212	Truck Trailer Manufacturing
3721		336411	Aircraft Manufacturing
3724		336412	Aircraft Engine and Engine Parts Manufacturing
3728		332912	Fluid Power Valve and Hose Fitting Manufacturing
3731	3731	336611	Ship Building and Repairing
3732		336612	Boat Building
3743		333911	Pump and Pumping Equipment Manufacturing
3751		336991	Motorcycle, Bicycle, and Parts Manufactures
3761		336414	Guided Missile and Space Vehicle Manufacturing

Primary Industry SIC Codes	SIC Codes Currently in TMP Program	N.A.I.C.S.	Categories *abbreviation "pt" means "part of"
3764	3764	336415	Guided Missile and Space Vehicle Propulsion Unit and Propulsion Unit Parts Manufacturing
3769		336419	Other Guided Missile and Space Vehicle Parts and Auxiliary Equipment Manufacturing
3792		336214	Travel Trailer and Camper Manufacturing (pt)
3795		336992	Military Armored Vehicle, Tank, and Tank Component Manufacturing
3799		336214	Travel Trailer and Camper Manufacturing (pt)
3812		334511	Search, Detection, Navigation, Guidance, Aeronautical, and Nautical System and Instrument Manufacturing
3821		339111	Laboratory Apparatus and Furniture Manufacturing
3822		334512	Automatic Environmental Control Manufacturing for Regulation Residential, Commercial, and Appliance Use
3823		334513	Instruments and Related Product Manufacturing for Measuring, Displaying, and Controlling Industrial Process Variables
3824		334514	Totalizing Fluid Meter and Counting Device Manufacturing
3825		334416	Electronic Coil, Transformer, and Other Inductor Manufacturing (pt)
3826		334516	Analytical Laboratory Instrument Manufacturing
3827		333314	Optical Instrument and Lens Manufacturing
3829		339112	Surgical and Medical Instrument Manufacturing (pt)
3841		339112	Surgical and Medical Instrument Manufacturing (pt)
3842		339113	Surgical Appliance and Supplies Manufacturing (pt)
3843		339114	Dental Equipment and Supplies Manufacturing
3844		334517	Irradiation Apparatus Manufacturing (pt)
3845		334517	Irradiation Apparatus Manufacturing (pt)
3851		339115	Ophthalmic Goods Manufacturing
3861		333315	Photographic and Photocopying Equipment Manufacturing
3873		334518	Watch, Clock, and Part Manufacturing (pt)
3911		339911	Jewelry (except Costume) Manufacturing (pt)
3914		339912	Silverware and Plated Ware Manufacturing (pt)
3915		339913	Jeweler's Material and Lapidary Work Manufacturing
3931		339992	Musical Instrument Manufacturing
3942		339931	Doll and Stuffed Toy Manufacturing
3944		336991	Motorcycle, Bicycle and Parts Manufacturing (pt)
3949		33992	Sporting and Athletic Good Manufacturing
3951		339941	Pen and Mechanical Pencil Manufacturing
3952		337127	Institutional Furniture Manufacturing (pt)

Primary Industry SIC Codes	SIC Codes Currently in TMP Program	N.A.I.C.S.	Categories *abbreviation "pt" means "part of"
3955		339944	Carbon Paper and Inked Ribbon Manufacturing
3961		339914	Costume Jewelry and Novelty Manufacturing
3991		339994	Broom, Brush and Mop Manufacturing
3993		33995	Sign Manufacturing
3995		339995	Burial Casket Manufacturing
3996		326192	Resilient Floor Covering Manufacturing
	4011	482111	Line-Haul Railroads
	4013	482112	Short Line Railroads
	4226	49312	Refrigerated Warehousing and Storage Facilities (pt)
	4231	48849	Other Support Activities for Road Transportation (pt)
	4463		Water Transportation
	4491	48831	Port and Harbor Operations (pt)
4911	4911	221111	Hydroelectric Power Generation (pt)
	4941		Drinking 'Water Supply
	4952	22132	Sewage Treatment Facilities
4953	4953	562111	Solid Waste Collection (pt)
	4961	22133	Steam and Air-Conditioning Supply
	5051	42151	Metals Service Centers and Offices
	5052	42152	Coal and Other Mineral and Ore Wholesalers
	5063	44419	Other Building Material Dealers (pt)
	5171	454311	Heating Oil Dealers (pt)
	5194	42294	Tobacco and Tobacco Product Wholesalers
	5541	44711	Gasoline Stations with Convenience Store (pt)
	5983	454311	Heating Oil Dealers (pt)
	7389	51224	Sound Recording Studios
	7542	811192	Car Washes
	7699	561622	Locksmiths
	8221	61131	Colleges, Universities and Professional Schools
	8922		Services, not elsewhere classified
	9223	92214	Correctional Institutions
	9661	92711	Space Research and Technology
	9711	928111	National Security

APPENDIX B

LABORATORY GUIDANCE

GUIDANCE FOR CONDUCTING & REPORTING

THE RESULTS OF TOXICITY TESTS

IN FULFILLMENT OF VPDES PERMIT REQUIREMENTS

Virginia Department of Environmental Quality
Office of Water Permit Programs
Toxics Management Program

Revised August 24, 2000

(Replaces July 1992 Revision)

INDEX

TABLES AND FORMS

GUIDANCE FOR CONDUCTING & REPORTING THE RESULTS OF TOXICITY TESTS IN FULFILLMENT OF VPDES PERMIT REQUIREMENTS

Virginia Department of Environmental Quality
Office of Water Permit Programs
Toxics Management Program

Revised June 27, 2000

I. INTRODUCTION

This document is intended to provide the guidelines and references for the aquatic toxicity tests that are required by Virginia Pollutant Discharge Elimination System (VPDES) permit holders with Toxics Management Program (TMP) Special Conditions, or Whole Effluent Toxicity (WET) limitations. The Department of Environmental Quality (DEQ) recommends that the EPA guidelines (which have been published as a final rule) for toxicity testing be followed to insure compliance with VPDES permits. This June 27, 2000 guidance document supercedes the previous July 1992 guidance document (same title), and incorporates the February 13, 1995 guidance document on reference toxicant tests. The guidance makes recommendations for testing and reporting for VPDES compliance tests. **All laboratories should be in compliance with this guidance by October 1, 2000.**

For assistance with developing protocols or questions on this guidance, contact Deborah L. DeBiasi of the Toxics Management Program at DEQs Central Office, 629 East Main Street, Richmond, Virginia 23219. The phone number is 804/698-4028, FAX number 804/698-4032, e-mail address is: dldebiasi@deq.state.va.us

II. REFERENCES

EPA amended 40 CFR 136.3 (Tables 1A and II) by adding the methods for measuring the acute and short-term chronic toxicity of effluents and receiving waters. The rulemaking was initiated at the request of the States to standardize the test methods. The final rule, published in Volume 60, No. 199 of the Federal Register on October 16, 1995 became effective November 15, 1995. Laboratory protocols should be based on the guidance offered by these listed EPA manuals, and any testing and reporting requirements stated in this DEQ OWPP-TMP guidance document dated June 27, 2000. Protocols should include the following in their list of reference documents, where applicable:

This form, or one that includes all required information, should be supplied with the VPDES permit application of each municipality the lab is performing work for:

http://www.epa.gov/owm/npdes.htm#forms

The WET testing methods are cited in the Federal Register, October 16, 1995, Volume 60, Number 199, Pages 53529-53544, and at the site below:

http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=1995_register&docid=fr16oc95-10

Technical corrections to the document above can be found in the Federal Register, February 2, 1999, Volume 64, number 21, Pages 4975-4978, and at the site below:

http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=1999_register&docid=fr02fe99-7

1. ***Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms***, Fourth Edition, EPA/600/4-90/027F, August 1993.

2. ***Short-term Methods to Estimate the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms***, Third Edition, EPA/600/4-91/002, July 1994.

3. ***Short-term Methods to Estimate the Chronic Toxicity of Effluents and Receiving Waters to Estuarine and Marine Organisms***, Second Edition, EPA/600/4-91/003, July 1994.

4. ***Technical Support Document for Water Quality Based Toxics Control***, EPA/505/2-90/001, March 1991.

5. ***Guidance for Conducting & Reporting the Results of Toxicity Tests in Fulfillment of VPDES Permit Requirements***, Virginia DEQ OWPP-TMP, June 27, 2000.

Sources for these documents are:

1. National Center for Environmental Publications and Information (NCEPI), 513/489-8190 or FAX 513/489-8695

2. EPA Office of Water Resource Center, 202/260-7786.

III. TESTS REQUIRED IN VPDES PERMITS

1. FRESHWATER TESTS

a. ACUTE TESTS (LC_{50} Endpoint)

48 Hour Static Acute Test with *Ceriodaphnia dubia*
48 Hour Static Acute Test with *Pimephales promelas*
48 Hour Static Acute Test with *Oncorhynchus mykiss*
96 Hour Static Acute Test with *Pimephales promelas* *
96 Hour Static Acute Test with *Oncorhynchus mykiss* *
96 Hour Static Renewal Acute Test with *Pimephales promelas*
96 Hour Static Renewal Acute Test with *Oncorhynchus mykiss*

b. ACUTE TESTS (NOAEC Endpoint)

48 Hour Static Acute Test with *Ceriodaphnia dubia*
48 Hour Static Acute Test with *Pimephales promelas*
48 Hour Static Acute Test with *Oncorhynchus mykiss*

c. CHRONIC TESTS

3-Brood Chronic Static Renewal Survival and Reproduction Test with *Ceriodaphnia dubia*
7-Day Chronic Static Renewal Larval Survival and Growth Test with *Pimephales promelas*

2. SALTWATER TESTS

a. ACUTE TESTS (LC_{50} Endpoint)

48 Hour Static Acute Test with *Mysidopsis bahia*
48 Hour Static Acute Test with *Cyprinodon variegatus*
96 Hour Static Acute Test with *Cyprinodon variegatus* *
96 Hour Static Renewal Acute Test with *Cyprinodon variegatus*

b. ACUTE TESTS (NOAEC Endpoint)

48 Hour Static Acute Test with *Mysidopsis bahia*
48 Hour Static Acute Test with *Cyprinodon variegatus*

c. CHRONIC TESTS

7-Day Chronic Static Renewal Survival, Growth and Fecundity Test with *Mysidopsis bahia*
7-Day Chronic Static Renewal Larval Survival and Growth Test with *Cyprinodon variegatus*

* Some of the older permits contain a requirement for the 96-hour static acute test. Since publication of the 4th Edition of EPA's Acute manual, the age of the larvae has been decreased from 1-90 days to 1-14 days old. EPA also recommends that the test organisms are fed and the test chambers cleaned of food debris if the test has a duration of more than 48 hours. Therefore, **all 96 hour long tests should be performed as static renewal tests.** The organisms should be fed 2 hours prior to renewal at 48 hours, and the test chambers should be renewed with a fresh aliquot of the original sample.

IV. LABORATORY PROTOCOLS

While the state of Virginia does not have a laboratory certification program at this time, we do recommend that the specific test and reporting procedures required by EPA, in conjunction with any specific requirements from DEQ be adhered to and documented in the protocols for that laboratory. The procedures utilized in the laboratory should be in accordance with the most current EPA guidance manuals (cited in Section II.) as well the DEQ OWPP-TMP guidance documents. All laboratories should be in compliance with this guidance by October 1, 2000. **Deviation from EPA test procedures or failure to meet minimum test acceptability criteria may render the test data unacceptable for VPDES compliance purposes and may subject the permittee to enforcement action.** Permittees should make every effort to ensure that the laboratory will provide acceptable test data. For assistance, contact the permit writer who developed the special condition for toxics monitoring, or DEQ Central Office Toxics Management Program.

The laboratory protocols should consist of the Standard Operating Procedures (SOP's) for sampling, culturing, testing, reporting, QA/QC with instrumentation, dilution water analyses and reference toxicant tests. Copies of forms used for bench sheets and for reports should be included.

Sample data forms that have spaces to record all required parameters are included with this guidance document and are recommended for use in reporting. Use of these forms would facilitate data review for the permit writers.

V. QA/QC AND REFERENCE TOXICANT TESTS

The water used for culturing and for the test diluent should be analyzed at least annually or whenever difficulty is encountered in meeting minimum acceptability criteria for control survival and reproduction or growth for toxic metals and organics. The concentration of the following metals (expressed as total metal) should not exceed 1 μg/L each: Al, As, Cr, Co, Cu, Fe, Pb, Ni, Zn. The concentration of Cd, Hg, and Ag should not exceed 100 ng/L each when expressed as total metal. Total organochlorine pesticides plus PCB's should be less than 50 ng/L (APHA 1992).

The EPA guidance manuals require that a laboratory test in-house cultured organisms monthly with a reference toxicant using the acute and chronic test procedures. Where acute or short-term chronic toxicity tests are performed with effluents using test organisms obtained from outside the test laboratory, concurrent toxicity tests of the same type must be performed with a reference toxicant. If a routine reference toxicant test fails to meet acceptability criteria, the test must be immediately repeated. If the failed reference toxicant test was being performed concurrently with an effluent test, both tests must be repeated.

All instruments used for routine measurements of chemical and physical parameters such as pH, DO, temperature, conductivity, salinity, alkalinity and hardness must be calibrated

and standardized prior to use each day. The calibration data should be recorded in a permanent log book.

The tests are to be performed in the same manner as effluent tests, with the same requirements. A minimum of 5 dilutions of a geometric series should be used with synthetic dilution water. All of the chemical parameters should be reported in the appropriate time frames.

While there are a variety of reference toxicants that can be used, it is preferred that either NaCl or KCl is used for testing. Either salt would present fewer hazards for the laboratory staff than SDS or cadmium chloride, and are relatively inexpensive.

Reference toxicant data should be submitted to DEQ on a quarterly basis, so that the integrity of the test lab (procedures, organism viability, etc.) can be monitored. The submissions should include:

1. Raw data with each test
2. Statistical printout
3. Control chart (graph) showing the most recent 20 test results with upper (+2SD) and lower (-2SD) control limits. A list of the test dates and results, the CV for the data, and the statistical method of determination should be provided with the chart.

The address for submission of the reference toxicant data is: Deborah L. DeBiasi, Toxics Management Program, Office of Water Permit Programs, Department of Environmental Quality, P.O. Box 10009, Richmond, Virginia 23240-0009.

VI. SAMPLING REQUIREMENTS

The type of sample and often, the method of collection will be site-specific for each permittee. The Toxics Management Program Special Condition will state the sample type and duration of collection, usually in the first paragraph of the first section on Biological Monitoring. Any specific sampling and documentation requirements will be elaborated upon in a separate section on Sample Requirements. **Samples for biological testing must be collected as specified in the permit or the resulting test will not be acceptable.** The types of samples in VPDES permits will include the following:

1. **GRAB** - A single effluent sample. The permit may specify the time at which the grab should be taken, such as "within 1 hour of the commencement of the discharge", or "within 3 hours of commencement of discharge from a storm event". Sometimes the sample is required to be taken when it is representative of an additive, such as "the sample shall be taken when (the additive) is present". Grab sampling may also be required when a particular process occurs, such as "sample should be collected within 1 hour of commencement of discharge from the (description) tank/basin/process". It may be necessary to record flow information. If the permit contains specific language

regarding the collection of a grab sample, verification of proper sample collection should be provided on the chain-of-custody. Flow or storm event information must be provided with the test report if required by the permit.

2. **COMPOSITE** - Series of grabs collected at regular time intervals over a stated period of time. The most common type of composite is for a 24-hour period. Unless the permit specifies otherwise, the 24-hour composite should consist of, at a minimum, 24 grabs collected hourly which are then composited. Some permits may require an 8-hour composite, which consists of grab samples collected hourly for an 8-hour period. The number, volume, and interval, in minutes or hours, between the collection of the individual grab samples comprising the composite must be documented. **Composite samples must be chilled to 4° C during collection.**

3. **24-HOUR FLOW-PROPORTIONAL COMPOSITE** - Series of grabs collected based on flow for 24-hour period. **Composite samples must be chilled to 4° C during collection.** This type of sample may be collected two ways, with documentation on the flow, subsample volumes, and number of subsamples (if appropriate) provided:

   a. Individual subsamples are collected at regular time intervals with the amount of the sample collected proportional to the flow. For example, if subsamples were to be collected hourly at a proportion of 100-ml sample/1000 gallon flow, then when 1200 gallons of flow have been discharged, 120 ml of sample would be collected.

   b. Set-volume subsamples are collected per set volume of flow. For example, for every 1000 gallons of flow, a 100-ml volume of sample is collected.

   Static acute and static renewal acute tests require only one sample. The static renewal tests are renewed with a fresh aliquot of the original sample.

   The chronic tests require a minimum of three samples, each with sufficient volume for sample renewals.

VII. EFFLUENT FIELD DATA

The following parameters should be measured prior to altering the condition of the sample for transport:

1. **Temperature** - Effluent temperature must be measured at the collection point:

   a. Grab - within 0-15 minutes of collection

   b. Composite

(1) from the last subsample at the end of the sampling period,

(2) from the sample contained in the sample collection device.

2. **pH** - Effluent pH must be measured at the collection point

   a. Grab - within 0-15 minutes of collection.

   b. Composite - **while it is not required**, it would be useful information for the test laboratory to have the pH of the composite upon completion of collection, prior to transport.

3. **Residual chlorine** - It should be noted on the chain-of-custody as to whether the effluent is chlorinated or dechlorinated, and what was used for dechlorination.

VIII. SAMPLE TRANSPORT

Sample preservation efforts should continue by packing the sample(s) in sufficient ice for shipping/transport so that the temperature upon arrival at the laboratory is $\leq 4^{\circ}$ C (but not frozen). Note that dry ice should not be used for preservation since frozen samples are not acceptable.

Samples received at temperatures $>4^{\circ}$ C may be considered not acceptable. Consideration will be given to grab samples that do not have sufficient time to cool, due to proximity of the site to the laboratory.

IX. SAMPLE HOLDING TIMES

The sample holding time begins when the last grab sample in a composite series has been taken.

The first use of any sample collected for toxicity (acute or chronic) testing must be within 36 hours of retrieval from the sample collection device. Samples held after the first use which are to be used in chronic test solution renewals, must not be used if more than 72 hours have elapsed since retrieval from the sample collection device. While the EPA guidance manuals suggest using the 3 samples for days 1-2, 3-4, 5-7 respectively, the lab may want to use one of the first two samples for a 3-day period to allow for a chronic test with *Ceriodaphnia* which may run to 8 days.

Sample collection, shipping, and test initiation time should be coordinated to adhere to the sample holding time requirements. Excursions of the sample holding times may result in the test being not acceptable.

X. EFFLUENT CONDITION UPON ARRIVAL AT LABORATORY

The condition of the sample(s) upon arrival at the laboratory should be described on the Test Summary Sheet or the sample chain-of-custody form. Confirm that the method of the shipment was indicated, whether ice was present in the cooler, if leakage of the sample container occurred, the volume (approximate) of sample received, and the date and time of arrival. The name of the person receiving the sample should be documented.

The sample temperature should be less than or equal to 4° C upon arrival at the laboratory. If there is a question as to whether a sample temperature exceeding 4° C would be acceptable, testing should not proceed until the DEQ regional office or DEQ OWPP-TMP has been consulted for guidance as to whether the resulting test would be considered acceptable.

After the sample has been warmed to test temperature ($12\pm1^{\circ}$ C for acute trout tests, $25\pm1^{\circ}$ C for all other acute tests, $26\pm1^{\circ}$ C for chronic *mysid* tests, $25\pm1^{\circ}$ C for all other chronic tests), the following parameters must be measured and recorded: pH, chlorine residual, dissolved oxygen (DO), conductivity or salinity, and total ammonia where toxicity may be contributed to by unionized ammonia (where total ammonia $\geq$5 mg/l). A visual/scent description of the sample should be made and recorded with the chemical parameters.

XI. SAMPLE ADJUSTMENTS

1. DO The dissolved oxygen (DO) concentration in the sample (and dilution water) should be at or below saturation prior to use. The DO saturation point should be determined from the table in Standard Methods. If the sample (or dilution water) is supersaturated, the DO level must be reduced by aeration, shaking, or stirring until the DO stabilizes at an acceptable level. Samples (or dilution water) that have a DO less than 4.0 mg/l for warm and salt water species, or less than 6.0 mg/l for cold water species must be aerated to increase the DO to acceptable levels prior to use in a test. **Tests that are set up with either the sample or dilution water greater than 100% saturation or less than 4.0 mg/l (or less than 6.0 mg/l for cold water species) may be considered not acceptable.**

2. pH Tests for compliance should be performed on the sample <u>without</u> pH adjustment, so as to better assess the effects of the effluent on the organisms. If the effluent is out of the pH 6-9 range, it is recommended that the lab check with the permittee to see if they want a parallel test set up with pH adjusted effluent and controls. This would enable the permittee to see if there are toxicants present without the effects of the "out of range" sample pH. Compliance will be determined from the unadjusted samples test result.

3. Chlorine Tests for compliance should be performed on the sample "as is", unless noted in the VPDES permit to dechlorinate, or if the VPDES permit has

a schedule for the facility to complete dechlorination. The chlorine residual should be reported for all effluent samples. Again, it may be to the permittee's benefit to run a parallel test to see if chlorine is the toxicant.

4. Samples that contain debris or organisms may be filtered through a sieve having 60 μm mesh openings prior to use.

5. Samples containing filamentous bacteria or fungi may be exposed to UV light prior to use.

XII. DILUTION WATER

Fresh water tests should use synthetic moderately hard water (SMHW) prepared with deionized water and either reagent grade chemicals or mineral water. The final water quality should be in the following ranges: pH 7.4 – 7.8 (pH 7.9 – 8.3 for mineral water), hardness 80 – 100 mg $CaCO_3$/L, alkalinity 60-70 mg $CaCO_3$/L.

Saltwater tests should use a standard synthetic reconstituted seawater (GP2) prepared with deionized water and either reagent grade chemicals or commercial sea salts which do not contain dechlorination agents. The salinity should be adjusted to 20±2 $^{o}/_{oo}$.

Source water for the deionizer can be groundwater or tap water. Deionized water is obtained from a MILLIPORE® MILLI-Q®, MILLIPORE® QPAK$^{TM}_2$, or equivalent system. It is advisable to provide preconditioned (deionized) feed water by using a CULLIGAN®, CONTINENTAL®, or equivalent system in front of the MILLIPORE® System to extend the life of the MILLIPORE® cartridges.

XIII. DATA REVIEW

Test reports will be reviewed by the DEQ regional staff to determine compliance with the test methods. The report should include bench sheets with all necessary data, test summary information, statistical printouts, and a data summary of biological testing for the facility. There are acute and chronic data review checklists at the end of this document that will be used for the reviews. The permittee should insure that the performance of the laboratory would result in acceptable data to report on the DMR. If a test is deemed unacceptable, a retest must be performed within the time frame scheduled in the VPDES permit or the permittee will be in non-compliance. Unacceptable data does not relieve the permittee of responsibility for DMR reporting.

Tests may be considered acceptable or not acceptable. We are no longer considering tests "conditionally acceptable" since the data results are reported on the DMR.

ACUTE LC_{50} AND NOAEC TEST REQUIREMENTS					
	Ceriodaphnia dubia	***Pimephales promelas***	***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Cyprinodon variegatus***
Organism Type	Invertebrate	Vertebrate	Vertebrate	Invertebrate	Vertebrate
Water Type	Fresh	Fresh	Fresh-Cold	Salt	Salt
Test Type and Duration	48 Hr Static	48 Hr Static 96 Hr Static Renewal	48 Hr Static 96 Hr Static Renewal	48 Hr Static	48 Hr Static 96 Hr Static Renewal
Dilution Water	SMHW 20% DMW	SMHW 20% DMW	SMHW 20% DMW	20 ±2 ppt Sea Salts	20 ±2 ppt Sea Salts
Test Temperature	25 ± 1 °C	25 ± 1 °C	12 ± 1 °C	25 ± 1 °C	25 ± 1 °C
Test Container Size (minimum)	30 ml	250 ml	5000 ml	250 ml	250 ml
Sample Volume (minimum)	15 ml	200 ml	4000 ml	200 ml	200 ml
Number of Replicates for LC_{50} test (minimum)	4	2	2	2	2
Number of Replicates for NOAEC test (minimum)	4	4	4	4	4
Number of Organisms per Replicate for LC_{50} test	5	10	10	10	10
Number of Organisms per Replicate for NOAEC test	5	5	5	5	5

ACUTE LC_{50} AND NOAEC TEST REQUIREMENTS					
	Ceriodaphnia dubia	***Pimephales promelas***	***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Cyprinodon variegatus***
Age of Organisms	<24 Hours	1-14 Days (within 24 hrs of age)	15-30 Days from swim up	1-5 Days (within 24 hrs of age)	1-14 Days (within 24 hrs of age)
Number of Geometric Derived Dilutions for LC_{50} test and Multi-dilution NOAEC test (Single dilution NOAEC test uses 100% and Control)	5 plus Control	5 plus Control	5 plus Control	5 plus Control	5 plus Control
Dissolved Oxygen Level	≥4.0 mg/l and less than saturation at 20°	≥4.0 mg/l and less than saturation at 20°	≥6.0 mg/l and less than saturation at 12°	≥4.0 mg/l and less than saturation at 20°	≥4.0 mg/l and less than saturation at 20°
Aeration during Test	No	Yes, if DO <4.0 mg/l	Yes, if DO <6.0 mg/l	Yes, if DO <4.0 mg/l	Yes, if DO <4.0 mg/l
Feeding	Prior to test initiation	Prior to test initiation; 96 hr test organisms fed prior to renewal at 48 hr	Prior to test initiation; 96 hr test organisms fed prior to renewal at 48 hr	Daily	Prior to test initiation; 96 hr test organisms fed prior to renewal at 46 hr
Food Type	YCT	*Artemia*	*Artemia*	*Artemia*	*Artemia*
Test Acceptability Criterion	≥90% Survival in Controls	≥90% Survival in Controls	≥90% Survival in Controls	≥90% Survival in Controls	≥90% Survival in Controls

REQUIREMENTS FOR ALL ACUTE TESTS
1. Sample temperature must be shipped on ice and 0-4° C upon receipt at the testing facility.
2. The pH, temperature and chlorine should be measured/recorded within 15 minutes of sample collection.
3. The pH, temperature, DO, chlorine residual and sample description (visual, scent) should be measured/recorded upon receipt.
4. The test must be initiated within 36 hours of sample retrieval from sample device.
5. The sample DO must be adjusted prior to use if it is above or below the acceptable test range.
6. If the DO drops below minimum required levels, all test chambers must be aerated (except for tests with *C. dubia).*
7. Test parameter checks/survival counts should be performed every 24±2 hour increments from test initiation.
8. Parameter checks should have the time noted and be initialed by the technician.
9. The test should be terminated 48±1 hour (or 96±1 hour) from test initiation.
10. Test chambers must be randomized at test initiation and the order maintained throughout test duration.
11. Daily photoperiod must be maintained at 16 hours light/8 hours dark.
12. All sample manipulations (adjustments by pH, aeration, dechlorination, filtration, etc.) must be recorded and reported.
13. Acute reference toxicant tests must be performed monthly for each organism cultured in-house. Submit to DEQ Quarterly.
14. Concurrent reference toxicant tests must be performed with the effluent test when organisms are procured from outside source.
15. The use of surrogate samples for chemical measurement is not acceptable.
16. Acute tests should report results as both LC_{50} and Tu_a. Single dilution NOAEC tests should be reported as NOAEC < or = 100% effluent. Multidilution NOAEC tests should be reported as NOAEC = the percent of no effect and the LC_{50} .

REPORTING PARAMETERS FOR ACUTE LC_{50} TESTS						
	0 Hours	**24 Hours**	**48 Hours**	**48 Hours Post-Renewal**	**72 Hours**	**96 Hours**
Ceriodaphnia dubia	**Survival** **DO** **pH** **Cond** **Temp** **Alk** **Hard** **Cl Resid**	**Survival** **Temp**	**Survival** **DO** **pH** **Cond** **Temp**			
Oncorhynchus mykiss ***Pimephales promelas***	**Survival** **DO** **pH** **Cond** **Temp** **Alk** **Hard** **Cl Resid**	**Survival** **DO** **pH** **Temp**	**Survival** **DO** **pH** **Cond** **Temp**	 **DO** **pH** **Cond**	**Survival** **DO** **pH** **Temp**	**Survival** **DO** **pH** **Cond** **Temp**
Mysidopsis bahia ***Cyprinodon variegatus***	**Survival** **DO** **pH** **Salinity** **Temp** **Alk** **Hard** **Cl Resid**	**Survival** **DO** **pH** **Temp**	**Survival** **DO** **pH** **Salinity** **Temp**	 **DO** **pH** **Salinity**	**Survival** **DO** **pH** **Temp**	**Survival** **DO** **pH** **Salinity** **Temp**

REPORTING PARAMETERS FOR ACUTE TESTS - WHERE TO MEASURE		
Parameter	**Replicate**	**Concentration**
Survival (Surv)	All Replicates	All Concentrations
DO (Dissolved Oxygen)	One Replicate	All Concentrations
pH	One Replicate	All Concentrations
Conductivity (Cond)	One Replicate	All Concentrations
Salinity	One Replicate	All Concentrations
Temperature (Temp)	One Replicate	All Concentrations
Alkalinity (Alk)		100% Concentration, Control
Hardness (Hard)		100% Concentration, Control
Chlorine Residual (Cl Resid)		100% Concentration, Control

NOTE: The use of surrogate samples to measure chemical parameters is not acceptable.

CHRONIC TEST REQUIREMENTS				
	Ceriodaphnia dubia	***Pimephales promelas***	***Mysidopsis bahia***	***Cyprinodon variegatus***
Organism Type	Invertebrate	Vertebrate	Invertebrate	Vertebrate
Chronic Endpoints	Survival Reproduction	Survival Growth	Survival Growth Fecundity	Survival Growth
Test Duration	6-8 Days (8 max) - Until 60% of Controls have 3rd brood	7 Days (168±1 hours)	7 Days (168±1 hours)	7 Days (168±1 hours)
Water Type	Fresh	Fresh	Salt	Salt
Dilution Water	SMHW 20% DMW	SMHW 20% DMW	20±2 ppt Sea Salts	20±2 ppt Sea Salts
Test Temperature	25 ± 1° C	25 ± 1° C	26 ± 1° C	25 ± 1° C
Test Container Size (minimum)	30 ml	500 ml	400 ml	600 ml
Sample Volume (minimum)	15 ml	250 ml	150	500 ml
Number of Replicates (minimum)	10	4	8	4
Number of Organisms per Replicate	1	10	5	10
Age of Organisms	<24 hrs old, within 8 hrs of age of each other	<24 Hours old; can be 24-48 Hours old if obtained off site	7 days old, within 24 hrs of age of each other	<24 Hours old; can be 24-48 Hours old if obtained off site
Number of Geometric Derived Dilutions	5 plus Controls	5 plus Controls	5 plus Controls	5 plus Controls
Dissolved Oxygen Level	≥4.0 mg/l and less than saturation at 25°	≥4.0 mg/l and less than saturation at 25°	≥4.0 mg/l and less than saturation at 26°	≥4.0 mg/l and less than saturation at 25°

Aeration during Test	No	Yes, if DO <4.0 mg/l	Yes, if DO <4.0 mg/l	Yes, if DO <4.0 mg/l
Feeding	0.1 ml YCT and 0.1 ml algae per day after renewal	0.15 ml *Artemia* a minimum of twice daily	*Artemia,* at a rate of 75 per mysid, a minimum of twice daily	Once per day using 0.1 g (wet weight) for days 0, 1 and 2; 0.15 g for days 3-6
Food Type	YCT and Selenastrum	<24 hr old *Artemia*	<24 hr old *Artemia*	<24 hr old *Artemia*
Test Acceptability	≥80% Survival in Controls; average of 15 neonates or more per surviving Control female	≥80% Survival in Controls; average dry weight of Control larvae ≥0.25 mg	≥80% Survival in Controls; average dry weight of Control mysids ≥0.20 mg	≥80% Survival in Controls; average dry weight of Control larvae ≥0.60 mg (unpreserved) or ≥0.50 mg (preserved)

REQUIREMENTS FOR ALL CHRONIC TESTS
1. A minimum of three samples must be used: The first use of a sample must be within 36 hours of retrieval from sampling device, and the last use must be within 72 hours of retrieval from sampling device.
2. The pH, temperature and chlorine should be measured/recorded within 15 minutes of sample collection.
3. The pH, temperature, DO, chlorine residual and sample description (visual, scent) should be measured/recorded upon receipt in the lab.
4. Hardness and alkalinity must be measured/recorded for each new sample and the Control.
5. Chlorine should be measured in each new sample and the Control.
6. The sample DO must be adjusted prior to use if it is above or below the acceptable test range.
7. If the DO drops below minimum required levels, all test chambers must be aerated (except for tests with *C. dubia*).
8. Test parameter checks/survival counts should be performed every 24±2 hour increments from test initiation.
9. Parameter checks should have the time noted and be initialed by the technician.
10. The tests using *P. promelas, M. bahia,* and *C. variegatus* should be terminated 168±1 hours from test initiation. The *C. dubia* test is terminated when 60% of the surviving Controls have had 3 broods, or at a maximum 8 days in duration.
11. Test chambers must be randomized at test initiation and the order maintained throughout test duration.
12. The neonates used to set up chronic *C. dubia* tests must be distributed in a blocked fashion.
13. Daily photoperiod must be maintained at 16 hours light/8 hours dark.
14. All sample manipulations (adjustments by pH, aeration, dechlorination, etc. where allowed) must be recorded and reported.
15. Monthly chronic reference toxicant tests must be performed and submitted to DEQ on a quarterly basis.
16. Concurrent reference toxicant tests must be performed with the effluent test when organisms are procured form outside source.
17. Chronic tests should report the NOEC for each endpoint, express also as Tu_c . Report the IC_{25}, and report the LC_{50} at 48 hours.

18. The use of surrogate samples to measure chemical parameters is not acceptable.
19. Where both acute and chronic tests are required in a permit, the acute tests should be set up with a sample other than the 1st sample of the chronic test.
20. Chronic tests with *C. dubia* should notate where males are present and where appropriate, delete the rows from statistical analysis.
20. The EPA guidance manuals to refer to are: ***Short-term Methods to Estimate the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms***, Third Edition, EPA/600/4-91/002, July 1994. ***Short-term Methods to Estimate the Chronic Toxicity of Effluents and Receiving Waters to Estuarine and Marine Organisms***, Second Edition, EPA/600/4-91/003, July 1994.

REPORTING PARAMETERS FOR CHRONIC TESTS				
	Prior to test	**Start of 24 Hr Period**	**End of 24 Hr Period**	**End of Test**
Ceriodaphnia dubia **Survival and Reproduction**	**Temperature** **pH** **DO** **Alkalinity** **Hardness** **Chlorine Residual** **Conductivity or Salinity** **Ammonia if suspect**	**Temperature** **pH** **DO** **Conductivity**	**Survival (and Reproduction)** **pH** **DO**	**Percent survival per dilution** **LC_{50} 48 hours** **IC_{25} and NOEC** **Survival – all tests** **Reproduction – *C. dubia*** **Fecundity – *M. bahia*** **Growth – *P. promelas*** ***C. variegatus*** ***M. bahia***
Pimephales promelas **Survival and Growth**				
Mysidopsis bahia **Survival, Growth and Fecundity**		**Temperature** **pH** **DO** **Salinity**	**Survival** **pH** **DO**	
Cyprinodon variegatus **Survival and Growth**				

Changes from previous guidance

1. New acute and chronic manuals

2. Field parameters for pH (last grab) and Cl residual (last grab) do not have to be reported to DEQ on chain-of-custody for composites. EPA manuals require that Cl be taken on last grab for all samples.

3. Total ammonia should be checked upon receipt of sample where unionized ammonia suspected to be present (total ammonia >5.0 mg/l).

4. Scent description has been added to visual description of sample upon arrival at lab.

5. The new EPA manuals explain that for a facility that needs a composite sample, 4 6-hour composites should be taken and the toxicity testing performed on each of the 4 samples. OWPP-TMP believes that this is an unnecessary burden to the permittee. The TMP portion of the permit should explicitly state the type of sample required, i.e. 24-hour composite, and after the section on biological testing, insert a section that defines how this sample should be taken. For example, a 24-hour composite might be defined as hourly grabs for the duration of the discharge, not to exceed a 24-hour period.

6. The growth endpoints for the chronic vertebrate tests should be calculated using the original number of organisms, instead of dividing by the number of survivors.

7. Acute tests for Virginia are now to be performed at $25 \pm 1^{\circ}C$.

8. Test temperature for the chronic test using *Mysidopsis bahia* has changed from 26-27°C to $26 \pm 1^{\circ}C$.

9. The IC_{25} should be calculated and reported along with the NOEC's for the chronic tests. Report also the 48 hour LC_{50} on all chronic tests.

10. Check the permit to see if sample dechlorination can be performed.

11. We no longer require pH adjustment for samples outside of the pH 6-9 range. It should be run "as is".

PERMITTEE: _____ OUTFALL: _____ NPDES#: _____

TEST DATES: _____ TO _____

SUMMARY OF ACTUAL TEST CONDITIONS

1. TEST TYPE:

2. TEST DURATION:

3. TEST ORGANISM:

4. ORGANISM AGE:

5. TEST TEMPERATURE (LOW TO HIGH):

6. LIGHT INTENSITY:

7. PHOTOPERIOD:

8. TEST CHAMBER SIZE:

9. TEST SOLUTION VOLUME:

10. # ORGANISMS/TEST CHAMBER:

11. # REPLICATES/CONCENTRATION:

12. FOOD TYPE, AMOUNT, FREQUENCY:

13. AERATION RATE/INITIATION TIME:

14. DILUTION WATER:

15. CULTURE WATER FOR TEST ORGANISMS:

16. TEST CONCENTRATIONS:

17. ACUTE TEST ACCEPTABILITY CRITERION OF ≥90% SURVIVAL IN CONTROLS:

18. CHRONIC TEST ACCEPTABILITY CRITERIA OF ≥80% SURVIVAL IN CONTROLS, AND AVERAGE 15 NEONATES/SURVIVING ADULT OR CONTROL LARVAE WEIGH ≥0.25 mg:

19. ACUTE TEST LC_{50}: _____ STATISTICAL PROGRAM/VERSION: _____
CONFIDENCE LIMITS: _____ NOAEC:_____

20. CHRONIC TEST NOEC SURVIVAL: _____ NOEC REPRO/GROWTH/FEC: _____

TEST NOEC: _____ TEST IC_{25} _____ LC_{50} AT 48 HOURS: _____

CHAIN-OF-CUSTODY

PERMITTEE: _____ NPDES#: _____

OUTFALL: _____ FLOW TYPE (CIRCLE ONE): CONTINUOUS INTERMITTENT BATCH STORMWATER OTHER:

SAMPLE INFORMATION

1. GRAB: ___ DATE/TIME COLLECTED: _____ AMOUNT: _____

2. TIME COMPOSITE: COLLECTED FROM (Date/Time): _____
TO (Date/Time): _____

NUMBER/VOL OF SUBSAMPLES: _____ TIME INCREMENT: _____ TOTAL AMOUNT: _____

3. FLOW-PROPORTIONAL COMPOSITE: _ COLLECTED FROM(Date/Time): _____
TO(Date/Time): _____

SET VOLUME SUBSAMPLE/VOLUME FLOW: _____ TOTAL AMOUNT: _____

FOR VARIABLE VOLUME SUBSAMPLES BASED ON FLOW/SET TIME INCREMENTS - ATTACH SAMPLE AND FLOW INFORMATION.

4. NAME OF SAMPLER: _____ AFFILIATION: _____

FIELD PARAMETERS

1. GRAB SAMPLES: pH: _____ TEMPERATURE: _____°C CHLORINE RESIDUAL: ____mg/l TIME: ____

2. COMPOSITES: TEMPERATURE: (last grab) _____°C CHLORINATED?: _____ DECHLORINATED?: _____
DECHLORINATION AGENT?: _____

pH (Composite): _____ TEMPERATURE (Composite): _____°C TIME: _____

3. NAME OF ANALYST: _____AFFILIATION: _____

4. METHOD OF SHIPMENT: _____ COOLANT USED: _____ SHIPMENT DATE/TIME: _____

RELINQUISHED BY:	RECEIVED BY:
_____ TIME/DATE	_____ TIME/DATE
_____ TIME/DATE	_____ TIME/DATE
_____ TIME/DATE	_____ TIME/DATE

EFFLUENT CONDITION UPON ARRIVAL AT LABORATORY

DATE: _____ TIME: _____ ICE PRESENT: Yes/No SAMPLE ID: _____ SAMPLE VOLUME: ____

TEMPERATURE: ____°C pH: ____ CL RESIDUAL: ____mg/l DO: ____mg/l CONDUCTIVITY: ____ìmhos

VISUAL/SCENT DESCRIPTION: _____ ANALYST: _____

48 HOUR STATIC ACUTE REFERENCE TOXICANT TEST

Laboratory Name: _____ Species: _____

Test Start Date/Time: _____/_____ Organism Source: _____

Test End Date/Time: _____/_____ Organism Age: _____ ID#: _____

Dilution Water: DM - Dilute Mineral Water
MH - Moderate Hard Synthetic
TW - Tap Water, Dechlor
EV - Evaporated Seawater
IO - Instant Ocean
ME - Marine Environment
HM - Hawaiian Marine Mix
FF - Forty Fathoms
GP - Modified GP2
Other - _____

Reference Toxicant: _____

Analytical Conc Ref Tox: _____ µg/l

	Alkalinity	Hardness	Chlorine
Diluent	_____ mg/l	_____ mg/l	_____ mg/l
Ref Tox 100%	_____ mg/l	_____ mg/l	

CONC & REP	SURVIVAL			DO, mg/l			pH			CONDUCTIVITY or SALINITY			TEMP, °C			PERCENT SURVIVAL PER CONC
	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	
Tech/ Time																

RESULT as LC_{50}: _____ STATISTICAL METHOD: _____ (Attach printout)

DIRECTIONS - ACUTE 96-HOUR STATIC RENEWAL TEST

1. Use either of the 2 forms included. One of the forms has shading for replicate B, to indicate that the parameter checks are all done from replicate A. If you do not do all of the parameter checks from replicate A, use the form that does not have the shading.

2. All 96-hour tests for Virginia should be performed as static renewal tests. A fresh aliquot of the original sample is used for renewal at 48 hours. "R" indicates the readings for the test samples after renewal.

3. A chain-of-custody form is included.

4. A test summary form is included to record any information that is not already on the acute test form.

5. A copy of the statistics should be attached.

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL: _____ LOCATION: _____

PURPOSE OF TEST: _____

GRAB SAMPLE DATE/TIME: _____ TEST ORGANISM: _____

COMPOSITE DATES/TIMES: ORGANISM AGE: _____

FROM: _____ TO: _____ ORGANISM SOURCE: _____

COMPOSITE SUBSAMPLE VOLUMES/INCREMENTS: TEST START DATE/TIME: _____

SAMPLE VOLUME/TIME INCREMENT: _____ TEST END DATE/TIME: _____

SAMPLE VOLUME/FLOW VOLUME: _____ DATE/TIME ORGANISMS FED: _____

DILUTION WATER: _____ SAMPLE CHLORINE RESIDUAL: _____

SAMPLE AMMONIA: _____

ACUTE 96-HOUR STATIC RENEWAL TEST

CONC/REP	SURVIVAL					DO 2 HRS DO 4 HRS DO 6 HRS DO 8 HRS DO (mg/l)						pH						ALK	HARD	CONDUCTIVITY (imhos) or SALINITY				TEMPERATURE ° C					% SURV PER CONC
	0	24	48	72	96	0	24	48	48 R	72	96	0	24	48	48 R	72	96	0	0	0	48	48 R	96	0	24	48	72	96	96
CONTROL A																													
CONTROL B																													
6.25% A																													
6.25% B																													
12.5% A																													
12.5% B																													
25% A																													
25% B																													
50% A																													
50% B																													
100% A																													
100% B																													
A																													
B																													
TIME																													
TECH																													

COMMENTS:

PERMITTEE: _____ LABORATORY: _____
NPDES#: _____ OUTFALL: _____ LOCATION: _____
PURPOSE OF TEST: _____
GRAB SAMPLE DATE/TIME: _____ TEST ORGANISM: _____
COMPOSITE DATES/TIMES: ORGANISM AGE: _____
FROM: _____ TO: _____ ORGANISM SOURCE: _____ COMPOSITE
SUBSAMPLE VOLUMES/INCREMENTS: TEST START DATE/TIME: _____
SAMPLE VOLUME/TIME INCREMENT: _____ TEST END DATE/TIME: _____ SAMPLE
VOLUME/FLOW VOLUME: _____ DATE/TIME ORGANISMS FED: _____
DILUTION WATER: _____ SAMPLE CHLORINE RESIDUAL: _____
SAMPLE AMMONIA: _____

ACUTE 96-HOUR STATIC RENEWAL TEST

CONC/REP	SURVIVAL					DO 2 HRS __ DO 4 HRS __ DO 6 HRS __ DO 8 HRS __ DO (mg/l)						pH						ALK	HARD	CONDUCTIVITY (imhos) or SALINITY				TEMPERATURE ° C					% SURV PER CONC
	0	24	48	72	96	0	24	48	48 R	72	96	0	24	48	48 R	72	96	0	0	0	48	48 R	96	0	24	48	72	96	96
CONTROL A																													
CONTROL B																													
6.25% A																													
6.25% B																													
12.5% A																													
12.5% B																													
25% A																													
25% B																													
50% A																													
50% B																													
100% A																													
100% B																													
A																													
B																													
TIME																													
TECH																													

COMMENTS:

DIRECTIONS - ACUTE 48-HOUR STATIC TEST

1. Two forms are included: one form is for the vertebrate test which has 2 replicates, and the other is for the invertebrate test which has 4 replicates.

2. A chain-of-custody form is included.

3. A test summary form is included to record any information that is not already on the acute test form.

4. A copy of the statistics should be attached.

PERMITTEE: _____ LABORATORY: _____
NPDES#: _____ OUTFALL: _____ LOCATION: _____
PURPOSE OF TEST: _____
GRAB SAMPLE DATE/TIME: _____ TEST ORGANISM: _____
COMPOSITE DATES/TIMES: ORGANISM AGE: _____
FROM: _____ TO: _____ ORGANISM SOURCE: _____
COMPOSITE SUBSAMPLE VOLUMES/INCREMENTS: TEST START DATE/TIME: _____
SAMPLE VOLUME/TIME INCREMENT: _____ TEST END DATE/TIME: _____
SAMPLE VOLUME/FLOW VOLUME: _____ SAMPLE CHLORINE RESIDUAL: _____ DILUTION WATER: _____ SAMPLE AMMONIA: _____

ACUTE 48-HOUR STATIC TEST

CONC/REP	SURVIVAL			DO 2 HRS DO 4 HRS DO 6 HRS DO 8 HRS DO (mg/l)			pH			ALK	HARD	COND (imhos)		TEMPERATURE ° C			% SURV PER CONC
	0	24	48	0	24	48	0	24	48	0	0	0	48	0	24	48	48
CONTROL A																	
CONTROL B																	
6.25% A																	
6.25% B																	
12.5% A																	
12.5% B																	
25% A																	
25% B																	
50% A																	
50% B																	
100% A																	
100% B																	
A																	
B																	
TIME																	
TECH																	

COMMENTS:

PERMITTEE: _____ LABORATORY: _____
NPDES#: _____ OUTFALL: _____ LOCATION: _____
PURPOSE OF TEST: _____ DATE PROTOCOLS APPROVED: _____
GRAB SAMPLE DATE/TIME: _____ TEST ORGANISM: _____
COMPOSITE DATES/TIMES: ORGANISM AGE: _____
FROM: _____ TO: _____ ORGANISM SOURCE: _____
COMPOSITE SUBSAMPLE VOLUMES/INCREMENTS: TEST START DATE/TIME: _____
SAMPLE VOLUME/TIME INCREMENT: _____ TEST END DATE/TIME: _____
SAMPLE VOLUME/FLOW VOLUME: _____ SAMPLE CHLORINE RESIDUAL: _____
DILUTION WATER: _____ SAMPLE AMMONIA: _____

ACUTE 48-HOUR STATIC TEST

CONC/REP	SURVIVAL			DO (mg/l)			pH			ALK	HARD	COND (imhos)		TEMPERATURE ° C			% SURV PER CONC
	0	24	48	0	24	48	0	24	48	0	0	0	48	0	24	48	48
CONTROL A																	
CONTROL B																	
CONTROL C																	
CONTROL D																	
6.25% A																	
6.25% B																	
6.25% C																	
6.25% D																	
12.5% A																	
12.5% B																	
12.5% C																	
12.5% D																	
25% A																	
25% B																	
25% C																	
25% D																	
50% A																	
50% B																	
50% C																	
50% D																	
100% A																	
100% B																	
100% C																	
100% D																	
A																	
B																	
C																	
D																	
TIME																	
TECH																	

COMMENTS:

PERMITTEE: _____ LABORATORY: _____
NPDES#: _____ OUTFALL: _____ LOCATION: _____
PURPOSE OF TEST: _____ DATE PROTOCOLS APPROVED: _____ GRAB SAMPLE
DATE/TIME: _____ TEST ORGANISM: _____
COMPOSITE DATES/TIMES: ORGANISM AGE: _____
FROM: _____ TO: _____ ORGANISM SOURCE: _____
COMPOSITE SUBSAMPLE VOLUMES/INCREMENTS: TEST START DATE/TIME: _____
SAMPLE VOLUME/TIME INCREMENT: _____ TEST END DATE/TIME: _____
SAMPLE VOLUME/FLOW VOLUME: _____ SAMPLE CHLORINE RESIDUAL: _____ DILUTION
WATER: _____ SAMPLE AMMONIA: _____

ACUTE 48-HOUR STATIC TEST

CONC/REP	SURVIVAL			DO (mg/l)			pH			ALK	HARD	COND (imhos)		TEMPERATURE °C			% SURV PER CONC
	0	24	48	0	24	48	0	24	48	0	0	0	48	0	24	48	48
CONTROL A																	
CONTROL B																	
CONTROL C																	
CONTROL D																	
6.25% A																	
6.25% B																	
6.25% C																	
6.25% D																	
12.5% A																	
12.5% B																	
12.5% C																	
12.5% D																	
25% A																	
25% B																	
25% C																	
25% D																	
50% A																	
50% B																	
50% C																	
50% D																	
100% A																	
100% B																	
100% C																	
100% D																	
A																	
B																	
C																	
D																	
TIME																	
TECH																	

COMMENTS:

CHRONIC STATIC RENEWAL 7-DAY SURVIVAL AND GROWTH TEST WITH PIMEPHALES PROMELAS

Test dilutions grouped by parameter (this method of reporting is preferred)

There are three forms to use; one has DO and pH on it, the second one has temperature and conductivity, and the third form has alkalinity, hardness, chlorine residual and sample holding time documentation.

1 The next form is for recording survival of the larvae daily, starting with test initiation (Day 0).

2 The next form is for recording the weights of the larvae.

3 A test summary sheet is included to record information not already included on the bench sheets.

4 There should be a minimum of 3 chain-of-custody forms (one per sample) to go with the samples required for a chronic test.

5 The statistics should be attached to the test report.

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th TEST START DATE/TIME: _____

COMPLIANCE ENDPOINT: _____ TEST END DATE/TIME: _____

WATER CHEMISTRY FOR CHRONIC SURVIVAL AND GROWTH TEST WITH PIMEPHALES PROMELAS														
PARAMETER: DISSOLVED OXYGEN, mg/l														
DATE														
DAY	DAY 0	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7
HOURS	0	24	0	24	0	24	0	24	0	24	0	24	0	24
CONTROL														
DIL #1														
DIL #2														
DIL #3														
DIL #4														
DIL #5														
ADJUSTMENTS:														
PARAMETER: pH														
CONTROL														
DIL #1														
DIL #2														
DIL #3														
DIL #4														
DIL #5														
COMMENTS:														
TECH/TIME:														

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th TEST START DATE/TIME: _____

COMPLIANCE ENDPOINT: _____ TEST END DATE/TIME: _____

WATER CHEMISTRY FOR CHRONIC SURVIVAL AND GROWTH TEST WITH PIMEPHALES PROMELAS														
PARAMETER: TEMPERATURE, °C														
DATE														
DAY	DAY 0	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7
HOURS	0	24	0	24	0	24	0	24	0	24	0	24	0	24
CONTROL														
DIL #1														
DIL #2														
DIL #3														
DIL #4														
DIL #5														
COMMENTS:														
PARAMETER: CONDUCTIVITY, µmhos														
CONTROL														
DIL #1														
DIL #2														
DIL #3														
DIL #4														
DIL #5														
COMMENTS:														
TECH/TIME:														

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th TEST START DATE/TIME: _____

COMPLIANCE ENDPOINT: _____ TEST END DATE/TIME: _____

WATER CHEMISTRY FOR CHRONIC SURVIVAL AND GROWTH TEST WITH PIMEPHALES PROMELAS														
PARAMETER: ALKALINITY, mg/l $CaCO_3$														
DATE														
DAY	DAY 0	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7
HOURS	0	24	0	24	0	24	0	24	0	24	0	24	0	24
CONTROL														
DIL #5														
PARAMETER: HARDNESS, mg/l $CaCO_3$														
CONTROL														
DIL #5														
PARAMETER: CHLORINE RESIDUAL, mg/l														
CONTROL														
DIL #5														
TECH/TIME														
COMMENTS:														

PARAMETER: SAMPLE HOLDING TIME DOCUMENTATION							
SAMPLE DATE(S) AND TIME(S)							
DATE/TIME OF USE							
AGE OF SAMPLE (HRS)							

COMMENTS:

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

URPOSE OF TEST: _____ TEST START DATE/TIME: _____

COMPLIANCE ENDPOINT: _____ TEST END DATE/TIME: _____

CHRONIC 7-DAY SURVIVAL AND GROWTH TEST WITH PIMEPHALES PROMELAS

		WEIGHT DATA FOR SURVIVING LARVAE					AVERAGE WEIGHT OF LARVAE PER CONC
		A WEIGHT OF BOAT (mg)	**B DRY WT BOAT AND LARVAE (mg)**	**B-A TOTAL DRY WT OF LARVAE (mg)**	**C # OF LARVAE PER REP**	**(B-A)/C MEAN DRY WT OF LARVAE (mg)**	
CONTROL	**A**						
	B						
	C						Average Wt of
	D						Surviving Controls:_____
CONC:	**A**						
	B						
	C						
	D						
ONC:	**A**						
	B						
	C						
	D						
CONC:	**A**						
	B						
	C						
	D						
CONC:	**A**						
	B						
	C						
	D						
CONC:	**A**						
	B						
	C						
	D						
CONC:	**A**						
	B						
	C						
	D						

TEST END DATE/TIME:_____ DATE WEIGHED: _____ OVEN TEMP: ___°C DRYING TIME: ___HRS TECH: ___

PERMITTEE: ____________________ LABORATORY: ____________________

NPDES#: ________ OUTFALL#: ________ LOCATION: ____________________

URPOSE OF TEST: ____________________ TEST START DATE/TIME: ____________________

COMPLIANCE ENDPOINT: ________________ TEST END DATE/TIME: ____________________

CHRONIC 7-DAY SURVIVAL AND GROWTH TEST WITH PIMEPHALES PROMELAS

Day of test / Date		NUMBER OF SURVIVING LARVAE PER DAY								# LARVAE PER CONC
		DAY 0	**DAY 1**	**DAY 2**	**DAY 3**	**DAY 4**	**DAY 5**	**DAY 6**	**DAY 7**	
CONTROL	**A**									
	B									
	C									
	D									
CONC:	**A**									
	B									
	C									
	D									
CONC:	**A**									
	B									
	C									
	D									
CONC:	**A**									
	B									
	C									
	D									
CONC:	**A**									
	B									
	C									
	D									
CONC:	**A**									
	B									
	C									
	D									
CONC:	**A**									
	B									
	C									
	D									
ECH/TIME										

COMMENTS:

CHRONIC STATIC RENEWAL 3-BROOD SURVIVAL AND REPRODUCTION TEST USING CERIODAPHNIA DUBIA

Test dilutions are grouped by parameter (this method of reporting is preferred).

There are three forms to use; one has DO and pH on it, the second one has temperature and conductivity, and the third form has alkalinity, hardness, chlorine residual and sample holding time documentation.

1 The next form is based on a brood board configuration (6 across, 10 down), with the top half of the brood board in the left-hand column and the bottom half in the right-hand column. The concentration can be noted next to the 'space' number after the randomization format is determined.

2 The next three forms are for grouping the replicates together as a test concentration. The column marked "# Adults Alive" should be used for determination of the LC_{50} at 48 hours (Day 2). The "Day 0" row is for test initiation, to correlate with the water chemistry forms.

3 A test summary form is included to record information not on the bench sheets.

Three chain-of-custody forms are included.

5 The statistics should be attached to the test report.

PERMITTL _____ ABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th ORGANISM SOURCE: _____

COMPLIANCE ENDPOINT: _____ TEST START (DATE/TIME): _____ TEST END (DATE/TIME: _____

WATER CHEMISTRY FOR CHRONIC 3-BROOD SURVIVAL AND REPRODUCTION TEST WITH CERIODAPHNIA DUBIA																
PARAMETER: DISSOLVED OXYGEN, mg/l																
DATE																
DAY	DAY 0	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7		DAY 8
HOURS	0	24	0	24	0	24	0	24	0	24	0	24	0	24	0	24
CONTROL																
DIL #1																
DIL #2																
DIL #3																
DIL #4																
DIL #5																
ADJUSTMENTS:																
PARAMETER: pH																
CONTROL																
DIL #1																
DIL #2																
DIL #3																
DIL #4																
DIL #5																
COMMENTS:																
TECH/TIME:																

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

COMPLIANCE ENDPOINT: _____ TEST START: _____ TEST END: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th

WATER CHEMISTRY FOR CHRONIC 3-BROOD SURVIVAL AND REPRODUCTION TEST WITH CERIODAPHNIA DUBIA

PARAMETER: TEMPERATURE, °C

DATE																
DAY	DAY 0	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7		DAY 8
HOURS	0	24	0	24	0	24	0	24	0	24	0	24	0	24	0	24
CONTROL																
DIL #1																
DIL #2																
DIL #3																
DIL #4																
DIL #5																
COMMENTS:																

PARAMETER: CONDUCTIVITY, imhos

CONTROL																
DIL #1																
DIL #2																
DIL #3																
DIL #4																
DIL #5																
COMMENTS:																
TECH/TIME:																

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th ORGANISM SOURCE: _____

COMPLIANCE ENDPOINT: _____ TEST START: _____ TEST END: _____

WATER CHEMISTRY FOR CHRONIC 3-BROOD SURVIVAL AND REPRODUCTION TEST WITH CERIODAPHNIA DUBIA																
PARAMETER: ALKALINITY, mg/l $CaCO_3$																
DATE																
DAY	DAY 0	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7		DAY 8
HOURS	0	24	0	24	0	24	0	24	0	24	0	24	0	24	0	24
CONTROL																
DIL #5																
PARAMETER: HARDNESS, mg/l $CaCO_3$																
CONTROL																
DIL #5																
PARAMETER: CHLORINE RESIDUAL, mg/l																
CONTROL																
DIL #5																
TECH/TIME																
COMMENTS:																

PARAMETER: SAMPLE HOLDING TIME DOCUMENTATION								
SAMPLE DATE(S) AND TIME(S)								
DATE/TIME OF USE								
AGE OF SAMPLE (HRS)								

COMMENTS:

PERMITTEE: _____ LABORATORY: _____

'PDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th TEST START DATE/TIME: _____

COMPLIANCE ENDPOINT: _____ TEST END DATE/TIME: _____

SURVIVAL AND REPRODUCTION DATA FOR 3-BROOD CHRONIC TEST WITH CERIODAPHNIA DUBIA														
CONTROL		REPLICATES										# NEONATES PER DAY	# ADULTS ALIVE	# FEMALES ALIVE
DATE/TIME		1	2	3	4	5	6	7	8	9	10			
	DAY 0													
	DAY 1													
	DAY 2													
	DAY 3													
	DAY 4													
	DAY 5													
	DAY 6													
	DAY 7													
	DAY 8													
TOTAL														

SURVIVAL AND REPRODUCTION DATA FOR 3-BROOD CHRONIC TEST WITH CERIODAPHNIA DUBIA														
CONC:		REPLICATES										# NEONATES PER DAY	# ADULTS ALIVE	# FEMALES ALIVE
DATE		1	2	3	4	5	6	7	8	9	10			
	DAY 0													
	DAY 1													
	DAY 2													
	DAY 3													
	DAY 4													
	DAY 5													
	DAY 6													
	DAY 7													
	DAY 8													
TOTAL														

✔ = Test Organism Alive
X = Test Organism Dead
0 = Live neonates
(-0) = Dead neonates
M = Lost or Missing
Y = Male ♂

PERMITTEE: _____ LABORATORY: _____

'PDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th TEST START DATE/TIME: _____

COMPLIANCE ENDPOINT: _____ TEST END DATE/TIME: _____

SURVIVAL AND REPRODUCTION DATA FOR 3-BROOD CHRONIC TEST WITH CERIODAPHNIA DUBIA

CONC:		REPLICATES										# NEONATES PER DAY	# ADULTS ALIVE	# FEMALES ALIVE
DATE		1	2	3	4	5	6	7	8	9	10			
	DAY 0													
	DAY 1													
	DAY 2													
	DAY 3													
	DAY 4													
	DAY 5													
	DAY 6													
	DAY 7													
	DAY 8													
TOTAL														

SURVIVAL AND REPRODUCTION DATA FOR 3-BROOD CHRONIC TEST WITH CERIODAPHNIA DUBIA

CONC:		REPLICATES										# NEONATES PER DAY	# ADULTS ALIVE	# FEMALES ALIVE
DATE		1	2	3	4	5	6	7	8	9	10			
	DAY 0													
	DAY 1													
	DAY 2													
	DAY 3													
	DAY 4													
	DAY 5													
	DAY 6													
	DAY 7													
	DAY 8													
TOTAL														

✔ = Test Organism Alive 0 = Live neonates M = Lost or Missing

X = Test Organism Dead (-0) = Dead neonates Y = Male ♂

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th TEST START DATE/TIME: _____

COMPLIANCE ENDPOINT: _____ TEST END DATE/TIME: _____

SURVIVAL AND REPRODUCTION DATA FOR 3-BROOD CHRONIC TEST WITH CERIODAPHNIA DUBIA

CONC:		REPLICATES										# NEONATES PER DAY	# ADULTS ALIVE	# FEMALES ALIVE
DATE		1	2	3	4	5	6	7	8	9	10			
	DAY 0													
	DAY 1													
	DAY 2													
	DAY 3													
	DAY 4													
	DAY 5													
	DAY 6													
	DAY 7													
	DAY 8													
TOTAL														

SURVIVAL AND REPRODUCTION DATA FOR 3-BROOD CHRONIC TEST WITH CERIODAPHNIA DUBIA

CONC:		REPLICATES										# NEONATES PER DAY	# ADULTS ALIVE	# FEMALES ALIVE
DATE		1	2	3	4	5	6	7	8	9	10			
	DAY 0													
	DAY 1													
	DAY 2													
	DAY 3													
	DAY 4													
	DAY 5													
	DAY 6													
	DAY 7													
	DAY 8													
TOTAL														

✔ = Test Organism Alive
X = Test Organism Dead
0 = Live neonates
(-0) = Dead neonates
M = Lost or Missing
Y = Male

Chart for Recording *Ceriodaphnia dubia* Survival and Neonate Production

Row A	1	11	21	31	41	51
Day 0						
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						
Day 8						
Total						

Row B	2	12	22	32	42	52
Day 0						
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						
Day 8						
Total						

Row C	3	13	23	33	43	53
Day 0						
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						
Day 8						
Total						

Row D	4	14	24	34	44	54
Day 0						
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						
Day 8						
Total						

Row E	5	15	25	35	45	55
Day 0						
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						
Day 8						
Total						

Row F	6	16	26	36	46	56
Day 0						
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						
Day 8						
Total						

Row G	7	17	27	37	47	57
Day 0						
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						
Day 8						
Total						

Row H	8	18	28	38	48	58
Day 0						
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						
Day 8						
Total						

Row I	9	19	29	39	49	59
Day 0						
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						
Day 8						
Total						

Row J	10	20	30	40	50	60
Day 0						
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						
Day 8						
Total						

✓ = Test Organism Alive 0 = Live Neonate M = Lost or Missing Template Number: _____

X = Test Organism Dead (0) = Dead Neonate Y = Male ♂

Permittee: _____ VPDES Number: _____ Outfall Number: _____

Test Start Date/Time: _____ Test End Date/Time: _____

CHRONIC 7-DAY STATIC RENEWAL SURVIVAL AND GROWTH TEST WITH
CYPRINODON VARIEGATUS

Test dilutions grouped by parameter (this method of reporting is preferred)

There are three forms to use; one has DO and pH on it, the second one has temperature and salinity, and the third form has alkalinity, hardness, chlorine residual and sample holding time documentation.

1 The next form is for recording survival of the larvae daily, starting with test initiation (Day 0).

2 The next form is for recording the weights of the larvae.

3 A test summary sheet is included to record information not already included on the bench sheets.

4 There should be a minimum of 3 chain-of-custody forms (one per sample) to go with the samples required for a chronic test.

5 The statistics should be attached to the test report.

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th ORGANISM SOURCE/ AGE: _____

COMPLIANCE ENDPOINT: _____ TEST START DATE/TIME: _____ TEST END DATE/TIME: _____

WATER CHEMISTRY FOR CHRONIC SURVIVAL AND GROWTH TEST WITH CYPRINODON VARIEGATUS

PARAMETER: DISSOLVED OXYGEN, mg/l

DATE														
DAY	DAY 0	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7
HOURS	0	24	0	24	0	24	0	24	0	24	0	24	0	24
CONTROL														
DIL #1														
DIL #2														
DIL #3														
DIL #4														
DIL #5														
ADJUSTMENTS:														

PARAMETER: pH

CONTROL														
DIL #1														
DIL #2														
DIL #3														
DIL #4														
DIL #5														
COMMENTS:														
TECH/TIME:														

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th ORGANISM SOURCE/AGE: _____

COMPLIANCE ENDPOINT: _____ TEST START DATE/TIME: _____ TEST END DATE/TIME: _____

WATER CHEMISTRY FOR CHRONIC SURVIVAL AND GROWTH TEST WITH CYPRINODON VARIEGATUS														
PARAMETER: TEMPERATURE, °C														
DATE														
DAY	DAY 0	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7
HOURS	0	24	0	24	0	24	0	24	0	24	0	24	0	24
CONTROL														
DIL #1														
DIL #2														
DIL #3														
DIL #4														
DIL #5														
COMMENTS:														
PARAMETER: SALINITY °/oo														
CONTROL														
DIL #1														
DIL #2														
DIL #3														
DIL #4														
DIL #5														
COMMENTS:														
TECH/TIME:														

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th ORGANISM SOURCE/AGE: _____

COMPLIANCE ENDPOINT: _____ TEST START (DATE/TIME): _____ TEST END (DATE/TIME): _____

WATER CHEMISTRY FOR CHRONIC SURVIVAL AND GROWTH TEST WITH CYPRINODON VARIEGATUS														
PARAMETER: ALKALINITY, mg/l $CaCO_3$														
DATE														
DAY	DAY 0	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7
HOURS	0	24	0	24	0	24	0	24	0	24	0	24	0	24
CONTROL														
DIL #5														
PARAMETER: HARDNESS, mg/l $CaCO_3$														
CONTROL														
DIL #5														
PARAMETER: CHLORINE RESIDUAL, mg/l														
CONTROL														
DIL #5														
TECH/TIME														
COMMENTS:														

PARAMETER: SAMPLE HOLDING TIME DOCUMENTATION							
SAMPLE DATE(S) AND TIME(S)							
DATE/TIME OF USE							
AGE OF SAMPLE (HRS)							

COMMENTS:

PERMITTEE: ______________________________ LABORATORY: ______________________

NPDES#: _________ OUTFALL#: ____________ LOCATION: ___________________________

PURPOSE OF TEST: ________________________ ORGANISM SOURCE/AGE: ______________

COMPLIANCE ENDPOINT: ___________________ TEST DATES: _________________________

CHRONIC 7-DAY SURVIVAL AND GROWTH TEST WITH CYPRINODON VARIEGATUS							
		WEIGHT DATA FOR SURVIVING LARVAE					AVERAGE WEIGHT OF LARVAE PER CONC
		A WEIGHT OF BOAT (mg)	**B DRY WT BOAT AND LARVAE (mg)**	**B-A TOTAL DRY WT OF LARVAE (mg)**	**C # OF LARVAE PER REP**	**(B-A)/C MEAN DRY WT OF LARVAE (mg)**	
CONTROL	**A**						Average Wt of Surviving Controls:________
	B						
	C						
	D						
CONC:	**A**						
	B						
	C						
	D						
CONC:	**A**						
	B						
	C						
	D						
CONC:	**A**						
	B						
	C						
	D						
CONC:	**A**						
	B						
	C						
	D						
CONC:	**A**						
	B						
	C						
	D						
CONC:	**A**						
	B						
	C						
	D						

TEST END DATE/TIME:_________ DATE WEIGHED: _____ OVEN TEMP: ___°C DRYING TIME: ___HRS

TECH: ______

PERMITTEE: ____________________ LABORATORY: ____________________

NPDES#: __________ OUTFALL#: __________ LOCATION: ____________________

PURPOSE OF TEST: ____________________ ORGANISM SOURCE/AGE: ____________________

COMPLIANCE ENDPOINT: ____________________ TEST DATES: ____________________

CHRONIC 7-DAY SURVIVAL AND GROWTH TEST WITH CYPRINODON VARIEGATUS										
Day of test		NUMBER OF SURVIVING LARVAE PER DAY								# LARVAE PER CONC
Date		**DAY 0**	**DAY 1**	**DAY 2**	**DAY 3**	**DAY 4**	**DAY 5**	**DAY 6**	**DAY 7**	
CONTROL	A									
	B									
	C									
	D									
CONC:	A									
	B									
	C									
	D									
CONC:	A									
	B									
	C									
	D									
CONC:	A									
	B									
	C									
	D									
CONC:	A									
	B									
	C									
	D									
CONC:	A									
	B									
	C									
	D									
CONC:	A									
	B									
	C									
	D									
TECH/TIME										

COMMENTS:

CHRONIC 7-DAY SURVIVAL, GROWTH AND FECUNDITY TEST WITH MYSIDOPSIS BAHIA

Test dilutions grouped by parameter (this method of reporting is preferred)

There are three forms to use; one has DO and pH on it, the second one has temperature and salinity, and the third form has alkalinity, hardness, chlorine residual and sample holding time documentation.

1 The next form is for recording survival of the mysids daily, starting with test initiation (Day 0).

2 The next form is for recording the weights of the mysids.

3 A test summary sheet is included to record information not already included on the bench sheets.

4 There should be a minimum of 3 chain-of-custody forms (one per sample) to go with the samples required for a chronic test.

5 The statistics should be attached to the test report.

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th ORGANISM SOURCE/AGE: _____

COMPLIANCE ENDPOINT: _____ TEST START DATE/TIME: _____ TEST END DATE/TIME: _____

WATER CHEMISTRY FOR CHRONIC SURVIVAL, GROWTH AND FECUNDITY TEST WITH MYSIDOPSIS BAHIA														
PARAMETER: DISSOLVED OXYGEN, mg/l														
DATE														
DAY	DAY 0	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7
HOURS	0	24	0	24	0	24	0	24	0	24	0	24	0	24
CONTROL														
DIL #1														
DIL #2														
DIL #3														
DIL #4														
DIL #5														
ADJUSTMENTS:														
PARAMETER: pH														
CONTROL														
DIL #1														
DIL #2														
DIL #3														
DIL #4														
DIL #5														
COMMENTS:														
TECH/TIME:														

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th ORGANISM SOURCE/AGE: _____

COMPLIANCE ENDPOINT: _____ TEST START DATE/TIME: _____ TEST END DATE/TIME: _____

WATER CHEMISTRY FOR CHRONIC SURVIVAL , GROWTH AND FECUNDITY TEST WITH MYSIDOPSIS BAHIA														
PARAMETER: TEMPERATURE, °C														
DATE														
DAY	DAY 0	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7
HOURS	0	24	0	24	0	24	0	24	0	24	0	24	0	24
CONTROL														
DIL #1														
DIL #2														
DIL #3														
DIL #4														
DIL #5														
COMMENTS:														
PARAMETER: SALINITY °/oo														
CONTROL														
DIL #1														
DIL #2														
DIL #3														
DIL #4														
DIL #5														
COMMENTS:														
TECH/TIME:														

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: Qt Semi/An An Retest 1st 2nd 3rd 4th 5th ORGANISM SOURCE/AGE: _____

COMPLIANCE ENDPOINT: _____ TEST START (DATE/TIME): _____ TEST END (DATE/TIME): _____

WATER CHEMISTRY FOR CHRONIC SURVIVAL , GROWTH AND FECUNDITY TEST WITH MYSIDOPSIS BAHIA														
PARAMETER: ALKALINITY, mg/l $CaCO_3$														
DATE														
DAY	DAY 0	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7
HOURS	0	24	0	24	0	24	0	24	0	24	0	24	0	24
CONTROL														
DIL #5														
PARAMETER: HARDNESS, mg/l $CaCO_3$														
CONTROL														
DIL #5														
PARAMETER: CHLORINE RESIDUAL, mg/l														
CONTROL														
DIL #5														
TECH/TIME														
COMMENTS:														

PARAMETER: SAMPLE HOLDING TIME DOCUMENTATION							
SAMPLE DATE(S) AND TIME(S)							
DATE/TIME OF USE							
AGE OF SAMPLE (HRS)							

COMMENTS:

PERMITTEE: _____ LABORATORY: _____

NPDES#: _____ OUTFALL#: _____ LOCATION: _____

JRPOSE OF TEST: _____ TEST START DATE/TIME: _____

COMPLIANCE ENDPOINT: _____ TEST END DATE/TIME: _____

CHRONIC 7-DAY SURVIVAL, GROWTH AND FECUNDITY TEST WITH MYSIDOPSIS BAHIA

		WEIGHT DATA FOR SURVIVING MYSIDS					AVERAGE WEIGHT OF MYSIDS PER CONC
		A **WEIGHT OF BOAT (mg)**	*B* **DRY WT BOAT AND MYSIDS (mg)**	*B-A* **TOTAL DRY WT OF MYSIDS (mg)**	*C* **# OF MYSIDS PER REP**	**(B-A)/C MEAN DRY WT OF MYSIDS (mg)**	
CONTROL	**A**						
	B						
	C						
	D						
	E						
	F						
	G						
	H						
CONC:	**A**						
	B						
	C						
	D						
	E						
	F						
	G						
	H						
CONC:	**A**						
	B						
	C						
	D						
	E						
	F						
	G						
	H						

CHRONIC 7-DAY SURVIVAL, GROWTH AND FECUNDITY TEST WITH MYSIDOPSIS BAHIA

		WEIGHT DATA FOR SURVIVING MYSIDS					AVERAGE WEIGHT OF MYSIDS PER CONC
		A **WEIGHT OF BOAT (mg)**	B **DRY WT BOAT AND MYSIDS (mg)**	B-A **TOTAL DRY WT OF MYSIDS (mg)**	C **# OF MYSIDS PER REP**	**(B-A)/C MEAN DRY WT OF SURV MYSIDS (mg)**	
CONC:	**A**						
	B						
	C						
	D						
	E						
	F						
	G						
	H						
CONC:	**A**						
	B						
	C						
	D						
	E						
	F						
	G						
	H						
CONC:	**A**						
	B						
	C						
	D						
	E						
	F						
	G						
	H						
CONC:	**A**						
	B						
	C						
	D						
	E						
	F						
	G						
	H						

TEST END DATE/TIME:_________ DATE WEIGHED: _____ OVEN TEMP: ___°C

RYING TIME: ___HRS TECH: ________

PERMITTEE: _____ LABORATORY: _____

PDES#: _____ OUTFALL#: _____ LOCATION: _____

PURPOSE OF TEST: _____ TEST START DATE/TIME: _____

COMPLIANCE ENDPOINT: _____ TEST END DATE/TIME: _____

CHRONIC 7-DAY SURVIVAL, GROWTH AND FECUNDITY TEST WITH MYSIDOPSIS BAHIA

Day of test Date		NUMBER OF SURVIVING MYSIDS PER DAY								FEMALE WITH EGGS	FEMALE NO EGGS	MALE	NOT MATURE
		DAY 0 / /	DAY 1 / /	DAY 2 / /	DAY 3 / /	DAY 4 / /	DAY 5 / /	DAY 6 / /	DAY 7 / /				
CONTROL	A												
	B												
	C												
	D												
	E												
	F												
	G												
	H												
Totals													
CONC:	A												
	B												
	C												
	D												
	E												
	F												
	G												
	H												
Totals													
CONC:	A												
	B												
	C												
	D												
	E												
	F												
	G												
	H												
Totals													

CHRONIC 7-DAY SURVIVAL, GROWTH AND FECUNDITY TEST WITH MYSIDOPSIS BAHIA

Day of test Date		NUMBER OF SURVIVING MYSIDS PER DAY								FEMALE WITH EGGS	FEMALE NO EGGS	MALE	NOT MATURE
		DAY 0 / /	DAY 1 / /	DAY 2 / /	DAY 3 / /	DAY 4 / /	DAY 5 / /	DAY 6 / /	DAY 7 / /				
CONC:	A												
	B												
	C												
	D												
	E												
	F												
	G												
	H												
Totals													
CONC:	A												
	B												
	C												
	D												
	E												
	F												
	G												
	H												
Totals													
CONC:	A												
	B												
	C												
	D												
	E												
	F												
	G												
	H												
Totals													
CONC:	A												
	B												
	C												
	D												
	E												
	F												
	G												
	H												
Totals													
CH/TIME													

ACUTE TEST DATA REVIEW CHECKLIST

ermit Number VA_____ Outfall_____ Permittee _____

Test Date _____ Period Reviewed: QT ___ SA ___ AN ___ Other_____
1st ___ 2nd ___ 3rd ___ 4th ___

Testing Laboratory _____

#	ACUTE DATA PARAMETER - (Some are organism specific)	YES	NO
1.	Was the test performed as per schedule?		
2.	Was the correct test performed?		
3.	**Was the correct type of sample used?**		
4.	Were pH, temp, Cl of sample checked at sample site (or within 15 minutes of sample retrieval)?		
5.	**Was the sample packed in ice and chilled to ≤4° C for transport? NOTE: Frozen samples are not valid!**		
6.	Were pH, DO, Cl, temperature and sample description recorded upon receipt?		
7.	Does description (visual, scent) of sample (when received at lab) seem typical for this type of facility?		
8.	**Was the test initiated within 36 hours of sample retrieval from sampler?**		
9.	a. Was the sample DO ≥ 4.0 mg/l and ≤ saturation at 25° C prior to test initiation? (applies to *D. ulex, C. dubia, M. bahia, P. promelas, C. variegatus*) b. Was the sample DO ≥ 6.0 mg/l and ≤ saturation at 12° C prior to test initiation? (applies to *O. ykiss*)		
10.	If 9 is "NO", was the DO adjusted to the acceptable range (see a. and b. above) prior to test initiation?		
11.	If the sample had a chlorine residual, was it dechlorinated?		
12.	**Did the permit allow for dechlorination of the sample? (Only if it contains a compliance schedule for Cl limit or for dechlorination)**		
13.	If the sample was dechlorinated, were controls treated with the same amount of dechlorination agent and run with untreated controls? (determines adverse effect of agent)		
14.	Was the sample pH within the 6.0 - 9.0 range?		
15.	**Was the age of the organisms in the correct range at test initiation?** ***a.*** ***P. promelas and C. variegatus* - 1-14 days old, within 24 hours of age of each other** ***b.*** ***O. mykiss* - 15-30 days old** ***c.*** ***D. pulex and C. dubia* - <24 hours old** ***d.*** ***M. bahia* - 1-5 days old, within 24 hours of age of each other**		
16.	Were 5 geometric test concentrations (preferably 0.5 series) and 1 control set up?		
17.	Was the test chamber size acceptable? *a.* *P. promelas, C. variegatus, M. bahia* - 250 ml minimum *b.* *O. mykiss* - 5000 ml minimum *c.* *D. pulex and C. dubia* - 30 ml minimum		
18.	Was the sample volume acceptable? *a.* *P. promelas, C. variegatus, M. bahia* - 200 ml minimum *b.* *O. mykiss* - 4000 ml minimum *c.* *D. pulex* - 25 ml minimum		

#	ACUTE DATA PARAMETER - (Some are organism specific)	YES	NO
	d. *C. dubia* - 15 ml minimum		
19.	Was the minimum number of replicates per concentration represented? *a.* 2 replicates - *P. promelas, O. mykiss* *C. variegatus, M. bahia* *b.* 4 replicates - *D. pulex, C. dubia*		
20.	Was the minimum number of organisms in each replicate? *a.* 10 organisms - *P. promelas, O. mykiss* *C. variegatus, M. bahia* *b.* 5 organisms - *D. pulex, C. dubia*		
21.	a. Was the dilution water synthetic moderately hard water or 20% DMW? (applies to freshwater pecies *P. promelas, O. mykiss, D. pulex, C. dubia)* b. Was the dilution water synthetic moderately hard water or 20% DMW that had been adjusted to 20 2 ppt, or the same salinity as the receiving water? (applies to salt water species, *C. variegatus, M. bahia)*		
22.	Was the dilution water hardness within the 80-100 mg $CaCO_3$/L?		
23.	Was the dilution water hardness within the 60-70 mg $CaCO_3$/L?		
24.	Was the dilution water pH within the range of 7.4 – 7.8 (7.9 – 8.3 for mineral water)?		
25.	**a. Was the test temperature 25±1° C upon initiation, and throughout the test? (applies to *P. romelas, D. pulex, C. dubia C. variegatus, M. bahia)*** **b. Was the test temperature 12±1° C upon initiation, and throughout the test? *(applies to O. ykiss)***		
26.	**Was the temperature measured daily in one replicate of each concentration?**		
27.	Was the DO measured daily in one replicate of each concentration? (Exceptions to this requirement are for tests using *D. pulex* or *C. dubia,* where the 24-hr DO reading can be omitted to prevent organism stress.)		
28.	If the DO dropped to <4.0 mg/l, was aeration initiated? (Exceptions to this requirement are for tests *using D. pulex or C. dubia,* where aeration is impractical.)		
29.	If aeration was necessary (and acceptable), were **all** test chambers aerated for the duration of the test, and the time at which aeration was initiated recorded?		
30.	If aeration was necessary (and acceptable), was it applied at a maximum rate of 100 bubbles/minute so as not to cause injury to the organisms?		
31.	Was pH measured at the beginning and end of the test (daily is optimal) for a 48-hour test, or at 0, 48 hours, after renewal, and at 96 hours for a 96-hour test in one replicate of each sample concentration?		
32.	a. For a freshwater test, was conductivity measured at the beginning and end (also at renewal for 96-our tests) of the test in one replicate of each concentration? (applies to freshwater species *P. promelas, O. ykiss, D. pulex, C. dubia)* b. For a saltwater test, was salinity measured at the beginning and end (also at renewal for 96-hour sts) of the test in one replicate of each concentration? (applies to salt water species, *C. variegatus, M. ahia)*		
33.	For freshwater tests, was the alkalinity measured in 100% effluent and the control at the beginning of the test?		
34.	For freshwater tests, was the hardness measured in 100% effluent and the control at the beginning of the test?		
35.	a. For a test using *Mysidopsis bahia*, were the mysids fed *Artemia* nauplii daily? b. For a 96-hour test using either *Pimephales promelas* or *Cyprinodon variegatus*, were the larvae ed prior to sample renewal at 48 hours?		
36.	For a 96-hour test using either *Pimephales promelas* or *Cyprinodon variegatus*, was the sample used for		

#	ACUTE DATA PARAMETER - (Some are organism specific)	YES	NO
	renewal the original sample?		
37.	Was the daily photoperiod 16 hours light/8 hours dark?		
38.	Were the surviving organisms counted daily in all test chambers?		
39.	Was the test terminated at 48±1 hours (less than 47 hours invalidates the test) or 96±1 hours (less than 95 hours invalidates the test)?		
40.	Was the percent survival in each concentration recorded at the end of the test?		
41.	**Was the percent survival in the controls ≥90%?**		
42.	Was the LC_{50} correctly determined?		
43.	If the acute test was run in conjunction with a chronic test using the same species, was the acute test initiated with the second or third sample pulled for the chronic test? (Any sample other than the same sample used to initiate the chronic test is acceptable.)		

Items in bold type (and shaded) are significant in that if they are answered "NO", the test is automatically deemed "not acceptable" and must be repeated to fulfill permit TMP requirements. Bold type items are numbers 3, 5, 8, 12, 15, 25, 26, and 41.

RESPONSE GUIDE

1. - 8.	Response should be "YES" or note the problem in the review
9. - 10.	If 9. is "NO", then 10. must be "YES" or the test is not acceptable
11. - 13.	If 11. is "YES", then 12. and 13. must be "YES" or the test is not acceptable
14. - 17.	If 14. is "NO", then 15., 16. and 17 must be "YES" or the test is not acceptable
18. - 43.	Response should be "YES" or note the problem in the review

RATING

ACCEPTABLE	NOT ACCEPTABLE

Comments

CHRONIC TEST DATA REVIEW CHECKLIST

Permit Number VA_____ Outfall _____ Permittee _____

Test Start Date _____ Period Reviewed: QT ___ SA ___ AN ___ Other_____
1st ___ 2nd ___ 3rd ___ 4th ___

Testing Laboratory _____

#	CHRONIC DATA PARAMETERS - (Some are organism specific)	YES	NO
1.	Was the test performed as per schedule?		
2.	Was the correct test performed?		
3.	**Was the correct type of sample collected at each sampling event?**		
4.	**Was a minimum of 3 samples collected?**		
5.	Were pH, temp, Cl of sample checked at sample site (or within 15 minutes of sample retrieval) for each sample?		
6.	**Was each sample packed in ice and chilled to ≤4° C for transport? NOTE: Frozen samples are not valid!**		
7.	Were pH, DO, Cl, temperature and sample description recorded upon receipt of each sample?		
8.	Does the description (visual, scent) of each sample (when received at lab) seem typical for this type of facility?		
9.	**Was the test initiated within 36 hours of sample retrieval from sampler? Was the first use of a sample for renewal within 36 hours?**		
10.	**Was the last use of the sample within 72 hours of retrieval from the sample device?**		
11.	a. Was the sample DO ≥ 4.0 mg/l and ≤ saturation at 25° C prior to test initiation? (applies *to C. dubia* and *P. promelas)* b. Was the sample DO ≥ 6.0 mg/l and ≤ saturation at 25° C prior to test initiation? (applies *to C. variegatus* and *M. bahia)*		
12.	If "11." is "NO", was the DO adjusted to the acceptable range (see a., and b. above) prior to test initiation?		
13.	If the sample had a chlorine residual, was it dechlorinated?		
14.	**Did the permit allow for dechlorination of the sample? (Only if it contains a compliance schedule for a chlorine limit or for dechlorination)**		
15.	If the sample was dechlorinated, were controls treated with the same amount of dechlorination agent and run with untreated controls? (This determines any adverse effect of the dechlorination agent.)		
16.	Was each sample pH within the 6.0 - 9.0 range?		
17.	**Was the age of the organisms in the correct range at test initiation?** ***a.*** ***P. promelas* and *C. variegatus* - <24 hours old (24-48 hours old is acceptable if the organisms were shipped in from an outside source)** ***c.*** ***C. dubia* - <24 hours old, within 8 hours of age of each other** ***d.*** ***M. bahia* - 7 days old, within 24 hours of age of each other**		
18.	Was a minimum of 5 geometric test concentrations and 1 control set up?		
19.	Was the test chamber size acceptable? *a.* *P. promelas* - 500 ml minimum *b.* *C. variegatus* - 300-1000 ml		

#	CHRONIC DATA PARAMETERS - (Some are organism specific)	YES	NO
	c. *M. bahia* - 400 ml *d.* *C. dubia* - 30 ml minimum		
20.	Was the sample volume acceptable? *a.* *P. promelas* - 250 ml minimum *b.* *C. variegatus* - 250-750 ml *c.* *M. bahia* - 150 ml *d.* *C. dubia* - 15 ml minimum		
21.	Was the minimum number of replicates per concentration represented? *a.* 3 replicates (4 preferred) - *P. promelas, C. variegatus* *b.* 8 replicates - *M. bahia* *c.* 10 replicates - *C. dubia*		
22.	Was the minimum number of organisms in each replicate? *a.* 10 organisms - *P. promelas, C. variegatus,* *b.* 5 organisms - *M. bahia* *c.* 1 organism - *C. dubia*		
23.	a. Was the dilution water synthetic moderately hard water or 20% DMW? (applies to freshwater species *P. promelas, C. dubia)* b. Was the dilution water synthetic moderately hard water or 20% DMW that had been adjusted to 20 ± 2 ppt, or the same salinity as the receiving water? *(*applies to salt water species, *C. variegatus, M. bahia)*		
24.	Freshwater - Was the dilution water hardness within the 80-100 mg $CaCO_3$/L?		
25.	Freshwater - Was the dilution water hardness within the 60-70 mg $CaCO_3$/L?		
26.	Freshwater - Was the dilution water pH within the range of 7.4 – 7.8 (7.9 – 8.3 for mineral water)?		
27.	Saltwater – was the salinity 20±2 ppt?		
28.	**a. Was the test temperature 25±1° C upon initiation, and throughout the test (applies to *P. promelas, C. dubia and C. variegatus)*?** **b. Was the test temperature 26±1° C upon initiation, and throughout the test (applies to *M. bahia)*?**		
29.	**Was the temperature measured daily in one replicate of each concentration?**		
30.	Was the DO measured daily, before and after renewal in one replicate of each concentration?		
31.	a. If the DO dropped to <4.0 mg/l in a test using *P. promelas*, was aeration initiated? For a test using *C. dubia*, a low DO sample should be aerated prior to test initiation or renewal, as aeration with the organisms present is impractical.) b. If the DO dropped to ≤6.0 mg/l in a saltwater test, was aeration initiated?		
32.	If aeration was necessary (and acceptable), were **all** test chambers aerated for the duration of the test, and the time at which aeration was initiated recorded? (Not applicable to tests using *C. dubia*)		
33.	If aeration was necessary (and acceptable), was it applied at a maximum rate of 100 bubbles/minute so as not to cause injury to the organisms?		
34.	Was pH measured at test initiation, and before and after sample renewal in one replicate of each concentration?		
35.	For salt water test using *M. bahia*, was ammonia and nitrite measured prior to renewal in one replicate of each concentration?		
36.	a. For a freshwater test, was conductivity measured at the beginning of each 24-hour period in one replicate of each concentration? *(*applies to freshwater species *P. promelas, C. dubia)* b. For a saltwater test, was the salinity measured at the beginning of each 24-hour period in one replicate of each concentration? *(*applies to salt water species, *C. variegatus, M. bahia)*		

#	CHRONIC DATA PARAMETERS - (Some are organism specific)	YES	NO
37.	For freshwater tests, was the alkalinity measured in 100% effluent and the control at test initiation, and for each new sample?		
38.	For freshwater tests, was the hardness measured in 100% effluent and the control at test initiation, and for each new sample?		
39.	a. For a test using *Mysidopsis bahia*, were the mysids fed *Artemia* nauplii (at a rate of 75/mysid) twice daily? b. For a test using *Pimephales promelas*, were the larvae fed 0.15 ml concentrated *Artemia* nauplii a minimum of twice daily? c. For a test using *Cyprinodon variegatus*, were the larvae fed *Artemia* nauplii once per day at a rate of 0.1 g (wet weight) for days 0-2, and 0.15 g (wet weight) for days 3-6? d. For a test using *Ceriodaphnia dubia*, were the organisms fed 0.1 ml YCT and 0.1 ml algae per day after renewal?		
40.	Was the sample data for the renewal days consistent with the data for the first use of that sample?		
41.	Was the daily photoperiod 16 hours light/8 hours dark?		
42.	Were the surviving organisms counted daily in all test chambers?		
43.	Were the number of young produced recorded daily for the *C. dubia* test?		
44.	Was the occurrence of males noted in the *C. dubia* test?		
45.	Were the daily renewals of chronic test solutions performed no earlier or later than subsequent 24±2 hour periods from test initiation?		
46.	**a. For tests using *P. promelas, C. variegatus*, or *M. bahia*, was the test terminated 7 days (this is interpreted as 7 24-hour periods) and within ± 1 hour of the time of day at which it was initiated?** **b. For tests using *C. dubia*, was the test terminated when 60% or more of the surviving females in the controls had produced their third brood within 8 days?**		
47.	Was the percent survival in each concentration recorded at the end of the test?		
48.	**Was the percent survival in the controls ≥80%?**		
49.	**Did the test meet the additional acceptability criteria?** **a. *P. promelas* - For tests initiated with larvae ≤ 24 hours old, was the average dry weight of the control larvae surviving at the end of the test ≥ 0.25 mg?** **b. *C. variegatus* - For tests initiated with larvae ≤ 24 hours old, was the average dry weight of control larvae ≥ 0.60 mg (unpreserved), or ≥ 0.50 mg (preserved)?** **c. *M. bahia* - Was the average weight of the controls ≥ 0.20 mg?** **d. *C. dubia* - Did reproduction in the controls average 15 or more young per surviving female?**		
50.	Were the data Arcsin transformed prior to statistical analysis (*M. bahia* – survival and growth, *C. variegatus* – survival, *P. promelas* – survival)?		
51.	Was the NOEC correctly determined using the appropriate statistical method?		
52.	Did the test result in a calculable NOEC (Result reported as "<" is not acceptable. Lower dilutions should have been added or the test rerun to determine the result.)		
53.	Was the IC_{25} reported for the test?		
54.	Was the LC_{50} at 48 hours reported for the test?		

Items in bold type (and shaded) are significant in that if they are answered "NO", the test is automatically invalidated and must be repeated to fulfill permit TMP requirements. Bold type items are numbers 3, 4, 6, 9, 10, 14, 17, 28, 29, 46, 48, and 49.

RESPONSE GUIDE

1. - 10. Response should be "YES" or note the problem in the review
11. - 12. If 11. is "NO", then 12. must be "YES" or the test is subject to invalidation
13. - 15. If 13. is "YES", then 14. and 15. must be "YES" or the test is subject to invalidation
20. - 54. Response should be "YES" or note the problem in the review

RESULTS

ACCEPTABLE	NOT ACCEPTABLE

COMMENTS:

APPENDIX C

DILUTION SERIES CHART

Appendix C

Either of the methods below can be used to determine test dilutions. If the Geometric method is used, find the endpoint/limit you want under the columns Conc 2, 3 or 4, and use the row of dilutions. For the RWC method, find the endpoint in Conc 3 column nd use the dilutions in that row. NOTE: The geometric series is preferred.
If you prefer, use the calculation at the bottom of this spreadsheet to put in your NOEC endpoint, and the dilutions will calculate for you. It also appears on page 3. of WETLIM10.xls

Geometric Dilution Series with 5 Concentrations

Series	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5
0.99	100	99	98	97	96
0.98	100	98	96	94	92
0.97	100	97	94	91	89
0.96	100	96	92	88	85
0.95	100	95	90	86	81
0.94	100	94	88	83	78
0.93	100	93	86	80	75
0.92	100	92	85	78	72
0.91	100	91	83	75	69
0.9	100	90	81	73	66
0.89	100	89	79	70	63
0.88	100	88	77	68	60
0.87	100	87	76	66	57
0.86	100	86	74	64	55
0.85	100	85	72	61	52
0.84	100	84	71	59	50
0.83	100	83	69	57	47
0.82	100	82	67	55	45
0.81	100	81	66	53	43
0.8	100	80	64	51	41
0.79	100	79	62	49	39
0.78	100	78	61	47	37
0.77	100	77	59	46	35
0.76	100	76	58	44	33
0.75	100	75	56	42	32
0.74	100	74	55	41	30
0.73	100	73	53	39	28
0.72	100	72	52	37	27
0.71	100	71	50	36	25
0.7	100	70	49	34	24
0.69	100	69	48	33	23
0.68	100	68	46	31	21
0.67	100	67	45	30	20
0.66	100	66	44	29	19
0.65	100	65	42	27	18
0.64	100	64	41	26	17
0.63	100	63	40	25	16
0.62	100	62	38	24	15
0.61	100	61	37	23	14
0.6	100	60	36	22	13
0.59	100	59	35	21	12
0.58	100	58	34	20	11
0.57	100	57	32	19	11
0.56	100	56	31	18	10
0.55	100	55	30	17	9
0.54	100	54	29	16	9
0.53	100	53	28	15	8
0.52	100	52	27	14	7
0.51	100	51	26	13	7
0.5	100	50	25	13	6
0.49	100	49	24	12	6

RWC Method for setting dilutions

		Endpoint		
Conc 1	Conc 2	Conc 3	Conc 4	Conc 5
100	98	95	48	24
100	97	94	47	24
100	97	93	47	23
100	96	92	46	23
100	96	91	46	23
100	95	90	45	23
100	95	89	45	22
100	94	88	44	22
100	94	87	44	22
100	93	86	43	22
100	93	85	43	21
100	92	84	42	21
100	92	83	42	21
100	91	82	41	21
100	91	81	41	20
100	90	80	40	20
100	90	79	40	20
100	89	78	39	20
100	89	77	39	19
100	88	76	38	19
100	88	75	38	19
100	87	74	37	19
100	87	73	37	18
100	86	72	36	18
100	86	71	36	18
100	85	70	35	18
100	85	69	35	17
100	84	68	34	17
100	84	67	34	17
100	83	66	33	17
100	83	65	33	16
100	82	64	32	16
100	82	63	32	16
100	81	62	31	16
100	81	61	31	15
100	80	60	30	15
100	80	59	30	15
100	79	58	29	15
100	79	57	29	14
100	78	56	28	14
100	78	55	28	14
100	77	54	27	14
100	77	53	27	13
100	76	52	26	13
100	76	51	26	13
100	75	50	25	13
100	75	49	25	12
100	74	48	24	12
100	74	47	24	12
100	73	46	23	12
100	73	45	23	11

Series	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5
0.48	100	48	23	11	5
0.47	100	47	22	10	5
0.46	100	46	21	10	4
0.45	100	45	20	9	4
0.44	100	44	19	9	4
0.43	100	43	18	8	3
0.42	100	42	18	7	3
0.41	100	41	17	7	3
0.4	100	40	16	6	3
0.39	100	39	15	6	2
0.38	100	38	14	5	2
0.37	100	37	14	5	2
0.36	100	36	13	5	2
0.35	100	35	12	4	2
0.34	100	34	12	4	1
0.33	100	33	11	4	1
0.32	100	32	10	3	1
0.31	100	31	9.6	3.0	0.9
0.3	100	30	9.0	2.7	0.8
0.29	100	29	8.4	2.4	0.7
0.28	100	28	7.8	2.2	0.6
0.27	100	27	7.3	2.0	0.5
0.26	100	26	6.8	1.8	0.5
0.25	100	25	6.3	1.6	0.4
0.24	100	24	5.8	1.4	0.3
0.23	100	23	5.3	1.2	0.3
0.22	100	22	4.8	1.1	0.2
0.21	100	21	4.4	0.9	0.2
0.2	100	20	4.0	0.8	0.2
0.19	100	19	3.6	0.7	0.1
0.18	100	18	3.2	0.6	0.1
0.17	100	17	2.9	0.5	0.1
0.16	100	16	2.6	0.4	0.1
0.15	100	15	2.3	0.3	0.1
0.14	100	14	2.0	0.3	0.0
0.13	100	13	1.7	0.2	0.0
0.12	100	12	1.4	0.2	0.0
0.11	100	11	1.2	0.1	0.0
0.1	100	10	1.0	0.1	0.0

Conc 1	Conc 2	Conc 3	Conc 4	Conc 5
100	72	44	22	11
100	72	43	22	11
100	71	42	21	11
100	71	41	21	10
100	70	40	20	10
100	70	39	20	10
100	69	38	19	10
100	69	37	19	9
100	68	36	18	9
100	68	35	18	9
100	67	34	17	9
100	67	33	17	8
100	66	32	16	8
100	66	31	16	8
100	65	30	15	8
100	65	29	15	7
100	64	28	14	7
100	64	27	14	7
100	63	26	13	7
100	63	25	13	6
100	62	24	12	6
100	62	23	12	6
100	61	22	11	6
100	61	21	11	5
100	60	20	10	5
100	60	19	10	5
100	59	18	9	5
100	59	17	9	4
100	58	16	8	4
100	58	15	8	4
100	57	14	7	4
100	57	13	7	3
100	56	12	6	3
100	56	11	6	3
100	55	10	5	3
100	55	9	5	2
100	54	8	4	2
100	54	7	4	2
100	53	6	3	2
100	52.5	5.0	2.5	1.3
100	52.0	4.0	2.0	1.0
100	51.5	3.0	1.5	0.8
100	51.0	2.0	1.0	0.5
100	50.5	1.0	0.5	0.3

Enter endpoint to get dilution factor and series!

Enter endpoint to meet: 75 This will be the middle dilution

Dilution factor to use: 0.86603

Dilution series to use:

100.0
86.6
75.0
65.0
56.3
48.7
42.2
36.5
31.6

APPENDIX D

WET LIMIT CALCULATION

APPENDIX D

Calculating WET Limits

In order to maintain consistency between the methods for derivation of limits for specific chemicals and for whole effluent toxicity (WET), this guidance will follow the statistical process for WET limit development. The following discussion provides the procedure for deriving WET limits. A sample data set is used to illustrate the calculations involved.

WET limits are written in permits in terms of maximum values. The units for the permit are Toxic Units (TU's), either acute (TU_a) or chronic (TU_c). Because the statistical approach evaluates both acute and chronic toxicity of the effluent, one limit can be used to protect from both acute and chronic toxicity. (Note that there may be occasions where both an acute and chronic limit are needed.) The limit is expressed only as a maximum daily limit (MDL) because the frequency of monitoring will typically be less than once per month. If the testing is to be monthly, then the MDL can also be expressed as an average monthly limit (AML). If the testing is more frequent than monthly, contact OWPP-TMP for guidance on establishing the AML. Appendix E gives guidance on how to show the WET limit in Part I.A of the permit and it contains examples of special conditions which should accompany this limit.

EPA has finalized their document titled "*Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System Program", June 2000, EPA 833-R-00-003,* http://www.epa.gov/owm/wet/variable/index.htm . EPA recommends the statistical method for limit development. We have utilized a lot of this approach, with a few small differences that will be footnoted in this Appendix and discussed more in section 13.

A. WET limits for streams

Note that the procedures in this illustration assume complete mix in flowing streams. If some percentage of mix is allowed, multiply the percent mix with the 7Q10 and then the 1Q10 to determine the stream flows used in the calculations. The permit writer is cautioned to read the guidance in paragraph A 12 regarding setting permit limits when there is any doubt about the validity of the complete mix assumption. Special considerations also apply when discharges are intermittent and when instream waste concentrations are less than 1% or less than 30%.

Assumptions:

$\mathbf{Q_d}$ = Flow of discharge (municipals = design flow, industries = 30 day maximum) = **2.0 MGD**

$\mathbf{Q_s}$ = Flow of Stream: **1Q10 = 1.0 MGD**
7Q10 = 1.5 MGD

Water Quality Criteria instream: 0.3 TU_a = acute
1.0 TU_c = chronic,

No background toxicity in stream
All data are lognormally distributed

1. Determine instream waste concentrations

Determine acute IWC, or IWC_a :

$$\mathbf{IWC_a} = \frac{Q_d}{Q_d+(1Q10)} \times 100 = \frac{2.0}{2.0+1.0} \times 100 = \mathbf{66.67\%}$$

Determine chronic IWC, or IWC_c:

$$\mathbf{IWC_c} = \frac{Q_d}{Q_d+(7Q10)} \times 100 = \frac{2.0}{2.0+1.5} \times 100 = \mathbf{57.14\%}$$

2. Determine the acute and chronic dilutions:

Acute dilution = $100/IWC_a$ = 100/66.67 = 1.5

Chronic dilution = $100/IWC_c$ = 100/57.14 = 1.75

3. Determine the Waste Load Allocations:

Acute WLA: WLA_a = Acute instream criterion X Acute dilution

= 0.3 TU_a X 1.5

WLA_a = 0.45 TU_a

Chronic WLA: WLA_c = Chronic instream criterion X Chronic dilution

= 1.0 TU_c X 1.75

WLA_c = 1.75 TU_c

In order to use the acute and chronic waste load allocations to derive long-term averages and the WET limit, we need to have both of them in the same units. We will use the Acute: Chronic Ratio (ACR) to convert the acute WLA in TU_a

(WLA_a) to an acute WLA in TU_c ($WLA_{a,c}$) so that both allocations will be directly comparable. Use the following equation to convert the WLA_a:

Acute WLA expressed as a chronic WLA = WLA_a X ACR (default ACR is 10)

= 0.45 X 10

$WLA_{a,c}$ = 4.5 TU_c

NOTE: If you decide to use WLA.EXE to determine whether limits are needed for a discharger with an IWC > 1%, you will need to use the $WLA_{a,c}$ and the WLA_c so that everything is expressed in terms of chronic Toxic Units (TU_c). A discharger who only tests for acute toxicity will use the WLA_a in WLA.EXE, and the data are entered as TU_a 's.

4. Determine the Acute:Chronic Ratio

The objective of the WET limit is to provide protection against both acute and chronic toxicity in the receiving stream. This requires us to consider both the acute and chronic waste load allocations whenever possible. To do otherwise would require making an assumption about the unknown relationship between acute and chronic toxicity in the effluent. This could result in a limit that would not protect against both acute and chronic toxicity. In order to avoid this situation, we should never use an arbitrary or default ACR value if data are available. Only by knowing the real ratio can we be sure that the WET limit will be fully protective.

The ACR relates acute toxicity to chronic toxicity as follows:

ACR = LC_{50} / NOEC or LC_{50}/IC_{25}

The ACR for an effluent should be determined by making a direct comparison between acute and chronic data for the same species with tests run on the same dates. (I.e. if there was an acute test run during or just before a chronic test, divide the LC_{50} by the NOEC (or IC_{25}) for that species for those tests and get an ACR.) If there is more than one data pair, calculate individual ACRs, convert the ACR to a logarithm, take the geometric mean of the values, and then the antilog. If you can calculate ACRs for more than one species, **use the lowest ACR value in WET calculations**. It is optimal to utilize acute data that are performed at the same temperature as the chronic tests; therefore, if separate acute tests are not available, derive the acute data endpoint (LC_{50}) from the first 48 hours of the chronic test.

EXAMPLES:

For one pair of data, divide the LC_{50} by the NOEC to get the ACR. 50/25 = 2

LC_{50}	NOEC	ACR	Log of ACR	Geo Mean	Antilog
50	25	2	0.6931472	0.6931472	2

For multiple data pairs of the same species, the results would look like this:

LC_{50}	NOEC	ACR	Log of ACR	Geo Mean	Antilog
88	50	1.76	0.5653138	0.5653138	1.76
45	25	1.8	0.5877867	0.5764407	1.7796927 ◀

See Tables 1. and 2. on Page 3 of WETLIM10.xls, which will calculate the ACR from entered data. The resulting ACR would recalculate the $WLA_{a,c}$. Using the WLA_a of 0.45 (calculated in step 3.), multiply it by the ACR of 1.7796927 to get a $WLA_{a,c}$ of 0.8008617.

An LC_{50} reported as >100% is not very useful in this calculation because the resulting ACR is not a specific number. The actual LC_{50} is something between 100% and 333%. LC_{50}'s can be calculated from the first 48 hours of survival data from a chronic test, if necessary. This may be useful if the acute data set contains only LC_{50}s >100%. If all the chronic tests show NOEC's ≥ IWC, but not all NOEC's = 100%, then the calculations should still address chronic toxicity and the WET limit should be derived using both of the WLA's. In this case, there must be some acute toxicity, either in acute tests or in chronic tests at concentrations above the IWC. Therefore, there is a good likelihood that an actual ACR can be derived from the effluent data. If all LC_{50}s are >100% and there is no way to get an ACR from chronic data, then you can either consider that the effluent is not acutely toxic and only calculate a WLA_c in order to set a WET limit, or use the default value of 10 for the ACR for the relationship of the acute and chronic toxicity. (If the default ACR of 10 is used, the WLA_c will drive the derivation of the chronic limit.)

When the discharge is continuous and the IWC > 1%, but for some reason only acute toxicity test data are available, the limit should be based on only the acute toxicity data and the WLA_a. This limit would be in TU_a. The permittee should be required to conduct chronic toxicity tests so that a full evaluation of the discharge can be made. This chronic data can be generated prior to the WET limit going into the permit, after the permit is issued with the acute WET limit but during the compliance period, or in fulfillment of a separate TMP that requires the chronic testing after reissuance. Once the chronic data are available, the WET limit should be recalculated.

5. Calculate the Coefficient of Variation associated with the effluent toxicity test data:

CV = Coefficient of Variation.

Assuming a lognormal distribution of the data, consistent with our assumption for chemical effluent data, the observed coefficient of variation of the effluent data (CV) can be calculated with the computer program (WLA.EXE) used to evaluate chemical data described in Guidance Memo 93-015 and subsequent updates. **Note that there must be more than 10 data points before the program will calculate a CV. Less than 10 data points will utilize the default value of 0.6 for the CV.** Acute test data expressed as LC_{50}'s and chronic IC_{25}'s are considered here because they are statistically derived point estimates from continuous data sets. Also, the LC_{50}s and IC_{25}'s must be quantifiable and not "<" or ">" if WETLIM10.xls is to be used. **If there are censored (data reported at "<" or ">") data points in the data set of 10 or more data, it would be best to rely on WLA.EXE for calculating the CV for that data set.** NOECs are determined based on the dilution series employed by the laboratory and do not give a specific effect concentration estimate. Therefore, they are from noncontinuous data sets and are not useful in statistically evaluating the variability of the effluent. The chronic data can be entered into the ICP statistical program and an IC_{25} calculated, which can be used to determine an effluent CV. It has be recommended to the test labs (See Appendix B) that they provide the IC_{25} endpoint when they report the chronic test NOEC.

When the CV is calculated from fewer than 10 data points the error associated with the calculated value is too great and it is better to use an estimate based on the universe of effluent toxicity variability. This approach is consistent with that used for chemical data. Do not pool LC_{50}s for more than one species to calculate a CV for an effluent. Species sensitivity to the effluent may vary and this would cause the CV to be unreliable. **If there are less than 10 data points for any one species, assume CV = 0.6 and go to Step 11 in these instructions.**

6. Calculate Long Term Averages for both the WLA_c and the $WLA_{a,c}$ and choose the most limiting (smaller) one for determining limits:

a. $\mathbf{LTA_{a,c} = WLA_{a,c} \times e^{A}}$

$A = .5\delta^2 - Z\delta$ (refer to Page 100, Step 2, TSD)

$WLA_{a,c} = 4.5\ TU_c$

$Z = 1.881$ (97% probability statistic, from table[1])

[1] The draft EPA document uses the 99th percentile in the calculations in 6. And 7., which would

$\delta^2 = \ln(CV^2 + 1) = \ln((.6 \text{ X } .6) + 1) = \ln(.36 + 1) = 0.307485$
$\delta = \sqrt{(0.307485)} = 0.554513$

$A = .5(0.307485) - 1.881(0.554513)$
$= 0.1537423 - 1.043039 = -0.889297$
(e^x = Inv ln x)

$e^A = e^{-.889297} = 0.4109447$

$LTA_{a.c} = 4.5 \text{ X } 0.4109447 = 1.8492512 \text{ TU}_c$

b. **$LTA_c = WLA_c \text{ x } e^B$**

$B = .5\delta_4^2 - Z\delta_4$ (refer to Page 100, Step 2, TSD[1])

$\delta_4^2 = \ln[CV^2/4 + 1] = \ln[.6^2/4 + 1] = \ln[.09] = .0861777$

$\delta_4 = \sqrt{\delta_4^2} = .2935604$

$B = .5(.0861777) - 1.881(.2935604) = -0.509098$

$e^B = e^{-.509098} = .6010373$

$LTA_c = 1.75 \text{ X}.6010373 = 1.0518153 \text{ TU}_c$

Use LTA_c for calculating the limit in Step 7 and Step 8 because
1.0518153<1.8492512

7. Calculate Maximum Daily Limit:

$MDL = LTA \text{ x } e^C$
$C = Z\delta - .5\delta^2$ (refer to Page 100, Step 4, TSD[1])

$\delta^2 = \ln(CV^2 + 1) = \ln((.6 \text{ X } .6) + 1) = \ln(.36 + 1) = 0.307485$
$\delta = \sqrt{(0.307485)} = 0.554513$

$C = 1.881(0.554513) - .5(.307485)$
$= 1.043039 - 0.15374235 = 0.8892967$

$e^C = e^{.8892967} = 2.4334175$

---

make Z = 2.326

MDL = 1.0518153 X 2.43 = 2.559506 TU_c = NOEC 39.070044

8. Calculate the maximum daily and average monthly permit limit

AML = LTA x e^d
$D = Z\delta_n - .5\delta_n^2$ (refer to Page 100, Step 4, TSD[2])

N = number of samples[3] per month (in most cases, it will be 1, so the AML = MDL

$\delta^2 = \ln(CV^2/n+ 1) = \ln((.6 \text{ X } .6)/1 + 1) = \ln(.36 + 1) = 0.307485$

$\delta = \sqrt{(0.307485)} = 0.554513$

$D = 1.881(0.554513) - .5(.307485)$
$= 1.043039 - 0.15374235 = 0.8892967$

$e^D = e^{.8892967} = 2.4334175$

AML = 1.0518153 X 2.43 = 2.559506 TU_c = NOEC 39.070044

9. Permit limits, with the exception of the acute NOAEC test, should be expressed in terms of TU. It is a good idea to clarify what the TU limit means for the benefit of the permittee by indicating that it equates to an LC_{50}, or NOEC, in percent effluent. There are a couple of things to remember when stating a limit:

   a. It is not practical to expect a bioassay laboratory to measure effluents more precise than 0.1 ml. Therefore, the calculated NOEC should be rounded "up" to the nearest whole number where feasible. NOEC's of 10 % or less can be rounded to the nearest tenth. The permit limit should then be back calculated by dividing 100/NOEC. The calculated TU will be slightly different from the TU calculated by the procedures above, but will prove more reliable when determining reasonable potential.

   b. You have the option of using the WLA.EXE program to evaluate data that you collect. WLA determines limits to six decimal places. If you were to round an NOEC "down", the resulting Tu_a would be less stringent. Even though the permittee might be compliant with the NOEC of the permit, WLA would evaluate the data and determine a limit was needed due to

[2] The draft EPA document uses the 95th percentile in the calculation in 8., which is Z = 1.645
[3] The draft EPA document follows the TSD's recommendation: "Where the sample frequency is monthly or less, the TSD recommends that 'n' be set equal to 4."

non-compliance. As an example, look at the limit that was calculated previously:

2.559506 TU_c = NOEC 39.070044

If you round the NOEC to 39, the resulting TU_c is 2.564102564. Since the data are entered into WLA as TU_c's, the program would determine reasonable potential for toxicity and show that a limit is needed. Therefore, it is recommended that the data are rounded "up" (to NOEC of 40) to avoid this problem.

Remember that because the % effluent is the reciprocal of the TU value, a limit that is a maximum in toxic units becomes a minimum when expressed as percent effluent.

10. WET limit shortcut:

If the effluent toxicity test data set has less than 10 values and the default CV of 0.6 is chosen, then the calculation of limits is greatly simplified. The multiplication factors for long term average and maximum daily limit become constants because we have removed the two variables that could make them change. The constants for e^A, e^B e^C and e^D are 0.41 (.4109447), 0.60 (.6010373), 2.43 (2.4334175) and 2.43 (2.4334175), respectively. These constants for e^A and e^B include the calculation of CV. This means that the long-term average equations can be simplified to:

$LTA_{a,c}$ = $WLA_{a,c}$ x .41

LTA_c = WLA_c x .60

MDL = LTA x 2.43 where LTA is the lower of $LTA_{a,c}$ or LTA_c

Under these circumstances, the only variables are the acute and chronic WLA's (see Steps 2. and 3.) and the ACR (Step 6.) that converts the acute WLA to the $WLA_{a,c}$.

Using the assumptions from the example, the WET limit for this facility can be calculated as follows:

WLA_c = 1.75 TU_c (Step 3)

$WLA_{a,c}$ = 4.5 TU_c (Step 3)

$LTA_{a,c}$ = 4.5 x .4109447 = 1.8492512

$LTA_c = 1.75 \times .6010373 = 1.0518153$

MDL = 1.0518153 X 2.4334175 = 2.559506 TU_c = NOEC 39.070044

Round the NOEC to 40% and the calculated TU_c is 2.53

Although in this case the MDL derived by the full statistics is comparable to the shortcut method number, the variation in effluent quality and toxicity test data depicted by the actual coefficient of variation should be included in the derivation of the WET limit whenever there are 10 or more data points to calculate a CV.

11. WETLIM10.xls spreadsheet and the WLA.EXE program

The process for calculating a WET limit can be time consuming, particularly if there are data with which to calculate an ACR or CV. In an effort to make the process less burdensome and to reduce possible human error, WETLIM10.xls was developed. The 3-page spreadsheet is in Microsoft Excel 97, and will self calculate as data are entered. README10.xls offers explanations as to what and where information should be entered. At a minimum, you will need to enter the facility flow, 1Q10 and 7Q10. Diffuser or modeling study dilution ratio's can be entered and worked into the calculation process. The second page of the spreadsheet will work with data to calculate a CV. The third page has tables to enter paired data for calculation of the ACR. There are additional tables on page three to assist with converting data to TU_c so that it can be used in WLA.EXE, as well as creating a dilution series to use with the derived endpoint or limit.

The "e" values are worked with in long form so that the endpoint/limit that is derived will be the same as what WLA.EXE calculates. WLA.EXE provides the limits out to six decimal points, so WETLIM10.xls has been adjusted to do the same for consistency.

WLA.EXE requires the acute WLA ($WLA_{a,c}$), chronic WLA (WLA_c), and at least one data point. The human health WLA can be omitted, and the quantification level can be entered as "1". In most cases, the number of samples per month is "1".

If you are only going to work with acute toxicity in WLA.EXE, enter the WLA_a for the acute WLA, and then enter the LC_{50} data as TU_a 's into the program.

12. Special considerations for permit limits:

a. Continuous Discharges:

1) Effluent only acutely toxic - There may be cases where a continuous

discharge shows acute toxicity but passes the chronic decision criterion. In these cases it may be useful to express the WET limit in terms of acute toxic units so we can evaluate the effluent in terms of the type of test that they can't pass now and which we want them to pass in the future. If the acute WLA drives the permit limit, the WET limit can be expressed in terms of TU_a so that compliance is by acute tests instead of by chronic tests. This is done by converting the MDL from TU_c to TU_a. This process will be illustrated using our sample data set.

Because in Step 3 we converted WLA_a to $WLA_{a,c}$ by this equation:

$WLA_{a,c} = ACR \times WLA_a$

We can convert the MDL in terms of TU_c to TU_a by this equation:

$TU_a = TU_c/ACR$

$= 4.5/10$

$= .45\ TU_a$

However, when the acute limit is less than 1 TU_a (i.e. 0.45 TU_a), the corresponding LC_{50} is greater than 100% ($LC_{50} = 100/TU_a = 100/.45 = 222.22\%$).

It is not possible to determine compliance with an acute WET limit of less than 1 TU_a because the acute toxicity test is only accurate to 100% effluent. If the converted value is less than 1 TU_a, then revert to a chronic WET limit in TU_c. If the conversion to an acute limit from a chronic limit yields a number of 1 TU_a or greater, then the acute test can accurately measure compliance. And if the effluent was not chronically toxic to begin with, the acute limit is more appropriate. Remember that this conversion should only be attempted when the effluent passed its chronic tests and failed the acute tests. Permit writers should contact OWPP-TMP for further advice if this situation arises.

2) Complete mix assumption not valid - By assuming that an effluent is completely mixed, we can set WET limits that protect instream organisms everywhere in the receiving waters. If complete mix is a valid assumption, then use the MDL calculated in Step 10 as the permit limit. This assumption may not be valid when the discharge is stream dominated and hugs the riverbank. This may result in an extended section of stream in which acute toxicity is above

acceptable levels until the mixing dilutes the effluent. In these cases, we need a way to prevent this acute toxicity in the effluent plume.

For continuous discharges with acute and chronic data which have an IWC less than 33%, the complete mix assumption may not be valid unless the discharge is through some sort of diffuser that maximizes mixing. In the absence of such a diffuser, an alternative MDL should be selected to protect from acute toxicity in the initial mixing zone. If the acute LTA ($LTA_{a,c}$) is used to derive the MDL, the MDL may be converted from TU_c to TU_a as discussed above. If the resulting MDL is greater than 1 TU_a, then the permit limit should be set at 1 TU_a in order to be sure that acute toxicity does not occur in the mixing zone. Only use the greater than 1 TU_a limit if the discharge configuration assures complete mixing. If the MDL is derived from the LTA_c, then use the calculated limit, set in terms of TU_c, and the chronic limit should prevent lethality in the mixing zone.

For continuous discharges with an IWC less than 1%, there probably will not be any chronic data, so you will not be able to get a chronic WLA. In these cases, the WET limit would be based on the acute WLA as follows:

Dilution is 100/IWC = 100/1 = 100 (from Step 2),

Then the WLA would be 100 x 0.3 = 30 TU_a.

There is no need to convert to TU_c because only the acute long-term average will be used to derive the limit.

If we assume a CV of 0.6, then the rounded default for e^A is 0.41 and e^C is 2.43

The LTA_a would be 30 x e^A = 30 x 0.41 = 12.328 TU_a (Step 9).

The MDL would be 12.328 x e^C = 12.328 x 2.43 = 30.0000007 TU_a (Step 10).

NOTE that the WLA_a is equal to the MDL with the $LTA_{a,c}$, expressed as TU_a. The WLA_a = acute dilution X instream criterion of 0.3.

This MDL translates to an LC_{50} of 3.34%. That means that the effluent would be acceptable if only half of the organisms survived

in 3.34% effluent and higher concentrations would be even more toxic. Even though there is a lot of water in the stream for the effluent to eventually mix with and unless the discharge is rapidly mixed, this very low LC_{50} could result in toxicity in the initial mixing zone. In order to prevent toxicity in the initial mixing area from occurring, **when the IWC of a continuous discharge is less than 1%, the WET limit should be set no higher than 1 TU_a (LC_{50} = 100%), unless a mixing zone analysis shows that the complete mix assumption is valid and that aquatic life can be protected with a less stringent (higher) limit.**

3) For continuous discharges to 7Q10 zero streams, the statistical procedures will calculate a WET limit in TU_c that will protect from both acute and chronic toxicity. Complete mix is assumed in these situations. If the limit is > 1 TU_c it should still be used in the permit. According to the statistics, this limit will still assure compliance with the calculated long-term average even though it is higher than the IWC.

4) If the MDL calculated by the statistical method is less than 1 TU_c, this translates to an NOEC greater than 100% effluent. This is an endpoint that the chronic test cannot measure. So, there is no way to accurately assure compliance with a WET limit of less than 1 TU_c. This situation generally comes up when the MDL is derived from the $LTA_{a,c}$. As discussed above, if this happens and we convert to TU_a because the IWC is 33% or less, then the problem is solved. When the MDL is less than 1 TU_c and the IWC is greater than 33%, then it is not appropriate to convert to TU_a and the limit should stay in TU_c so that the instream aquatic life is protected from chronic toxicity as well as acute toxicity. In order to do this and still have a test endpoint we can accurately measure, we recommend that the MDL be set at 1 TU_c (NOEC = 100%).

b. Intermittent discharges (no chronic data):

1) If the 1Q10 is zero (IWC_a = 100%).

The dilution factor is 100/IWC = 100/100 = 1 (Step 2).

So the $WLA_a = 0.3 \times 1 = 0.3$.

For simplicity of this example we will again assume a CV of 0.6, then $e^A = 0.41$ and $e^C = 2.43$ (Step 7),

The $LTA_a = .3 \times e^A = .3 \times 0.41 = .123$ TU_a (Step 9).

The MDL would be .123 x e^C = .123 x 2.43 = .30 TU_a (Step 10).

As discussed above, the acute test cannot measure compliance with an acute WET limit lower than 1 TU_a. In this case the limit of 1 TU_a would not protect aquatic life during drought flow because all of the stream would be effluent and a 1 TU_a limit would allow half of the organisms to be killed (LC_{50} = 100%). Therefore, in order to assure that the discharge does not cause toxicity in the receiving stream during drought periods, a more restrictive endpoint must be established in the permit. The limit should be expressed in terms of the No Observed Adverse Effect Concentration (NOAEC) and it will be set at NOAEC = 100%. This WET limit will require that the discharge be nontoxic without dilution. The test organisms must be able to survive in 100% effluent as well as they survive in the control solution. If there is any significant difference between mortality/survival in the 100% effluent solution versus the control, then the discharge fails to meet the limit. See Appendix E for an example of this type of WET limit.

2) <u>If the 1Q10 of the receiving stream is greater than zero ($IWC_a \leq$ 100%).</u>

Most of the discharges which fall into this category will be storm water dependant and they will only discharge into the receiving stream when there is some flow of storm water to dilute the effluent. It may be possible to estimate an IWC for these discharges based on the drainage area of the watershed above the discharge point and the area drained by the discharge itself, but an accurate value may be difficult to obtain. If the IWC can be determined with some confidence, in many of these cases the MDL will be calculated as < 1 TU_a. This may be true until the IWC falls to about 40. Because in these cases the receiving stream provides some dilution, we will not hold these discharges to the same stringent NOAEC endpoint as those where the IWC = 100%. In order to have an endpoint that can be measured using the standard acute toxicity test procedures, we will assume that an effluent in this category that passes the test at $LC_{50} \geq$ 100% (1 TU_a) will be diluted enough by the water in the stream to prevent acute toxicity. %. If a discharge is contaminated stormwater, acute toxicity testing should be performed by the discharger to see how effective their stormwater prevention program is. **When the IWC of an intermittent discharge is less than 100% and the MDL is calculated to be < 1 TU_a, set the MDL at 1 TU_a.**

c. WET limits for discharges to lakes, marshes and swamps

The procedures for developing WET limits for discharges to lakes, marshes and swamps is the same as for discharges to flowing streams except that the WLA is set equal to the instream criterion. This is done because assumptions about dilution available through initial turbulent mixing that are applied to flowing streams are not applicable in these environments. This is consistent with the approach for chemical specific limits given in Guidance Memo 93-015 and subsequent updates.

$WLA_a = .3\ TU_a$ and $WLA_c = 1\ TU_c$

Actual WLA's may be calculated when there is specific information available that defines the actual mixing zone for the discharge. In those cases, a site-specific WLA can be calculated based on the dilution available: WLA = instream criterion x dilution.

d. WET limits for discharges to estuarine embayments or tidal estuaries

Because of the difference in mixing characteristics, the waste load allocations for discharges to estuarine waters are different from those for discharges to flowing streams. Once the WLA has been determined, however, the procedures and calculations are the same as in Part A.

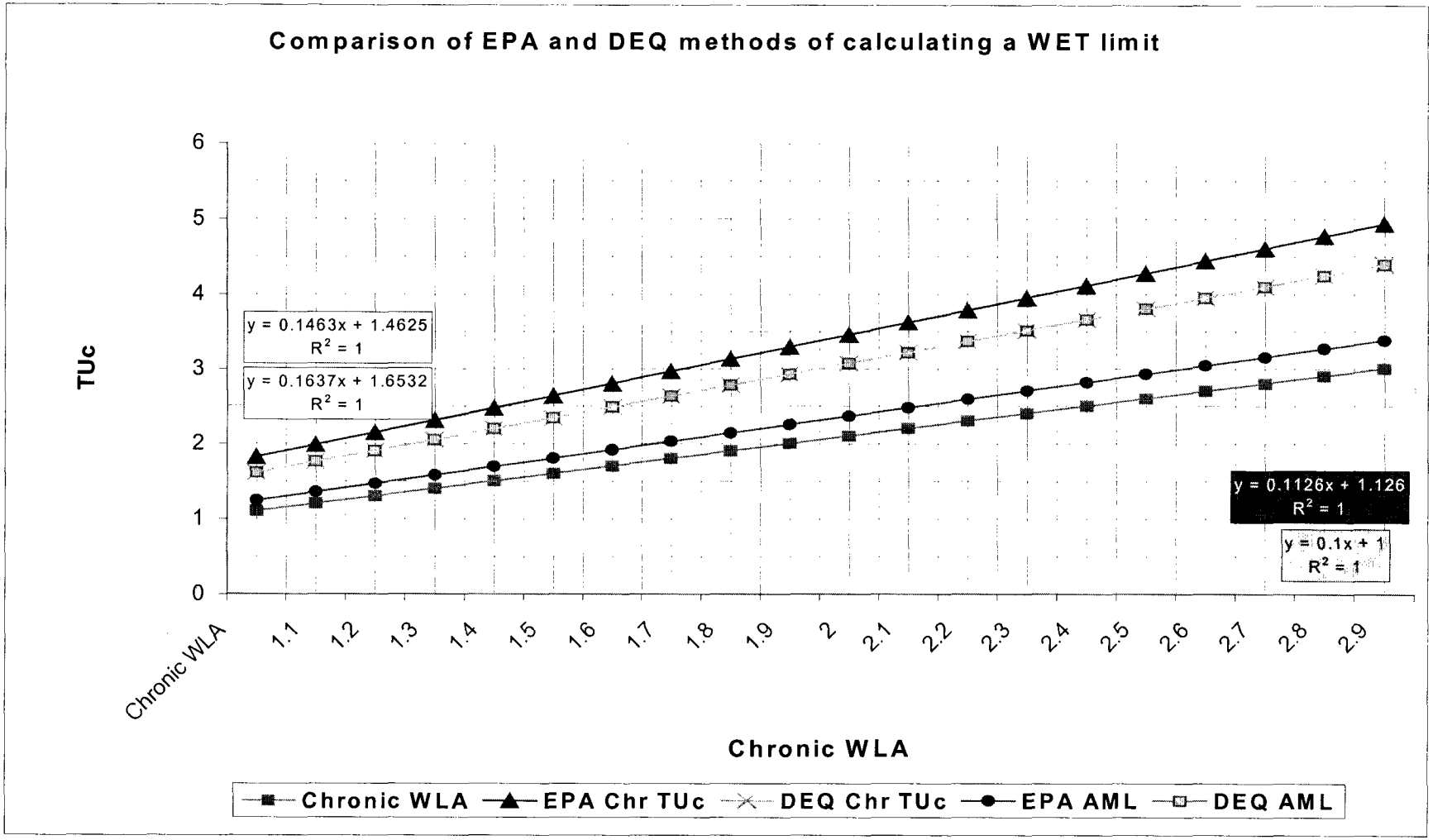
For surface discharges into these environments, unless there is information regarding initial mixing at the discharge point, the acute waste load allocation (WLA_a) should be set at 2x the instream criterion. $WLA_a = 0.6\ TU_a$. This recognizes some limited initial dilution, but it provides some assurance that lethality in the allocated impact zone will be prevented. Initial mixing at subsurface discharges should be determined through a model study. Then the WLA_a can be set by the equation WLA_a = instream criterion x dilution = .3 TU_a x dilution.

The chronic waste load allocation should ideally be based on site-specific mixing information. When a model study has determined a chronic mixing zone dilution, the WLA_c is determined by the equation: WLA_c = instream criterion x dilution = 1.0 TU_c x dilution. **If there is no information on the actual dilution available for the chronic mixing zone, a 50:1 dilution ratio can be assumed. This results in a WLA_c of 50 TU_c.**

13. Comparison of EPA's approach and DEQ's approach on developing WET endpoints.

EPA uses the 99th percentile for calculation of the "e" values, which are used in the TSD. For the AML, they use the 95th percentile, the logic being that the Average Monthly Limit should be lower than the daily limit, so that the daily limit is not exceeded. The other part of the calculation that is different from the way that DEQ is using is with the number of samples used to calculation the AML. The TSD states that if there is 1 or less samples taken per month, that "4" should be used in the calculation for the number of samples per month. The end result of these differences in EPA's approach and this June 27, 2000 revision of the Toxics Management Program guidance can be seen below in the table and on the graph on the following page. The EPA method calculates a less stringent TU_c, but a more stringent AML. The DEQ method calculates the TU_c and AML to be the same, unless more than 1 sample is actually used.

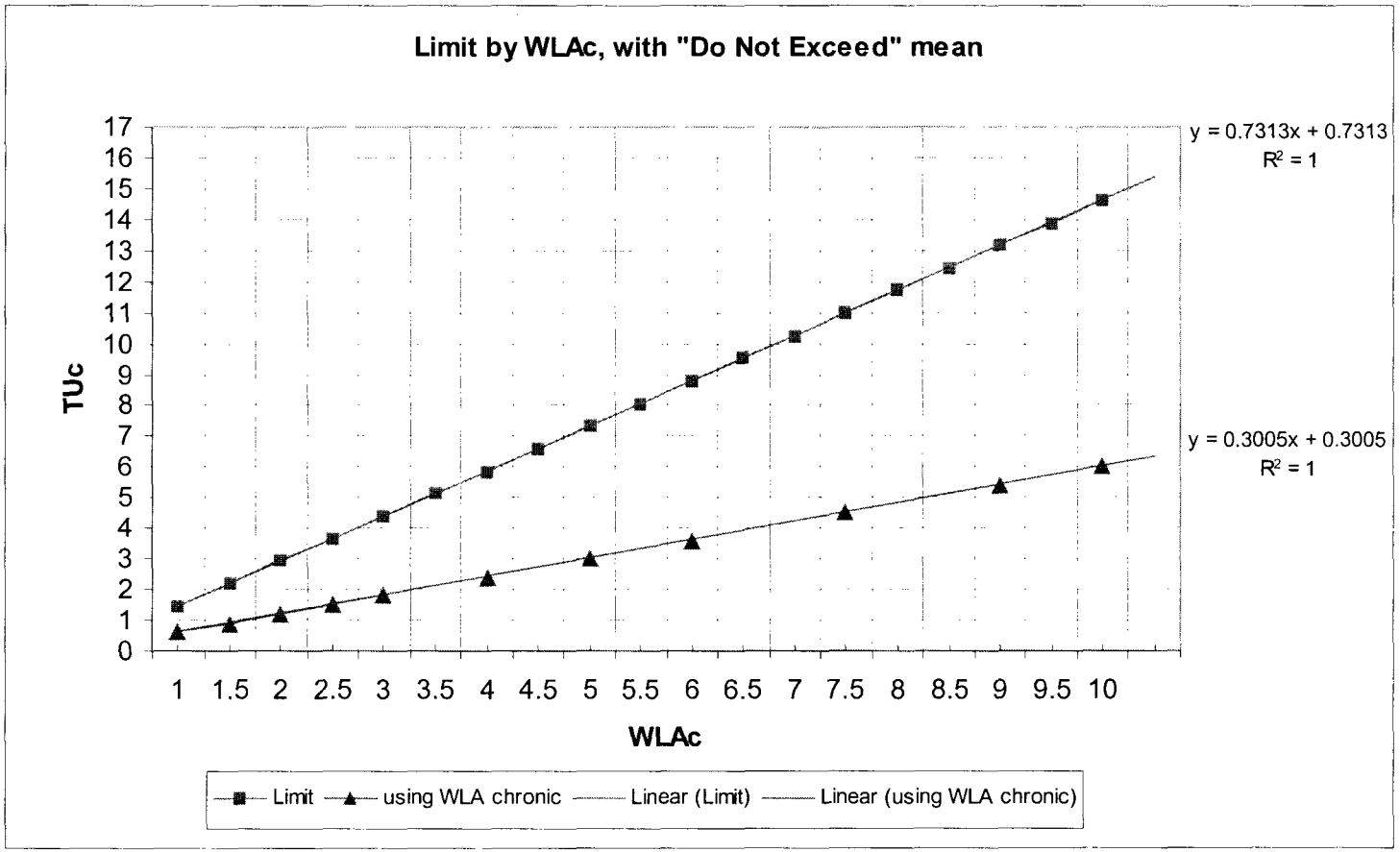
Acute WLA	Chronic WLA	EPA Chr TUc	DEQ Chr TUc	Both TUc	EPA AML	DEQ AML
3.3	1.1	1.8169	1.6088	3.3	1.2386	1.6088
3.6	1.2	1.980621818	1.75505697	3.6	1.35120545	1.755056
3.9	1.3	2.144343636	1.901313939	3.9	1.46381090	1.901313
4.2	1.4	2.308065455	2.047570909	4.2	1.57641636	2.047570
4.5	1.5	2.471787273	2.193827879	4.5	1.68902181	2.193827
4.8	1.6	2.635509091	2.340084848	4.8	1.80162727	2.340084
5.1	1.7	2.799230909	2.486341818	5.1	1.91423272	2.486341
5.4	1.8	2.962952727	2.632598788	5.4	2.02683818	2.632598
5.7	1.9	3.126674545	2.778855758	5.7	2.13944363	2.778855
6	2	3.290396364	2.925112727	6	2.25204909	2.925112
6.3	2.1	3.454118182	3.071369697	6.3	2.36465454	3.071369
6.6	2.2	3.61784	3.217626667	6.6	2.47726	3.217626
6.9	2.3	3.781561818	3.363883636	6.9	2.58986545	3.363883
7.2	2.4	3.945283636	3.510140606	7.2	2.70247090	3.510140
7.5	2.5	4.109005455	3.656397576	7.5	2.81507636	3.656397
7.8	2.6	4.272727273	3.802654545	7.8	2.92768181	3.802654
8.1	2.7	4.436449091	3.948911515	8.1	3.04028727	3.948911
8.4	2.8	4.600170909	4.095168485	8.4	3.15289272	4.095168
8.7	2.9	4.763892727	4.241425455	8.7	3.26549818	4.241425
9	3	4.927614545	4.387682424	9	3.37810363	4.387682


Comparison of EPA and DEQ methods of calculating a WET limit
TUc
Chronic WLA
y = 0.1463x + 1.4625
R² = 1
y = 0.1637x + 1.6532
R² = 1
y = 0.1126x + 1.126
R² = 1
y = 0.1x + 1
R² = 1
Chronic WLA
EPA Chr TUc
DEQ Chr TUc
EPA AML
DEQ AML

Discussion of the development of the data "Mean", which if exceeded may trigger a limit with WLA.EXE

Management made the decision to use WLA.EXE for evaluating the biological test data submitted in compliance of the Toxics Management Program. With this implementation, monitoring compliance can not be determined as readily as before, when "not to exceed" types of endpoints were established in the permit. The calculation below is offered as a helpful tool for the permittee.

Data are entered into WLA.EXE and the program determines statistically whether a limit is needed or not. The point that triggers this determination is the mean of the data that have been entered. If all of the data are defined data points (not "<" or ">"), the calculation for the mean is: (exp(((ln(WLA_c))+0.153742)-1.043039)) This is calculated for you at the top of WETLIM10.xls. When censored data are present, the data mean is adjusted depending on the number of censored data points there are. The permittee can now compare the mean of his data (in TU_c) to this calculated mean to see if a limit will be necessary when the data are evaluated.



APPENDIX E

WETLIM10.XLS SPREADSHEET
README10.XLS FILE

README for Spreadsheet for determination of WET test endpoints or WET limits, WETLIM10.xls.

This spreadsheet has been prepared with Excel 97 for Windows, and will self-calculate when the data are entered. You should also run MIX.EXE to determine whether complete mix is allowed using the full 7Q10 or 1Q10, or if a percentage should be used.

When you pull up the spreadsheet, you will notice that the information will appear in various colors. Data should be entered where the type is blue - these are unprotected cells. The black type is in protected cells and cannot be altered without removing the protection from the file. The green type highlights the calculated endpoints. Red type highlights information of note.

BASIC INFORMATION TO ENTER:

PAGE 1

	Entry Date (Entered as the current date automatically)		
C12	Facility Name		
C13	VPDES #		
C14	Outfall #		
C16	Plant Flow		
C17	1Q10	E17	Acute MIX
C18	7Q10	E18	Chronic MIX
J16	Diffuser/Mixing study? Y/N		Mixing ratios won't work unless "Y" is checked for diffuser
J17	Acute Dilution		
J18	Chronic Dilution		
E20	Are data available to calculate the CV? Y/N		Data won't be used unless "Y" is checked
E21	Are data available to calculate an ACR? Y/N		Data won't be used unless "Y" is checked

PAGE 2 **SITE SPECIFIC COEFFICIENT OF VARIATION (CV)**

If you have at least 10 pieces of acute toxicity data using the same species, you may be able to calculate a CV (Coefficient of Variation) for the effluent data to use in place of the default 0.6 CV. Chronic data can be used, but must have the endpoints recalculated as IC_{25}'s - NOEC's cannot be used. Enter data in column "G" or "J", rows 61-80, as appropriate. The CV will calculate and be placed in cell C70. The eA, eB, eC and eD values will recalculate and appear where needed on the spreadsheet. Note that eC and eD will be the same, unless the number of samples required per month, "n", is changed from 1. In this event, which would be rare, "n" is changed in cell C98.

DO NOT USE:	**DO USE:**
NOEC endpoints	IC_{25}'s instead of NOEC's
Less than 10 data points	10 or more data points
LC_{50}'s >100%	LC_{50}'s less than 100%
Different species	Same species

PAGE 3 **SITE SPECIFIC ACUTE TO CHRONIC RATIO (ACR), Table 1. and Table 2.**

If you have paired data of the same species that meets the usability criteria, a site specific ACR can be developed. The most likely data will come from a chronic test where the NOEC for survival was around 50% effluent or less. Calculate an LC_{50} at 48 hours, and if it is something other than an LC_{50} >100%, it can be used. The chronic test NOEC, if less than the LC_{50}, is entered in the next column. The ACR will calculate and be entered into the appropriate places on the spreadsheet. If you have data where the LC_{50} and the NOEC are equal, and it is the only usable data you have, the ACR will be 1 (LC_{50}/NOEC). Do not put it in the spreadsheet because it will cause all error messages due to the log of 1 being "0".

DO NOT USE:

LC_{50}'s >100%

LC_{50}'s < NOEC's

LC_{50} = NOEC (unless it is the only usable data pair you have)

DO USE:

LC_{50}'s > NOEC's

The endpoints calculated by the spreadsheet will appear at the top right of Page 1 in a box. If you need endpoints for monitoring, use the separate acute and chronic endpoints. At the conclusion of the test period, the data should be evaluated by WLA.EXE to see if a limit is needed. Use professional judgement to determine if one limit will be sufficiently protective for both acute and chronic toxicity.

Dilution Series to Recommend, Table 4.

This table will develop a geometric dilution series from the NOEC endpoints calculated on this spreadsheet. There is nothing to enter on this table. The dilution series has been created to have the "target" as the middle dilution. If the NOEC endpoint is for monitoring, use the dilution series which uses the mean of the data for WLA.EXE. If the NOEC endpoint is for a WET limit, use the dilution series which has the limit as the midpoint. Additional low dilutions have been provided in the event of a toxic effluent, to avoid a result of "<".

Convert LC_{50}'s and NOEC's to Chronic Toxic Units (TU_c), Table 3.

The program WLA.EXE can be used with WET data, as long as it has been converted to chronic toxic units, expressed as TU_c. Chronic NOEC's convert easily by using the calculation: 100/NOEC. The acute LC_{50} data is converted by a similar calculation, $100/LC_{50}$ to be expressed as acute toxic units (TU_a). The TU_a is then multiplied by the ACR (default is 10) to convert to chronic toxic units, or TU_c. Table 3. does all of that for you. Also, if you need to convert the TUc from WLA.EXE to TUa, enter it in cell K145. If you are working with only acute data and the WLAa, convert the acute data to TUa by entering data in the NOEC column. The calculation is the same.NOTE: For each use of WLA.EXE, all data used must be for the same species.

The NOEC's in the limits box will appear without decimal points, rounded "up" to the nearest whole number. The purpose is that it is difficult for a lab to be much more accurate than 1 ml for actual measuring purposes. Additionally, a derived limit of 1.462575 TU_c correlates to an NOEC of 68.37257. If the NOEC were rounded to an NOEC of 68, that would back calculate to a TU_c of 1.470588Z. This TU_c is greater than the derived TUc, and if entered into WLA.EXE, would result in a limit. Therefore, the NOEC that corresponds to the derived TU_c was given a 'correction factor' by adding 0.5, and then rounding it to the nearest whole number which in this case, was an NOEC = 69%.

PRINTING:

The orientation of the spreadsheet is portrait, and it is 3 pages total. Unless you have entered data on pages 2 or 3, it would only be necessary to print page 1. You can do that by specifying print page 1, or by putting in the print range of A1..M52. For page 2, the range is A53..M103, and page 3, the range is A104..N165.

It is advisable to change the name of this table whenever you use it, and save it under the name selected. That way, you can pull the original table up each time you need it, and not have to worry about previously entered data.

If you have problems with this spreadsheet, call Deborah L. DeBiasi, OWPP-TMP, 804/698-4028.

Cell: A20
Comment:

Cell: A21
Comment: Enter the flow expressed as MGD

Cell: D21
Comment: Enter the number without the % sign - for example - for 80%, enter 80

Cell: A22
Comment: Enter the flow expressed as MGD

Cell: D22
Comment: Enter the number without the % sign - for example - for 80%, enter 80

Cell: A24
Comment: Case doesn't matter

Cell: A25
Comment: Enter the first number of the acute mix ratio - example, for a 20:1 dilution, enter 20

Cell: A26
Comment: Enter the first number of the chronic mix ratio - example, for a 12:1 dilution, enter 12

Cell: A28
Comment:

If you know that you have sufficient data to calculate a CV, put a "Y" in this cell. If not, you can come back to it, and to any of the other cells that ask for a "Y" or "N". Effluent specific data that you enter on page 2 for the CV and page 3 for the ACR will be used in the WET calculations only if the "Y" is entered - otherwise, the defaults are used.

Cell: A29
Comment:

If you know that you have usable data to calculate an ACR, put a "Y" in this cell. If not, you can come back to it, and to any of the other cells that ask for a "Y" or "N". Effluent specific data that you enter on page 2 for the CV and page 3 for the ACR will be used in the WET calculations only if the "Y" is entered - otherwise, the defaults are used.

Spreadsheet for determination of WET test endpoints or WET limits

Excel 97
Revision Date: 08/24/00
File: WETLIM10.xls
(MIX.EXE required also)

Enter data in the cells with blue type:

Entry Date:	08/24/00	
Facility Name:	Facility	
VPDES Number:	VA0000000	
Outfall Number:	000	
Plant Flow:	1	MGD
Acute 1Q10:	0	MGD
Chronic 7Q10:	0	MGD

Acute Endpoint/Permit Limit — **Use as LC_{50} in Special Condition, as TUa on DMR**

ACUTE	100% =	NOAEC	LC_{50} =	NA	% Use as	NA	TUa
ACUTE WLAa		0.3	Note: Inform the permittee that if the mean of the data exceeds this TUa:	1.0	a limit may result using WLA.EXE		

Chronic Endpoint/Permit Limit — **Use as NOEC in Special Condition, as TUc on DMR**

CHRONIC	1.462574684	TU_c	NOEC =	69	% Use as	1.44	TU_c
BOTH*	3.000000074	TU_c	NOEC =	34	% Use as	2.94	TU_c
AML	1.462574684	TU_c	NOEC =	69	% Use as	1.44	TU_c

ACUTE WLAa,c	3	Note: Inform the permittee that if the mean	
CHRONIC WLAc	1	of the data exceeds this TUc:	1.0
* Both means acute expressed as chronic		a limit may result using WLA.EXE	

% Flow to be used from MIX.EXE

Acute 1Q10:	100	%
Chronic 7Q10:	100	%

Difuser /modeling study?

Enter Y/N	N
Acute	1 :1
Chronic	1 :1

Are data available to calculate CV? (Y/N) N (Minimum of 10 data points, same species, needed) Go to Page 2
Are data available to calculate ACR? (Y/N) N (NOEC<LC50, do not use greater/less than data) Go to Page 3

IWC_a	100	%	Plant flow/plant flow + 1Q10
IWC_c	100	%	Plant flow/plant flow + 7Q10
Dilution, acute	1		100/IWCa
Dilution, chronic	1		100/IWCc

NOTE: If the IWCa is >33%, specify the NOAEC = 100% test/endpoint for use

WLA_a	0.3	Instream criterion (0.3 TUa) X's Dilution, acute
WLA_c	1	Instream criterion (1.0 TUc) X's Dilution, chronic
$WLA_{a,c}$	3	ACR X's WLA_a - converts acute WLA to chronic units
ACR -acute/chronic ratio	10	LC50/NOEC (Default is 10 - if data are available, use tables Page 3)
CV-Coefficient of variation	0.6	Default of 0.6 - if data are available, use tables Page 2)
Constants eA	0.4109447	Default = 0.41
eB	0.6010373	Default = 0.60
eC	2.4334175	Default = 2.43
eD	2.4334175	Default = 2.43 (1 samp)

**The Maximum Daily Limit is calculated from the lowest LTA, X's eC. The LTAa,c and MDL using it are driven by the ACR.

						Rounded NOEC's	
$LTA_{a,c}$	1.2328341	WLAa,c X's eA					
LTA_c	0.6010373	WLAc X's eB					
MDL** with $LTA_{a,c}$	3.00000007	TU_c	NOEC =	33.333333	(Protects from acute/chronic toxicity)	NOEC =	34 %
MDL** with LTA_c	1.46257468	TU_c	NOEC =	68.372577	(Protects from chronic toxicity)	NOEC =	69 %
AML with lowest LTA	1.46257468	TU_c	NOEC =	68.372577	Lowest LTA X's eD	NOEC =	69

IF ONLY ACUTE ENDPOINT/LIMIT IS NEEDED, CONVERT MDL FROM TU_c to TU_a

							Rounded LC50's	
MDL with $LTA_{a,c}$	0.30000001	TU_a	LC50 =	333.333325	%	Use NOAEC=100%	LC50 =	NA %
MDL with LTA_c	0.14625747	TU_a	LC50 =	683.725769	%	Use NOAEC=100%	LC50 =	NA

Page 2 - Follow the directions to develop a site specific CV (coefficient of variation)

IF YOU HAVE AT LEAST 10 DATA POINTS THAT ARE QUANTIFIABLE (NOT "<" OR ">") FOR A SPECIES, ENTER THE DATA IN EITHER COLUMN "G" (VERTEBRATE) OR COLUMN "J" (INVERTEBRATE). THE 'CV' WILL BE PICKED UP FOR THE CALCULATIONS BELOW. THE DEFAULT VALUES FOR eA, eB, AND eC WILL CHANGE IF THE 'CV' IS ANYTHING OTHER THAN 0.6

Coefficient of Variation for effluent tests

CV = 0.6 (Default 0.6)

δ^2 = 0.3074847
δ = 0.55451303

Using the log variance to develop eA
(P. 100, step 2a of TSD)
Z = 1.881 (97% probability stat from table
A = -0.8892967
eA = 0.41094469

Using the log variance to develop eB
(P. 100, step 2b of TSD)
δ_4^2 = 0.0861777
δ_4 = 0.29356038
B = -0.5090982
eB = 0.60103733

Using the log variance to develop eC
(P. 100, step 4a of TSD)

δ^2 = 0.3074847
δ = 0.55451303
C = 0.88929666
eC = 2.43341753

Using the log variance to develop eD
(P. 100, step 4b of TSD)
n = 1 This number will most likely stay as "1", for 1 sample/month.
δ_n^2 = 0.3074847
δ_n = 0.55451303
D = 0.88929666
eD = 2.43341753

	Vertebrate IC_{25} Data or LC_{50} Data **********	LN of data	Invertebrate IC_{25} Data or LC_{50} Data **********	LN of data
1	0		0	
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
St Dev	NEED DATA	NEED DATA	St Dev	NEED DATA NEED DATA
Mean	0	0	Mean	0 0
Variance	0	0.000000	Variance	0 0.000000
CV	0		CV	0

Page 3 - Follow directions to develop a site specific ACR (Acute to Chronic Ratio)

To determine Acute/Chronic Ratio (ACR), insert usable data below. Usable data is defined as valid paired test results, acute and chronic, tested at the same temperature, same species. The chronic NOEC must be less than the acute LC_{50}, since the ACR divides the LC_{50} by the NOEC. LC_{50}'s >100% should not be used.

Table 1. ACR using Vertebrate data

Set #	LC_{50}	NOEC	Test ACR	Logarithm	Geomean	Antilog	ACR to Use
1	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
2	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
3	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
4	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
5	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
6	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
7	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
8	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
9	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
10	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA

ACR for vertebrate data: 0

Table 1. Result:	Vertebrate ACR	0
Table 2. Result:	Invertebrate ACR	0
	Lowest ACR	Default to 10

Table 2. ACR using Invertebrate data

Set #	LC_{50}	NOEC	Test ACR	Logarithm	Geomean	Antilog	ACR to Use
1	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
2	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
3	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
4	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
5	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
6	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
7	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
8	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
9	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
10	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA

ACR for vertebrate data: 0

Convert LC_{50}'s and NOEC's to Chronic TU's for use in WLA.EXE

Table 3. ACR used: 10

	Enter LC_{50}	TUc	Enter NOEC	TUc
1		NO DATA		NO DATA
2		NO DATA		NO DATA
3		NO DATA		NO DATA
4		NO DATA		NO DATA
5		NO DATA		NO DATA
6		NO DATA		NO DATA
7		NO DATA		NO DATA
8		NO DATA		NO DATA
9		NO DATA		NO DATA
10		NO DATA		NO DATA
11		NO DATA		NO DATA
12		NO DATA		NO DATA
13		NO DATA		NO DATA
14		NO DATA		NO DATA
15		NO DATA		NO DATA
16		NO DATA		NO DATA
17		NO DATA		NO DATA
18		NO DATA		NO DATA
19		NO DATA		NO DATA
20		NO DATA		NO DATA

If WLA.EXE determines that an acute limit is needed, you need to convert the TUc answer you get to TUa and then an LC50.

enter it here:	NO DATA	$\%LC_{50}$
	NO DATA	TUa

DILUTION SERIES TO RECOMMEND

Table 4.

	Monitoring		Limit	
	% Effluent	TUc	% Effluent	TUc
Dilution series based on data mean	100	1 0		
Dilution series to use for limit			69	1.4492754
Dilution factor to recommend:	0 5		0 8306624	
Dilution series to recommend:	100 0	1 00	100 0	1 00
	50 0	2 00	83.1	1.20
	25 0	4 00	69 0	1.45
	12.5	8 00	57.3	1.74
	6 25	16 00	47.6	2 10
Extra dilutions if needed	3.12	32.05	39.5	2.53
	1.56	64.10	32.9	3.04

Cell: I9
Comment:

Cell: K18
Comment: This is assuming that the data are Type 2 data (none of the data in the data set are censored - "<" or ">").

Cell: J22
Comment: Remember to change the "N" to "Y" if you have ratios entered, otherwise, they won't be used in the calculations.

Cell: C40
Comment:
If you have entered data to calculate an ACR on page 3, and this is still defaulted to "10", make sure you have selected "Y" in cell E21

Cell: C41
Comment: If you have entered data to calculate an effluent specific CV on page 2, and this is still defaulted to "0.6", make sure you have selected "Y" in cell E20

Cell: L48
Comment:
See Row 151 for the appropriate dilution series to use for these NOEC's

Cell: G62
Comment:
Vertebrates are:
Pimephales promelas
Oncorhynchus mykiss
Cyprinodon variegatus

Cell: J62
Comment:
Invertebrates are:
Ceriodaphnia dubia
Mysidopsis bahia

Cell: C117
Comment: Vertebrates are:

Pimephales promelas
Cyprinodon variegatus

Cell: M119
Comment: The ACR has been picked up from cell C34 on Page 1. If you have paired data to calculate an ACR, enter it in the tables to the left, and make sure you have a "Y" in cell E21 on Page 1. Otherwise, the default of 10 will be used to convert your acute data.

Cell: M121
Comment: If you are only concerned with acute data, you can enter it in the NOEC column for conversion and the number calculated will be equivalent to the TUa. The calculation is the same: 100/NOEC = TUc or 100/LC50 = TUa.

Cell: C138
Comment: Invertebrates are:

Ceriodaphnia dubia
Mysidopsis bahia

Spreadsheet for determination of WET test endpoints or WET limits

Excel 97
Revision Date: 08/24/00
File: WETLIM10.xls
(MIX.EXE required also)

Acute Endpoint/Permit Limit — **Use as LC_{50} in Special Condition, as TUa on DMR**

ACUTE	100% =	NOAEC	LC_{50} =	NA	% Use as	NA	TUa
ACUTE WLAa		0.3	Note: Inform the permittee that if the mean of the data exceeds this TUa:	1.0	a limit may result using WLA.EXE		

Chronic Endpoint/Permit Limit — **Use as NOEC in Special Condition, as TUc on DMR**

CHRONIC	1.462574684	TU_c	NOEC =	69	% Use as	1.44	TU_c
BOTH*	3.000000074	TU_c	NOEC =	34	% Use as	2.94	TU_c
AML	1.462574684	TU_c	NOEC =	69	% Use as	1.44	TU_c

ACUTE WLAa,c	3	Note: Inform the permittee that if the mean
CHRONIC WLAc	1	of the data exceeds this TUc: 1.0
* Both means acute expressed as chronic		a limit may result using WLA.EXE

Enter data in the cells with blue type:

Entry Date:	08/24/00
Facility Name:	Facility
VPDES Number:	VA0000000
Outfall Number:	000

		% Flow to be used from MIX.EXE	**Difuser /modeling study?**	
Plant Flow:	1 MGD		Enter Y/N	N
Acute 1Q10:	0 MGD	100 %	Acute	1 :1
Chronic 7Q10:	0 MGD	100 %	Chronic	1 :1

Are data available to calculate CV? (Y/N) N (Minimum of 10 data points, same species, needed) Go to Page 2
Are data available to calculate ACR? (Y/N) N (NOEC<LC50, do not use greater/less than data) Go to Page 3

IWC_a	100 %	Plant flow/plant flow + 1Q10	**NOTE: If the IWCa is >33%, specify the NOAEC = 100% test/endpoint for use**
IWC_c	100 %	Plant flow/plant flow + 7Q10	
Dilution, acute	1	100/IWCa	
Dilution, chronic	1	100/IWCc	

WLA_a	0.3	Instream criterion (0.3 TUa) X's Dilution, acute
WLA_c	1	Instream criterion (1.0 TUc) X's Dilution, chronic
$WLA_{a,c}$	3	ACR X's WLA_a - converts acute WLA to chronic units

ACR -acute/chronic ratio		10	LC50/NOEC (Default is 10 - if data are available, use tables Page 3)
CV-Coefficient of variation		0.6	Default of 0.6 - if data are available, use tables Page 2)
Constants	eA	0.4109447	Default = 0.41
	eB	0.6010373	Default = 0.60
	eC	2.4334175	Default = 2.43
	eD	2.4334175	Default = 2.43 (1 samp)

**The Maximum Daily Limit is calculated from the lowest LTA, X's eC. The LTAa,c and MDL using it are driven by the ACR.

							Rounded NOEC's	
$LTA_{a,c}$	1.2328341	WLAa,c X's eA						
LTA_c	0.6010373	WLAc X's eB						
MDL** with $LTA_{a,c}$	3.00000007	TU_c	NOEC =	33.333333	(Protects from acute/chronic toxicity)		NOEC =	34 %
MDL** with LTA_c	1.46257468	TU_c	NOEC =	68.372577	(Protects from chronic toxicity)		NOEC =	69 %
AML with lowest LTA	1.46257468	TU_c	NOEC =	68.372577	Lowest LTA X's eD		NOEC =	69

IF ONLY ACUTE ENDPOINT/LIMIT IS NEEDED, CONVERT MDL FROM TU_c to TU_a

							Rounded LC50's	
MDL with $LTA_{a,c}$	0.30000001	TU_a	LC50 =	333.333325 %	Use NOAEC=100%		LC50 = NA	%
MDL with LTA_c	0.14625747	TU_a	LC50 =	683.725769 %	Use NOAEC=100%		LC50 = NA	

Page 2 - Follow the directions to develop a site specific CV (coefficient of variation)

IF YOU HAVE AT LEAST 10 DATA POINTS THAT ARE QUANTIFIABLE (NOT "<" OR ">") FOR A SPECIES ENTER THE DATA IN EITHER COLUMN "G" (VERTEBRATE) OR COLUMN "J" (INVERTEBRATE). THE 'CV' WILL BE PICKED UP FOR THE CALCULATIONS BELOW. THE DEFAULT VALUES FOR eA, eB, AND eC WILL CHANGE IF THE 'CV' IS ANYTHING OTHER THAN 0.6

	Vertebrate IC_{25} Data or LC_{50} Data **********	LN of data	Invertebrate IC_{25} Data or LC_{50} Data **********	LN of data
1	0		0	
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

Coefficient of Variation for effluent tests

CV = 0.6 (Default 0.6)

δ^2 = 0.3074847

δ = 0.55451303

Using the log variance to develop eA
(P. 100, step 2a of TSD)

Z = 1.881 (97% probability stat from table

A = -0.8892967

eA = 0.41094469

Using the log variance to develop eB
(P. 100, step 2b of TSD)

δ_4^2 = 0.0861777

δ_4 = 0.29356038

B = -0.5090982

eB = 0.60103733

	St Dev	NEED DATA	NEED DATA	St Dev	NEED DATA	NEED DATA
	Mean	0	0	Mean	0	0
	Variance	0	0.000000	Variance	0	0.000000
	CV	0		CV	0	

Using the log variance to develop eC
(P. 100, step 4a of TSD)

δ^2 = 0.3074847

δ = 0.55451303

C = 0.88929666

eC = 2.43341753

Using the log variance to develop eD
(P. 100, step 4b of TSD)

n = 1 This number will most likely stay as "1", for 1 sample/month.

δ_n^2 = 0.3074847

δ_n = 0.55451303

D = 0.88929666

eD = 2.43341753

Page 3 - Follow directions to develop a site specific ACR (Acute to Chronic Ratio)

To determine Acute/Chronic Ratio (ACR), insert usable data below. Usable data is defined as valid paired test results, acute and chronic, tested at the same temperature, same species. The chronic NOEC must be less than the acute LC_{50}, since the ACR divides the LC_{50} by the NOEC. LC_{50}'s > 100% should not be used.

Table 1. ACR using Vertebrate data

Set #	LC_{50}	NOEC	Test ACR	Logarithm	Geomean	Antilog	ACR to Use
1	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
2	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
3	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
4	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
5	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
6	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
7	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
8	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
9	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
10	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA

ACR for vertebrate data: 0

Table 1. Result: Vertebrate ACR 0
Table 2. Result: Invertebrate ACR 0
Lowest ACR Default to 10

Table 2. ACR using Invertebrate data

Set #	LC_{50}	NOEC	Test ACR	Logarithm	Geomean	Antilog	ACR to Use
1	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
2	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
3	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
4	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
5	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
6	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
7	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
8	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
9	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA
10	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	NO DATA

ACR for vertebrate data: 0

Convert LC_{50}'s and NOEC's to Chronic TU's for use in WLA.EXE

Table 3. ACR used: 10

	Enter LC_{50}	TUc	Enter NOEC	TUc
1		NO DATA		NO DATA
2		NO DATA		NO DATA
3		NO DATA		NO DATA
4		NO DATA		NO DATA
5		NO DATA		NO DATA
6		NO DATA		NO DATA
7		NO DATA		NO DATA
8		NO DATA		NO DATA
9		NO DATA		NO DATA
10		NO DATA		NO DATA
11		NO DATA		NO DATA
12		NO DATA		NO DATA
13		NO DATA		NO DATA
14		NO DATA		NO DATA
15		NO DATA		NO DATA
16		NO DATA		NO DATA
17		NO DATA		NO DATA
18		NO DATA		NO DATA
19		NO DATA		NO DATA
20		NO DATA		NO DATA

If WLA.EXE determines that an acute limit is needed, you need to convert the TUc answer you get to TUa and then an LC50, enter it here: NO DATA $\%LC_{50}$
NO DATA TUa

DILUTION SERIES TO RECOMMEND

Table 4.

	Monitoring		Limit	
	% Effluent	TUc	% Effluent	TUc
Dilution series based on data mean	100	1.0		
Dilution series to use for limit			69	1.4492754
Dilution factor to recommend:	0.5		0.8306624	
Dilution series to recommend:	100.0	1.00	100.0	1.00
	50.0	2.00	83.1	1.20
	25.0	4.00	69.0	1.45
	12.5	8.00	57.3	1.74
	6.25	16.00	47.6	2.10
Extra dilutions if needed	3.12	32.05	39.5	2.53
	1.56	64.10	32.9	3.04

Cell: I9
Comment:

Cell: K18
Comment: This is assuming that the data are Type 2 data (none of the data in the data set are censored - "<" or ">").

Cell: J22
Comment: Remember to change the "N" to "Y" if you have ratios entered, otherwise, they won't be used in the calculations.

Cell: C40
Comment:
If you have entered data to calculate an ACR on page 3, and this is still defaulted to "10", make sure you have selected "Y" in cell E21

Cell: C41
Comment: If you have entered data to calculate an effluent specific CV on page 2, and this is still defaulted to "0.6", make sure you have selected "Y" in cell E20

Cell: L48
Comment:
See Row 151 for the appropriate dilution series to use for these NOEC's

Cell: G62
Comment:
Vertebrates are:
Pimephales promelas
Oncorhynchus mykiss
Cyprinodon variegatus

Cell: J62
Comment:
Invertebrates are:
Ceriodaphnia dubia
Mysidopsis bahia

Cell: C117
Comment: Vertebrates are:

Pimephales promelas
Cyprinodon variegatus

Cell: M119
Comment: The ACR has been picked up from cell C34 on Page 1. If you have paired data to calculate an ACR, enter it in the tables to the left, and make sure you have a "Y" in cell E21 on Page 1. Otherwise, the default of 10 will be used to convert your acute data.

Cell: M121
Comment: If you are only concerned with acute data, you can enter it in the NOEC column for conversion and the number calculated will be equivalent to the TUa. The calculation is the same: 100/NOEC = TUc or 100/LC50 = TUa.

Cell: C138
Comment: Invertebrates are:

Ceriodaphnia dubia
Mysidopsis bahia

APPENDIX F

SAMPLE PERMIT LANGUAGE

1. The following list shows the possible test types and species to use in the TMP Special Condition:

Fresh Water Acute Test Types

- ☐ 48 Hour Static Acute LC_{50} Test with *Ceriodaphnia dubia* (Invertebrate)
- ☐ 48 Hour Static Definitive NOAEC Test with *Ceriodaphnia dubia* (Invertebrate)

- ☐ 48 Hour Static Acute LC_{50} Test with *Oncorhynchus mykiss* (Cold Water Vertebrate)
- ☐ 48 Hour Static Definitive NOAEC Test with *Oncorhynchus mykiss* (Cold Water Vertebrate)
- ☐ 96 Hour Static Renewal Acute LC_{50} Test with *Oncorhynchus mykiss* (Cold Water Vertebrate)
- ☐ 48 Hour Static Acute LC_{50} Test with *Pimephales promelas* (Vertebrate)
- ☐ 48 Hour Static Definitive NOAEC Test with *Pimephales promelas* (Vertebrate)
- ☐ 96 Hour Static Renewal Acute LC_{50} Test with *Pimephales promelas* (Vertebrate)

Fresh Water Chronic Test Types

- ☐ Chronic Static Renewal 3-Brood Survival and Reproduction Test with *Ceriodaphnia dubia* (Invertebrate)
- ☐ Chronic Static Renewal 7-Day Survival and Growth Test with *Pimephales promelas (Vertebrate)*

Salt Water Acute Test Types

- ☐ 48 Hour Static Acute LC_{50} Test with *Mysidopsis bahia* (Invertebrate)
- ☐ 48 Hour Static Definitive NOAEC Test with *Mysidopsis bahia* (Invertebrate)

- ☐ 48 Hour Static Acute LC_{50} Test with *Cyprinodon variegatus* (Vertebrate)
- ☐ 48 Hour Static Definitive NOAEC Test with *Cyprinodon variegatus* (Vertebrate)
- ☐ 96 Hour Static Renewal Acute LC_{50} Test with *Cyprinodon variegatus* (Vertebrate)

Salt Water Chronic Test Types

- ☐ Chronic Static Renewal 7-Day Survival, Growth and Fecundity Test with *Mysidopsis bahia*
- ☐ Chronic Static Renewal 7-Day Survival and Growth Test with *Cyprinodon variegatus*

"Less than" (<) NOEC results are not acceptable for chronic tests. A retest must be performed with lower dilutions. For additional test requirements, refer to APPENDIX B of this guidance, which references the EPA document below:

Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fourth Edition, EPA/600/4-90/027F, August 1993.

Short-term Methods to Estimate the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Third Edition, EPA/600/4-91/002, July 1994.

Short-term Methods to Estimate the Chronic Toxicity of Effluents and Receiving Waters to Estuarine and Marine Organisms, Second Edition, EPA/600/4-91/003, July 1994.

2. Samples of language for Special Conditions

Industry Monitoring - Quarterly - Freshwater - Acute and Chronic

There are several options for modifying these special conditions. For example, 1.a. could end the first sentence with: ...for a period of 1 year, for a period of 2 years, for a period of 3 years, for the duration of the permit, within 6 months of commencement of the discharge, within 6 months of receipt of a CTO, etc. The sample frequency can be monthly, bi-monthly, or quarterly at a minimum. The sample type can change to 24-hour composite samples, a grab sample, or a series of grab samples taken during some event. If the language involving sampling is lengthy, make it section 2. of this condition, and the schedule can be 3. Any of the LC_{50} *(use tests with Oncorhynchus mykiss only where the receiving stream is considered trout waters) and NOEC tests listed on page 1. of this section can be substituted into the language. The NOAEC tests have some different language in part 1.a. and can be seen in a later example.*

1. Biological Monitoring:

   a. In accordance with the schedule in 2. below, the permittee shall conduct quarterly acute and chronic toxicity tests until there are a minimum of 10 for each test required. The permittee should collect 24-hour flow-proportioned composite samples of final effluent from outfall _. The acute tests to use are:

      48 Hour Static Acute test using *Ceriodaphnia dubia*
      48 Hour Static Acute test using *Pimephales promelas*

      These acute tests shall be performed with a minimum of 5 dilutions[1], derived geometrically, for calculation of a valid LC_{50}. Express as the results as TU_a (Acute Toxic Units) by dividing $100/LC_{50}$ for DMR reporting.

      The chronic tests to use are:

      Chronic 3-Brood Static Renewal Survival and Reproduction Test using *Ceriodaphnia dubia*
      Chronic 7-Day Static Renewal Survival and Growth Test using *Pimephales promelas*

      These chronic tests shall be conducted in such a manner and at sufficient dilutions (minimum of five dilutions[2], derived geometrically) to determine the "No Observed Effect Concentration" (NOEC) for survival and reproduction or growth. Results which cannot be quantified (i.e., a "less than" NOEC value) are not acceptable, and a retest

[1] There may be cases where you will want to specify the dilution series. The sentence would then be changed to read "The acute tests shall be performed with the following dilutions: 100%, 50%, etc."
[2] There is a suggested chronic dilution series for monitoring at the bottom of the WETLIM10 spreadsheet that should be recommended to the permittee, but does not have to be put into the permit.

will have to be performed. Express the test NOEC as TU_c (Chronic Toxic Units), by dividing 100/NOEC for DMR reporting. Report the LC_{50} at 48 hours and the IC_{25} with the NOEC's in the test report.

The permittee may provide additional samples to address data variability during the period of initial data generation. These data shall be reported and may be included in the evaluation of effluent toxicity. Test procedures and reporting shall be in accordance with the WET testing methods cited in 40 CFR 136.3[3].

b. The test dilutions should be able to determine compliance with the following endpoints[4]:

(1) Acute LC_{50} of_____ equivalent to a TU_a of _____

(2) Chronic NOEC of _____ equivalent to a TU_c of _____

c. The test data will be evaluated by WLA.EXE for reasonable potential at the conclusion of the test period. The data may be evaluated sooner if requested by the permittee, or if toxicity has been noted. Should evaluation of the data indicate that a limit is needed, a WET limit and compliance schedule will be required and the toxicity tests of 1.a. may be discontinued.

d. If after evaluating the data, it is determined that no limit is needed, the permittee shall continue acute and chronic toxicity testing (both species) of the outfall annually, as on the reporting schedule in 2.

e. All applicable data will be reevaluated for reasonable potential at the end of the permit term.

2. Reporting Schedule:

The permittee shall report the results on the DMR and supply (2 for majors, 1 for minors) copies of the toxicity test reports specified in this Toxics Management Program in accordance with the following schedule:

Period	Compliance Periods	DMR/Report Submission Dates
Quarter 1	By 03/31/1999	04/10/1999
Quarter 2	By 06/30/1999	07/10/1999
Quarter 3	By 09/30/1999	10/10/1999
Quarter 4	By 12/31/1999	01/10/2000
Quarter 5	BY 03/31/2000	04/10/2000

[3] The permit writer should reference this guidance and particularly Appendix B in the fact sheet.
[4] Use the endpoints determined by WETLIM10 for this section.

Quarter 6	By 06/31/2000	07/10/2000
Quarter 7	By 09/30/2000	10/10/2000
Quarter 8	By 12/31/2000	01/10/2001
Annual 1	By 12/31/2001	01/10/2002
Annual 2	By 12/31/2002	01/10/2003
Annual 3	By 12/31/2003	01/10/2004

Annual Monitoring – Freshwater – Acute and Chronic

Some permit writers prefer to specify the time frame for the annual sampling to be performed. Sample language could include: "The permittee should collect (sample type) from outfall (number) during the time period of July 1 through August 30. Any retest of a non-acceptable test must be performed during that same time period." As with the previous example, the sample type and collection method can be worded for the particular situation. Remember that municipal facilities are required to perform testing using both species; it is recommended that industrial facilities also use both species (see exemptions below[5]).

1. Biological Monitoring:

   a. In accordance with the schedule in 2. below, the permittee shall conduct annual acute and chronic toxicity tests for the duration of the permit. The permittee should collect 24-hour flow-proportioned composite samples of final effluent from outfall __. The acute tests to use are:

   48 Hour Static Acute test using *Ceriodaphnia dubia*
   48 Hour Static Acute test using *Pimephales promelas*

   These acute tests shall be performed with a minimum of 5 dilutions, derived geometrically, for calculation of a valid LC_{50}. Express as the results as TU_a (Acute Toxic Units) by dividing $100/LC_{50}$ for DMR reporting.

   The chronic tests to use are:

   Chronic 3-Brood Static Renewal Survival and Reproduction Test using *Ceriodaphnia dubia*
   Chronic 7-Day Static Renewal Survival and Growth Test using *Pimephales promelas*

   These chronic tests shall be conducted in such a manner and at sufficient dilutions (minimum of five dilutions, derived geometrically) to determine the "No Observed Effect Concentration" (NOEC) for survival and reproduction or growth[6]. Results which

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[5] Exceptions to this recommendation may be considered on a case by case basis if either of the following conditions is met:
a. The replicate average percent survival in 100% effluent for <u>all</u> the acceptable acute tests during a permit term (or that are being evaluated as representative of the effluent) with a particular species is ≥ 90%, or
b. The replicate average percent survival in 100% effluent for all of the acceptable chronic tests during a permit term (or that are being evaluated as representative of the effluent) with a particular species is ≥ 80% and the secondary endpoint for reproduction, growth, or fecundity is an NOEC=100%.

[6] If the permit specifies the saltwater tests using *Mysidopsis bahia*, then the NOEC will be determined for survival, growth and fecundity.

cannot be determined (i.e., a "less than" NOEC value) are not acceptable, and a retest will have to be performed. Express the test NOEC as TU_c (Chronic Toxic Units), by dividing 100/NOEC for DMR reporting. Report the LC_{50} at 48 hours and the IC_{25} with the NOEC's in the test report.

The permittee may provide additional samples to address data variability during the period of initial data generation. These data shall be reported and may be included in the evaluation of effluent toxicity. Test procedures and reporting shall be in accordance with the WET testing methods cited in 40 CFR 136.3

b. The test dilutions should be able to determine compliance with the following endpoints[7]:

(1) Acute LC_{50} of_____ equivalent to a TU_a of _____

(2) Chronic NOEC of _____ equivalent to a TU_c of _____

c. The test data will be evaluated by WLA.EXE for reasonable potential at the conclusion of the test period. The data may be evaluated sooner if requested by the permittee, or if toxicity has been noted. Should evaluation of the data indicate that a limit is needed, a WET limit and compliance schedule will be required and the toxicity tests of 1.a. may be discontinued.

2. Reporting Schedule:

The permittee shall report the results on the DMR and supply (2 for majors, 1 for minors) copies of the toxicity test reports specified in this Toxics Management Program in accordance with the following schedule:

Period	Compliance Periods	DMR/Report Submission Dates
Annual 1	By 12/31/2001	01/10/2002
Annual 2	By 12/31/2002	01/10/2003
Annual 3	By 12/31/2003	01/10/2004
Annual 4	By 12/31/2004	01/10/2005
Annual 5	By 12/31/2005	01/10/2006

[7] Use the endpoints as determined by WETLIM10.xls.

Freshwater – Acute NOAEC test language

1. Biological Monitoring:

   a. In accordance with the schedule in 2. below, the permittee shall conduct quarterly acute toxicity tests for the term of the permit using 24-hour flow-proportioned composite samples of final effluent from outfall __. The acute multi-dilution NOAEC tests to use are:

      48-Hour Static Acute test using *Ceriodaphnia dubia*
      48-Hour Static Acute test using *Pimephales promelas*

      These acute tests are to be conducted using 5 geometric dilutions of effluent with a minimum of 4 replicates, with 5 organisms in each. The NOAEC[8] (No Observed Adverse Effect Concentration), as determined by hypothesis testing, shall be reported on the DMR converted to TU_a (100/NOAEC). The LC_{50} should also be determined and noted on the submitted report. Tests in which control survival is less than 90% are not acceptable.

      The permittee may provide additional samples to address data variability during the period of initial data generation. These data shall be reported and may be included in the evaluation of effluent toxicity. Test procedures and reporting shall be in accordance with the WET testing methods cited in 40 CFR 136.3

   b. The test data will be evaluated by WLA.EXE for reasonable potential at the conclusion of the test period. The data may be evaluated sooner if requested by the permittee, or if toxicity has been noted. Should evaluation of the data indicate that a limit is needed, a WET limit and compliance schedule will be required and the toxicity tests of 1.a. may be discontinued.

2. Reporting Schedule

3. Sample of language for WET limits

WET limit - Acute Freshwater, TU_a endpoint

1. Whole Effluent Toxicity (WET) Limitation and Monitoring Requirements

   a. The Whole Effluent Toxicity limitation of __ TU_a ($LC_{50} \geq$ __) in Part I.A. is a final

[8] NOAEC = the highest percent concentration where there was no significant difference when compared to the controls. (Note: This is interpreted as the highest percent concentration where there is no significant difference when compared to the controls, and below which there is no statistically significant adverse effect.)

limit with an effective date of _____.

b. Commencing within three (3) months of the effective date of the limit, the permittee shall conduct quarterly acute toxicity tests using 24-hour flow-proportioned composite samples of final effluent from outfall __. The acute tests to use are:

48 Hour Static Acute test using *Ceriodaphnia dubia*
48-Hour Static Acute test using *Pimephales promelas*

These acute tests shall be performed with a minimum of 5 dilutions, derived geometrically, for calculation of a valid LC_{50} and corresponding acute Toxic Units (TU_a). Express as TU_a (Acute Toxic Units) by dividing $100/LC_{50}$ for DMR reporting. Two (2 for majors, 1 for minors) copies of the toxicity test results shall be submitted with the DMR. Test procedures and reporting shall be in accordance with the WET testing methods cited in 40 CFR 136.3

c. If after a minimum of four quarters of tests have been reviewed, it is determined that tests with one of the species in 1.b. meets the criterion below, testing may be reduced to using only one species:

Survival of $\geq$ 90% of the organisms of a particular species in 100% effluent in each of the tests considered.

NOTE: Item c. is optional – if the effluent is variable, you may want to leave both species in the permit for the permit term.

d. The permit may be modified or revoked and reissued to include pollutant specific limits in lieu of a WET limit should it be demonstrated that toxicity is due to specific parameters. The pollutant specific limits must control the toxicity of the effluent.

e. Reporting Schedule:

WET Limit effective date 01/01/2000

Period	Compliance Periods	DMR/Report Submission Dates
Quarter 1	By 03/31/2000	04/10/2000
Quarter 2	By 06/30/2000	07/10/2000
Quarter 3	By 09/30/2000	10/10/2000
Quarter 4	By 12/31/2000	01/10/2001
Quarter 5	By 03/31/2001	04/10/2001
Quarter 6	By 06/30/2001	07/10/2001
Quarter 7	By 09/30/2001	10/10/2001
Quarter 8	By 12/31/2001	01/10/2002
Quarter 9	By 03/31/2002	04/10/2002

Quarter 10	By 06/30/2002	07/10/2002
Quarter 11	By 09/30/2002	10/10/2002
Quarter 12	By 12/31/2002	01/10/2003
Quarter 13	By 03/31/2003	04/10/2003
Quarter 14	By 06/30/2003	07/10/2003
Quarter 15	By 09/30/2003	10/10/2003
Quarter 16	By 12/31/2003	01/10/2004

WET limit - Acute Freshwater, NOAEC endpoint

1. Whole Effluent Toxicity (WET) Limitation and Monitoring Requirements

   a. The Whole Effluent Toxicity limitation of NOAEC = 100% effluent in Part I.A. is a final limit with an effective date of _____.

   b. Commencing within one (1) month of the effective date of the limit, the permittee shall conduct monthly (bimonthly, quarterly) acute toxicity tests using 24-hour flow-proportioned composite samples of final effluent from outfall __. The acute tests to use are:

      48 Hour Static Acute Test using *Ceriodaphnia dubia*
      48 Hour Static Acute Test using *Pimephales promelas*

      These acute tests are to be conducted using a minimum of 4 replicates, with 5 organisms each, for the control and 100% effluent. The NOAEC (No Observed Adverse Effect Concentration) shall be reported as either 100% or <100% (less than 100%). The effluent will be in compliance if the survival of the test organisms in both the control and 100% effluent exposures equals or exceeds 90%. If the survival in the effluent is less than 90% and this value is significantly different form the control survival, as determined by hypothesis testing, the NOAEC is less than 100% and the effluent is not in compliance. Tests in which control survival is less than 90% are not acceptable.

      Two copies of the toxicity test results shall be submitted with the DMR. Test procedures and reporting shall be in accordance with the WET testing methods cited in 40 CFR 136.3

   c. If after a minimum of four quarters of tests have been reviewed, it is determined that tests with one of the species in 1.b. meets the criterion below, testing may be reduced to using only one species:

      Survival of ≥ 90% of the organisms of a particular species in 100% effluent in each of the tests considered.

      NOTE: Item c. is optional – if the effluent is variable, you may want to leave both species in the permit for the permit term.

   d. The permit may be modified or revoked and reissued to include pollutant specific limits in lieu of a WET limit should it be demonstrated that toxicity is due to specific parameters. The pollutant specific limits must control the toxicity of the effluent.

WET limit - Chronic Freshwater, TU_c endpoint

1. Whole Effluent Toxicity (WET) Limitation and Monitoring Requirements

   a. The Whole Effluent Toxicity limitation of __ TU_c (NOEC ≥ __) in Part I.A. is a final limit with an effective date of _____.

   b. Commencing within one (1) month of the effective date of the limit, the permittee shall conduct quarterly chronic toxicity tests using 24-hour flow-proportioned composite samples of final effluent from outfall __. The chronic tests to use are:

      Chronic 3-Brood Static Renewal Survival and Reproduction Test using *Ceriodaphnia dubia*
      Chronic 7-Day Static Renewal Survival and Growth Test using *Pimephales promelas*

      These chronic tests shall be conducted in such a manner and at sufficient dilutions (minimum of five dilutions, derived geometrically) to determine the "No Observed Effect Concentration" (NOEC) for survival and reproduction. The test endpoint (limit) must be represented by a dilution, and if other than 100%, should be bracketed by at least one dilution above and one dilution below it[9]. Express the test NOEC as TU_c (Chronic Toxic Units), by dividing 100/NOEC for DMR reporting. The IC_{25} should be included on the submitted test reports. Two copies of the toxicity test results shall be submitted with the DMR. Test procedures and reporting shall be in accordance with the WET testing methods cited in 40 CFR 136.3

   c. If after a minimum of four quarters of tests have been reviewed, it is determined that tests with one of the species in 1.b. meets the criterion below, testing may be reduced to using only one species:

      Survival of ≥ 80% of the organisms in 100% effluent in each of the tests considered, and the secondary NOEC endpoint for reproduction or growth is an NOEC = 100% effluent.

      NOTE: Item c. is optional – if the effluent is variable, you may want to leave both species in the permit for the permit term.

   d. The permit may be modified or revoked and reissued to include pollutant specific limits in lieu of a WET limit should it be demonstrated that toxicity is due to specific parameters. The pollutant specific limits must control the toxicity of the effluent.

[9] For limitations, you may want to specify the dilution series to use. Table 4 at the bottom of the WETLIM10 spreadsheet will calculate a series with the limitation as the middle dilution.

4. Other language that may be useful

For samples that are rainfall dependent:

2. Sampling Technique for Outfall(s) __ and Additional Information to be Submitted with the Results of Biological Tests Performed in Accordance with Section 1. above:

   a. Sampling of each outfall shall, if at all possible, be within the first three hours following the initiation of a rainwater discharge event. If this action can not be accomplished as required, the sample(s) shall be taken as soon as possible, but not later than 24 hours after the rainwater discharge commences. The permittee shall submit the following information with the results of the toxicity tests:

      (1) An actual measurement or estimate of the effluent flow at the time of sampling.

      (2) The time the storm event began, the time the effluent was sampled, and the duration of the storm event.

      (3) The duration between the storm event sampled and the end of the previous storm event.

For municipal facilities undergoing an UPGRADE

1. In accordance with the schedule in 2. below, commencing within six months **(need to give upgraded plant time to reach equilibrium)** from the issuance of the certificate to operate (CTO) for the **(fill in flow for upgraded plant)** MGD facility, the permittee shall conduct...

For industries undergoing an UPGRADE

1. In accordance with the schedule in 2. below, commencing within six months from the initiation of a discharge from the upgraded treatment works, the permittee shall conduct...

In cases where the PERMIT CONTAINS A FINAL CHLORINE LIMITATION, the first paragraph of the TMP should include the following language:

Effluent samples shall not be dechlorinated prior to use in toxicity tests.

In cases where the PERMIT HAS AN INTERIM CHLORINE LIMIT AND A COMPLIANCE SCHEDULE FOR MEETING A FINAL CHLORINE LIMIT, the TMP should start with the following language.

Beginning with the effective date of this permit (MODIFICATION) and continuing until

achievement of compliance with the final chlorine limitation as specified in (ENTER SECTION OF PERMIT WHICH CONTAINS IMPLEMENTATION SCHEDULE), effluent samples should be dechlorinated prior to use in toxicity testing.

Subsequent to the effective date of the final chlorine effluent limit, effluent samples shall not be dechlorinated prior to their use in toxicity tests.

FOR NONCONTACT COOLING WATER OUTFALLS ONLY:

d. Following completion of the testing of outfall(s) _ as above, the permittee may discontinue toxicity testing of a particular outfall if it is determined to be non-toxic when evaluated for reasonable potential.

For discharges from BULK OIL STORAGE FACILITIES WHICH CONTAIN TANK BOTTOM WATERS

Effluent samples collected from outfall _ shall be collected at a time when tank bottom waters are being discharged through this outfall. The relative amounts of stormwater and tank bottom waters contributing to the discharge shall be reported with the biological tests. The permittee shall maintain a record concerning the relative amounts of storm water and tank bottom waters predicted to comprise each discharge through outfall . This information shall be reported with the monthly DMRs submitted for the outfall.

EFFLUENTS FOR WHICH SAMPLE COLLECTION MAY NOT BE POSSIBLE DURING A PARTICULAR SAMPLING PERIOD:

In the event that sampling of a particular outfall as in (SPECIFY BIOLOGICAL SECTIONS) above, is not possible due to the absence of effluent flow during a particular testing period, the permittee shall provide written notification to the Department's (FILL IN REGIONAL OFFICE) Regional Office with the DMR submitted for the month following the period in which the toxicity tests were to have been conducted. In such cases, the reporting schedule in (FILL IN SCHEDULE SECTION) below shall be adjusted. The requirement for sampling of the outfall shall continue until the required number of toxicity tests have been performed.

Additional information to be submitted for EFFLUENTS COMPRISED OF MORE THAN ONE INTERNAL WASTESTREAM.

(1) The permittee shall list in chronological order all activities (e.g. washing, maintenance, cooling, processes, etc.) or events (weather) which contributed wastewater to the outfall during the 24-hour period prior to (or during depending on the nature of the outfall) sample collection including:

(a) The time at which the activity/event began and ended.

(b) The amount of wastewater generated by the activity.

(2) The permittee shall report the actual or estimated effluent flow at the time of sampling and the relative contributions of the individual wastewater source(s) above to this flow.

EFFLUENT SAMPLING PROCEDURES AND ADDITIONAL INFORMATION TO BE PROVIDED WITH THE RESULTS of toxicity tests performed using samples from STORM WATER DISCHARGES[10].

1. Sampling of each outfall shall, if at all possible, be within three hours after the rainwater discharge commences. Additional information which should be submitted for each outfall includes:

   a. An actual measurement or estimate of effluent flow at the time of sampling.

   b. The time the storm event began, the time the effluent was sampled, and the duration of the storm event.

   c. The duration between the storm event sampled and the end of the previous storm event.

EFFLUENT SAMPLING PROCEDURES AND ADDITIONAL INFORMATION TO BE SUBMITTED FOR INTERMITTENT DISCHARGES WHICH CAN BE INITIATED BY THE PERMITTEE.

1. The permittee shall collect composite samples of effluent from outfall _ for biological testing. Each composite sample shall consist of grab samples collected hourly during the period of discharge or, during the initial 24 hours of discharge, should the duration of the discharge exceed 24 hours. Effluent sampling shall begin as soon as possible following the initiation of the discharge.

2. The permittee shall include with the results of the biological tests performed with a particular sample:

   (a) An estimate of the total volume discharged through outfall _ and the duration of the discharge.

[10] Toxicity testing can be put into the permit for stormwater discharges for the purpose of seeing the effectiveness of facility BMP's for reducing toxicity.

(b) The time at which the discharge was initiated.

(c) The time at which sampling was initiated.

For use with effluents generated during the HYDROSTATIC TESTING

(b) Outfall _(Hydrostatic Test Water): In the event that the facilities are pressure tested with water, the subsequent discharge(s) shall be monitored for toxicity. During each hydrostatic test period, one set of acute toxicity tests shall be conducted using a composite of hourly grab samples collected over the duration of the discharge (not to exceed 24 hours). Information regarding discharge duration (i.e., beginning and end of discharge), volume discharged, and tank (or pipe) contents prior to the hydrostatic tests shall also be submitted. If less than 4 sets of data are collected, the results of these tests shall be used in the determination of the need for further testing requirements.