Whole Effluent Toxicity Testing (Biomonitoring)

Applicability

Whole effluent toxicity (WET) testing, also known as biomonitoring, is required in permits for dischargers whose effluent has a significant potential to exert toxicity in the receiving water (See § 307.6(e)(2)(A) of the Standards). WET testing directly measures a discharge’s aggregate toxic effect by exposing surrogate sensitive test species to effluent at the critical dilution of the receiving water. Thus, it is an integral tool in the assessment of water quality for the protection of aquatic life and part of EPA’s integrated strategy that includes the use of three control approaches (the other two being chemical-specific limits and biological criteria).

Domestic Dischargers

The TCEQ requires WET testing of domestic wastewater dischargers that have either or both of the following conditions:

- classified as an EPA major domestic discharger, whether due to (a design flow of 1 MGD or greater (or an interim or final phase design flow of 1 MGD or greater)), or due to any individual WWTP with an approved pretreatment program with significant industrial users discharging into its collection system.

Permittees with more than one flow phase in their permit begin WET testing upon expansion to 1 MGD or greater.

Industrial Dischargers

The TCEQ requires WET testing of industrial dischargers that have any of the following conditions:

- classified as an EPA major industrial discharger;
- a continuous discharge of process treated wastewater; or
- a discharge with the potential to exert toxicity in the receiving water.

Although the TCEQ generally does not require WET testing of EPA-classified minor industrial dischargers, the TCEQ may require WET testing of such dischargers in any of the following situations:

- the permittee applies water treatment
the TCEQ determines that the continuous treated process wastewater effluent which has the potential to exert significant toxicity in the receiving water.

• the permit requires effluent limits based on aquatic life water quality criteria because the effluent analysis exceeds the screening criteria.

Chapter Outline

The rest of this chapter covers the following topics:

• types of WET tests (chronic and 48-hour acute—page 3404; 24-hour acute—page 19148)

• test acceptability criteria (chronic and 48-hour acute—page 5405; 24-hour acute—page 20119)

• statistical interpretation of test results (chronic and 48-hour acute only—page 7407)

• test frequencies (chronic and 48-hour acute—page 7408; 24-hour acute—page 20419)

• dilution series, dilution water, and type of WET tests—page 9410

• reasonable potential determination (chronic and 48-hour acute only—page 12413)

• toxicity reduction evaluations (chronic and 48-hour acute—page 15415; 24-hour acute—page 22424)

• toxicity control measures (chronic and 48-hour acute—page 17416; 24-hour acute—page 23422)

• toxicity caused by some specific pollutants—dissolved salts (page 23422) and ammonia (page 29428), and Diazinon (page 129).
Chronic and 48-Hour Acute Tests

The TCEQ may require permittees to conduct 7-day chronic or 48-hour acute WET tests to measure compliance with the requirements of §307.6(e) of the Standards.

For hypothesis testing, toxicity in these tests is defined as a statistically significant difference (usually at the 95% confidence level) between the survival, reproduction, or growth of the test organisms at a specified effluent dilution (the critical dilution) compared to the survival, reproduction, or growth of the test organisms in the control (0% effluent).

For point estimate testing, toxicity is defined as a 25% or greater difference (the inhibition concentration) between the survival, reproduction, or growth of the test organisms at a specified effluent dilution (the critical dilution) compared to the survival, reproduction, or growth of the test organisms in the control (0% effluent).

The TCEQ will use point estimate testing as the default methodology. Hypothesis testing may be used at the request of the permittee. Both methods are referenced in the EPA method manuals (see below, “Test Types”).

Test Types

The permit will specify that tests be conducted using the latest version of the appropriate EPA method. These methods can be found in the following publications (or their most recent versions):


In addition, information on interpreting non-monotonic test results and percent minimum significant difference (PMSD) values can be found in the following publications:
The permittee must use a revised promulgated method if one becomes available during the term of the permit. The permittee may also use alternate test methods subject to EPA review and approval. An example of an approved alternate test method is the use of the water flea, *Daphnia magna*, in a chronic test in accordance with “Development and Validation of a *Daphnia magna* Four-Day Survival and Growth Test Method”, Environmental Toxicology and Chemistry, Volume 28, Number 5, 2009. Depending on the type of receiving water, the permit will specify chronic or 48-hour acute tests to assess toxicity to freshwater or saltwater organisms. The test organisms used for each type of test are listed below.

**FRESHWATER STREAMS AND LAKES (SALINITY < 12 PPT)**

**CHRONIC**
- 3-brood *Ceriodaphnia dubia* (water flea) survival and reproduction test
- 7-day *Pimephales promelas* (fathead minnow) larval survival and growth test

**ACUTE**
- 48-hour *Daphnia pulex* or *Ceriodaphnia dubia* (water fleas) survival test
- 48-hour *Pimephales promelas* (fathead minnow) survival test

**MARINE RECEIVING WATER (SALINITY ≥ 12 PPT)**

**CHRONIC**
- 7-day *Americamysis bahia* (mysid shrimp, formerly *Mysidopsis bahia*) survival and growth test
- 7-day *Menidia beryllina* (inland silverside) larval survival and growth test

**ACUTE**
- 48-hour *Americamysis bahia* (mysid shrimp) survival test
- 48-hour *Menidia beryllina* (inland silverside) survival test

Permittees may substitute other EPA approved tests and species if they obtain approval from the TCEQ during the permit application process (see the sections of this document entitled “Toxicity Attributable to Dissolved Salts” on page 23422 and “Site-Specific Standards for Total Toxicity” on page Error! Bookmark not defined.207).
Typically, if the segment criterion for total dissolved solids (TDS) or the site-specific TDS concentration in the receiving water is too high to support *Ceriodaphnia dubia* or *Daphnia pulex, Daphnia magna* (another water flea) will be substituted as the invertebrate freshwater test organism after the need to make the substitution is demonstrated. The permittee may submit evidence substantiating the need for an alternative species before or during the application process. However, draft permits with alternate tests, alternate species, or testing requirements that exclude a species are subject to EPA review and approval.

**Test Acceptability Criteria**

A toxicity test, whether using hypothesis or point estimate statistical analysis, which fails to meet any of the following acceptability criteria is considered invalid, and the permittee will have to repeat the test. Other factors may also invalidate a test. All test results, valid or invalid, are to be submitted to the TCEQ.

**Chronic Freshwater**

- a mean survival of 80% or greater in the control.
- a mean number of 15 or greater water flea neonates per surviving adult in the control.
- a mean dry weight of 0.25 mg or greater for surviving fathead minnow larvae in the control.
- a coefficient of variation percent (CV%) of 40 or less between replicates in the control and in the critical dilution for:
  - the young of surviving females in the water flea reproduction and survival test; and
  - the growth and survival endpoints in the fathead minnow growth and survival test.

However, if statistically significant lethal or sublethal effects are exhibited, a CV% greater than 40 does not invalidate the test.

- a PMSD of 47 or less for the water flea and a PMSD of 30 or less for the fathead minnow. However, if statistically significant sublethal effects are exhibited, a PMSD in excess of that specified above does not invalidate the test.
- a test population of < 20% males in a single concentration or < 20% males in a whole test for the water flea reproduction test.
**Chronic Saltwater**

- a mean survival of 80% or greater in the control.
- a mean dry weight of 0.20 mg or greater for surviving mysid shrimp in the control.
- a mean dry weight in the control of 0.50 mg or greater for surviving unpreserved inland silverside and 0.43 mg or greater for surviving preserved inland silverside.
- a CV% of 40 or less in the control and in the critical dilution in the growth and survival tests. However, if statistically significant lethal or sublethal effects are exhibited, a CV% greater than 40 does not invalidate the test.

- a PMSD of 37 or less for the mysid shrimp and a PMSD of 28 or less for the inland silverside. However, if statistically significant sublethal effects are exhibited, a PMSD in excess of that specified above does not invalidate the test.

**48-hour Acute Freshwater and Saltwater**

- a mean survival of 90% or greater in the control.
- a CV% of 40 or less in the control and in the critical dilution.

However, if significant lethality is demonstrated, a CV% greater than 40 does not invalidate the test.

**Once-Through Cooling Water Facilities**

Once-through cooling water facilities that use intake water as the control do not have to retest and report a valid test for each test species during the reporting period if the test is invalid because the control fails to meet acceptability criteria. This exception recognizes that running additional tests is not useful when the source waterbody itself is already toxic to one or both test organisms due to total dissolved solids (TDS), pathogenic bacteria, or toxic algae blooms.
**Statistical Interpretation of Test Results**

For hypothesis testing, if significant lethality is demonstrated (that is, if there is a statistically significant difference in survival at the critical dilution when compared to the control), but the conditions of test acceptability are met and the survival endpoint equals or exceeds the acceptability criteria at the critical dilution and all dilutions below that, then the permittee may report a survival NOEC of not less than the critical dilution.

While the nominal error rate (alpha) used for hypothesis testing in WET data is 0.05 (95% confidence interval), the alpha level for sublethal statistical analysis may be modified in accordance with EPA guidelines under appropriate conditions.\(^1\)

While the method manuals list a range for PMSDs, a value below that range does not invalidate the test. If no significant sublethal effects are indicated, the NOEC should be reported as is. However, if the test indicates statistically significant sublethal effects, additional calculations should be performed in order to determine the NOEC.\(^2\)

**Test Frequencies**

**General**

Figure 4 on page 109 illustrates the chronic and 48-hour acute testing frequencies for facilities with WET requirements. Testing is typically performed quarterly for both the vertebrate and the invertebrate test species for the first year of the permit term. EPA requires quarterly testing for at least one year to assess the variability and toxic potential of effluents.

If no significant effects are demonstrated in the first year of quarterly testing, the permittee may request a testing frequency reduction to once per six months for the more sensitive species (usually the invertebrate) and once per year for the less sensitive species (usually the vertebrate) for the remainder of the permit term. If one species is eligible for the reduction but the other isn’t, the one that is eligible shall only be required to test annually.

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\(^2\) Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System Program, EPA 833-R-00-003, June 2000.
After passing four consecutive tests with no lethal or sublethal failures, permittee may request a reduced testing frequency.

Pass all lethal and sublethal

Yes

No

Initial quarterly testing

Sublethal failure only (not a lethal failure)?

Yes

Two monthly retests

Continue or resume quarterly testing until four consecutive tests show no lethal or sublethal effects.

No

Lethal failure—two monthly retests

Did both tests

Did one or both tests pass?

Yes

No

Continue or resume quarterly testing.

TRE recommended

Figure 4. Chronic and 48-Hour WET Testing Frequencies
If significant toxicity lethality is demonstrated in the first year of quarterly testing, that species is not eligible for the testing frequency reduction and the permittee must then test quarterly for the permit term. If significant sublethality is demonstrated in the first year of quarterly testing, the permittee will not be eligible for the testing frequency reduction for that species until no significant effects are demonstrated for four consecutive quarterly tests.

If a testing frequency reduction has been granted for a species, but that species subsequently demonstrates significant toxicity lethality, the quarterly testing frequency for that species will be resumed for the permit term. If a testing frequency reduction has been granted for a species, but that species subsequently demonstrates significant sublethality, the quarterly testing frequency for that species will be resumed until four consecutive quarterly tests demonstrate no significant effects.

**With a WET Limit**

Permitees will be required to perform quarterly testing for at least three years when a WET limit is added to the permit. This frequency only applies to the species with the WET limit. Best professional judgment (BPJ) will be used to establish testing frequencies when a chemical-specific limit or best management practice (BMP) is placed in the permit to control effluent toxicity.

**Dilution Series, Dilution Water, and Type of WET Test**

**Dilution Series**

Chronic and some 48-hour acute tests are based on the critical dilution in the receiving water. The critical dilution represents the percentage of effluent at the edge of the mixing zone during critical low-flow (that is, the 7Q2 or appropriate critical low-flow for spring-fed streams) or critical mixing conditions. Some 48-hour acute tests are based on the percentage of effluent at the edge of the zone of initial dilution (ZID). The dilution series consists of five effluent concentrations besides the control.

For hypothesis testing, the test results at the critical dilution are statistically compared with the test results at the control dilution (0% effluent) to assess toxicity lethality and measure compliance. The permit specifies the critical dilution and the dilution series as well as the type of WET tests required. The dilution series consists of five effluent concentrations besides the control.

For point estimate testings, the standard dilution series may be used; it may or may not contain the critical dilution but will normally have an upper dilution of 100% so that the 24-hour acute results may be extracted from the chronic or 48-hour acute results without the need to run additional/separate tests. The critical dilution may or may not be part of the dilution series. The IC25 will assess
The dilution series consists of five or effluent concentrations besides the control in addition to the critical dilution. For domestic dischargers, the design flow is normally used to calculate the critical dilution. For industrial dischargers who are renewing permits, the highest monthly average flow from the preceding two years is normally used to calculate the critical dilution. For new or expanding industrial facilities, the design flow is used to calculate the critical dilution.

**Dilution Water**

As specified in the permit, receiving water unaffected by the discharge should be used as the control and as dilution water for at least the first series of WET tests performed after a new permit is issued. This condition may be waived for safety reasons or if the defined sampling location isn’t accessible (e.g., a lake significantly downstream from the outfall). The waiver should be requested during the permit application process so that the requirement can be addressed in the draft permit. If not, the waiver request must be made in writing.

If the receiving water demonstrates pre-existing instream toxicity (by failing to meet the appropriate test acceptability criteria in the control), the test is considered invalid, and a repeat test has to be performed unless a “performance control” using synthetic dilution water was run at the same time and no toxic effects were demonstrated.

Upon demonstrating that the receiving water is toxic, the permittee may substitute synthetic dilution water, prepared in accordance with the method manuals, may for receiving water be used as the control and as dilution water in all subsequent tests unless the permittee specifically requests the use of receiving water for that permit term. The physical and chemical properties (for example, pH, hardness, TSS, alkalinity) of the synthetic dilution water should be similar to those of the receiving water.

**Type of Test**

The TCEQ determines what type of WET test (freshwater or marine, acute or chronic) to place in the permit based on the salinity and critical conditions of the receiving waters. In general, TCEQ staff considers salinities at or above 12,000 mg/L (12.0 ppt) to represent saltwater conditions.

If the TCEQ determines that WET testing is required for a storm water discharge, TCEQ staff may use an analysis of the watershed to determine runoff volumes for dilution estimates. In addition, the TCEQ may require WET testing or other methods to protect water bodies with endangered species.

**INTERMITTENT STREAMS WITH MINIMAL AQUATIC LIFE USE**

Permittees that discharge into intermittent streams with a minimal aquatic life use
will conduct 48-hour acute testing with a critical dilution of 100% effluent.

**INTERMITTENT STREAMS WITH PERENNIAL POOLS**
Permittees that discharge into intermittent streams with perennial pools will conduct chronic testing with a critical dilution of 100% effluent.

**INTERMITTENT STREAMS WITH SEASONAL AQUATIC LIFE USES**
TCEQ may require dischargers to conduct chronic testing to protect intermittent streams that may have seasonal aquatic life uses. TCEQ determines the critical dilution from the typical flows in the season in which the use occurs.

**INTERMITTENT STREAMS WITHIN THREE MILES OF A PERENNIAL FRESHWATER STREAM**
Permittees that discharge into intermittent streams that flow into a perennial stream within a moderate distance downstream (normally 3 miles) will conduct either a 48-hour acute or a chronic test. The type of test depends on the size of the discharge relative to the flow of the perennial water downstream.

If the effluent flow equals or exceeds 10% of the low-flow of the perennial water, the permittee will conduct chronic testing with a critical dilution representative of the percentage of effluent in the perennial stream during low-flow. If the effluent flow is less than 10% of the low-flow in the perennial stream, the permittee will conduct 48-hour acute toxicity tests with a critical dilution of 100% effluent. The TCEQ generally requires permittees that discharge into intermittent streams within 3 miles of a bay, estuary, or tidal river to conduct chronic marine testing.

**PERENNIAL FRESHWATER STREAMS**
Permittees that discharge directly into perennial freshwater streams or rivers with a designated or limited, intermediate, high, or exceptional aquatic life use will conduct chronic testing; the critical dilution will be based on the effluent flow and critical low-flow of the stream or river. If the critical dilution is less than 5%, the TCEQ requires 48-hour acute testing and uses an acute-to-chronic ratio (ACR) of 10:1 to determine the appropriate critical dilution. The ACR is the ratio of the acute toxicity of an effluent or toxicant to its chronic toxicity. It is used to estimate the chronic toxicity based on acute toxicity results. An ACR of 10 represents the upper 90th percentile of the ACR data available to EPA in 1991.

**LAKES**
Permittees that discharge to a lake will normally conduct chronic WET tests with a critical dilution of 15% if the effluent flow is less than or equal to 10 MGD and the mixing zone is 100 feet wide. If the effluent flow is greater than 10 MGD or if the mixing zone is less than 100 feet wide, the TCEQ typically uses the horizontal Jet Plume equation (see page Error! Bookmark not defined. 74) to determine the percentage of effluent at the edge of the mixing zone. In these cases the critical dilution is generally greater than 15%. The TCEQ assigns a critical dilution of 100% effluent for discharges greater than 100 MGD.
**BAYS, ESTUARIES, AND WIDE TIDAL RIVERS**
Permittees that discharge into bays, estuaries, and wide tidal rivers (≥ 400 feet across) will normally conduct chronic WET tests with a critical dilution of 8% if the effluent flow is less than or equal to 10 MGD. If the effluent flow is greater than 10 MGD, the TCEQ uses the horizontal Jet Plume equation (see page Error! Bookmark not defined.74) to determine the percentage of effluent at the edge of the mixing zone. The TCEQ assigns a critical dilution of 100% effluent for discharges greater than 100 MGD.

**NARROW TIDAL RIVERS**
Permittees that discharge into narrow tidal rivers (< 400 feet across) will normally conduct chronic WET tests with the critical dilution based on upstream flow whenever flow information is available. In the absence of site-specific data such as dispersion dye studies or nearby flow measurements, the critical dilution typically is not less than 8% to ensure the same level of protection given to other marine waters. If upstream flows are not available, the horizontal Jet Plume equation (see page Error! Bookmark not defined.74) is used to determine the critical dilution at the edge of the mixing zone. Critical dilutions calculated in this way are greater than 8% because the mixing zone size is less than 200 feet.

**Diffusers**
An effluent diffuser installed at the end of a discharge pipe may increase mixing and lower critical dilutions. See the section of this document entitled “Diffusers” on page Error! Bookmark not defined.82 for more information. The effluent percentage at the edge of the mixing zone for a diffuser discharge is usually determined through modeling. This effluent percentage, if determined to be appropriate, is normally used as the critical dilution for chronic WET testing. If the critical dilution is less than 5%, the TCEQ may instead require 48-hour acute testing using an ACR of 10:1 to determine the appropriate critical dilution.

**Reasonable Potential Determination**
Permit applications that meet the applicability criteria for WET testing will be screened to determine if the discharge has a reasonable potential (RP) to cause significant toxicity. A reasonable potential analysis is performed in order to determine whether an effluent can reasonably be expected to cause or contribute to an exceedance of a state water quality standard or criterion within that standard.

For renewed or amended permit applications, screening for RP will be based on representative data from the previous three years of WET testing. New permit applications will not be screened for RP, since there will be no data from previous WET testing. Toxicity for new permits will be assessed by routine, periodic WET testing after the permits are issued.
Toxicity is presumed if a test fails for the lethal or sublethal endpoint. A test is considered to have failed if a statistically significant difference occurs between the control and the critical dilution for hypothesis testing or if the inhibition concentration (IC$_{25}$) is less than the critical dilution for point estimate testing.
In accordance with federal regulations, the TCEQ will make an RP determination for toxicity. The determination will be based on best professional judgment as well as additional factors, such as duration and magnitude, as agreed upon by the TCEQ and the EPA. Each test species will be evaluated separately.

When a final determination of RP is made, the permit will be issued for a five-year term, including an initial one-year investigative period for the permittee to conduct an initial toxicity investigation. The investigative period will be followed by up to a three-year compliance period to allow for assessment of the cause and/or elimination of toxicity prior to the effective date of the WET limit.

If appropriate, the permittee may apply for a permit amendment to remove the WET limit by replacing it with a chemical-specific limit or a best management practice (BMP) prior to the end of the compliance period (see below). If there are no further demonstrations of toxicity during the compliance period, the WET limit will not become effective. With a WET limit does become effective, the permittee may, after three years of compliance with an effective WET limit, submit a major amendment application to request removal of the WET limit and resumption of routine WET testing.

**Addressing WET Limit Violations**

If the permittee fails a WET test (that is, demonstrates significant toxicity at the critical dilution) while the limit is in effect, the testing frequency for the species increases to monthly until the permittee passes (does not demonstrate significant toxicity at the critical dilution) three consecutive tests, after which the permittee may resume quarterly testing.

However, if the permittee fails two tests during the increased monthly testing period, the permittee will be considered in significant noncompliance with the WET limit and will receive a Notice of Enforcement (NOE), and will be referred to TCEQ’s Enforcement Division for formal enforcement action. This process is illustrated in Figure 5 on page 117.

**Chemical-Specific Limit**

In order to be eligible for a chemical-specific limit in lieu of a WET limit, the permittee has to demonstrate that one or more known pollutants caused the toxicity and should attempt to determine a specific concentration of the pollutant that will not cause toxicity. A chemical-specific limit may be inadequate to address toxicity in the following situations:

- failure to identify the toxicant or toxicants.
- presence of multiple toxicants.
- lack of a routine test method capable of detecting a pollutant at levels causing persistent significant toxicity.
**BMP**

In terms of WET testing, BMPs are defined as a practice or combination of practices that remove toxicity from the effluent by eliminating the source of toxicity. In order to be eligible for a BMP in lieu of a WET limit, the permittee has to demonstrate that such a provision can adequately address toxicity. If successful, the BMP becomes an enforceable part of the permit. A BMP does not include making changes to operations or housekeeping practices to reduce toxicity. In these cases, the source of toxicity still remains.

**Toxicity Reduction Evaluations (TREs)**

**When is a TRE Performed?**

The TCEQ suggests that a permittee initiate a TRE when persistent significant lethality toxicity occurs during routine WET testing. A TRE may allow the permittee to avoid a WET limit as the toxicity control measure. A TRE may also be required if numerous demonstrations of significant toxicity (lethal or sublethal) occur during the course of a permit term.

If a permittee fails a WET test for lethality, that is, statistically significant lethality toxicity occurs at the critical dilution, the permittee will conduct two retests with that test species. The retests are to be conducted monthly during the next two consecutive months. If persistent significant lethality toxicity is demonstrated by a lethal failure of one or both retests, the permittee may wish to perform a TRE. A second retest is not required if the first retest confirms persistent lethality toxicity.

**TRE Purpose and Content**

The purpose of the TRE is to determine the cause and source of toxicity, and to determine methods to reduce or eliminate the toxicity. Components of a TRE may include, but are not limited to:

- chemical analyses
- effluent characterization tests (physical/chemical properties)
- WET tests on effluent before and after characterization test manipulations
- WET tests on effluent after chemical/physical separations
- source identification evaluation or toxicity source evaluation
- instream WET tests
• chemical identification after chemical/physical separations of toxic phase
• assessment of treatment technology available to remove the toxic substance from the effluent.
For more information on methods used in TREs, see the following documents (or their most recent versions):


Figure 5. Procedure for Addressing WET Limit Violations

Permittee performing quarterly testing under a WET limit

- Initial test failure
  - Increase test frequency to monthly for species that failed test.
  - Two additional test failures during period of increased test frequency

- Pass

- Permittee continues monthly testing until 3 consecutive tests are passed.

- Noncompliance with WET limit has been demonstrated:
  - NOE and Mandatory Enforcement

- Return to quarterly
24-Hour Acute (100% End-of-Pipe) Tests

In addition to conducting chronic or 48-hour acute tests, dischargers are required to conduct 24-hour acute tests using 100% effluent. This end-of-pipe test measures compliance with § 307.6(e)(2)(B) of the Standards, which requires that greater than 50% of the test organisms survive exposure to 100% effluent for 24 hours. This provision is designed to ensure that water in the state will not be acutely toxic to aquatic life passing through the ZID.

In addition to facilities mentioned previously in the section “Applicability” (see page 1102), the TCEQ may require 24-hour acute testing for intermittent process water outfalls and/or storm water outfalls with the potential for causing significant toxicity. Dischargers with multiple outfalls will test each outfall that has the potential to cause significant toxicity. Multiple outfall samples may not be composited for this test.

Test Types

The permit will specify that the tests be conducted using the latest version of the appropriate EPA method. The 24-hour acute test species and methods are the same as those for 48-hour acute testing and can be found in the manual listed on page 1104. Depending on the type of receiving water, the permit will specify 24-hour acute tests to assess toxicity to freshwater or saltwater organisms. The test organisms for each type of test are as follows:

**Freshwater streams and lakes (salinity < 12 ppt):**
- 24-hour *Daphnia pulex* or *Ceriodaphnia dubia* (water fleas) survival test
- 24-hour *Pimephales promelas* (fathead minnow) survival test

**Marine receiving water (salinity ≥ 12 ppt):**
- 24-hour *Americamysis bahia* (mysid shrimp) survival test
- 24-hour *Menidia beryllina* (inland silverside) survival test

Permittees may substitute other EPA-approved tests and species if they obtain approval from the TCEQ before or during the permit application process (see the sections in this document entitled “Toxicity Attributable to Dissolved Salts” on page 23422 and “Site-Specific Standards for Total Toxicity” on page Error! Bookmark not defined.207).
Typically, if the segment TDS criterion or site-specific TDS concentration in the receiving water is too high to support *Ceriodaphnia dubia* or *Daphnia pulex*, *Daphnia magna* (water flea) is substituted as the invertebrate test organism. However, draft permits with alternate tests, alternate species, or testing requirements that exclude a species are subject to EPA review and approval.

**Test Acceptability Criterion**

The permittee will have to repeat any toxicity test if the mean survival of the control is less than 90%. Any toxicity test that fails to meet the acceptability criterion is considered invalid.

**Test Frequencies**

The standard frequency for 24-hour acute WET testing is once per six months unless otherwise specified.

**Toxicity Reduction Evaluations (TREs)**

Failing a 24-hour acute WET test (demonstrating 50% or greater mortality) necessitates two retests over consecutive weeks (unless retesting concurrently with chronic test failure; in such a case, the permittee may defer to the chronic monthly retest schedule). If both retests pass (demonstrate greater than 50% survival), the permittee continues testing at the original frequency designated in the permit.

If one or both of the retests fail, the permittee has demonstrated persistent significant mortality, and the permittee is required to perform a TRE. From the date that persistent mortality is confirmed, the permittee has three years to comply with 30 TAC § 307.6(e)(2)(B) of the Standards.

**TRE Purpose and Content**

The purpose of the TRE is to determine the cause and source of toxicity, and to determine methods to reduce or eliminate the toxicity. Components of a TRE are the same as described in the chronic/48-hour acute section.

**TRE Plan**

The permit requires the discharger to submit a general outline for performing a TRE within 45 days of the retest that confirms persistent mortality. The outline should describe the preparations the permittee will take to develop and implement a TRE.
Within 90 days of the retest that confirms persistent mortality, the permit requires
the discharger to submit a detailed TRE plan. The TRE plan should describe the
specific approach and methodology the permittee will use during the TRE and
include schedules for chemical and biological testing, specific activities, a
sampling plan, a quality assurance plan, and project organization. The TRE
schedule and approach may be modified as necessary during the process.

Toxicity attributable to dissolved salts and ammonia are discussed in the sections
of this document entitled:

- “Toxicity Attributable to Dissolved Salts” (see page 23)
- “Ammonia Toxicity” (see page 29)

**Quarterly Reports**
The permittee must submit quarterly reports to TCEQ that describe TRE progress
and results. The permit also requires the permittee to complete the TRE and
submit a final report within 18 months of the retest that confirms lethality.
Permittees may request an extension to the 18-month time limit. The extension,
however, must be warranted, and approval is contingent upon permittees
demonstrating (1) due diligence in pursuit of the TRE and (2) the existence of
circumstances beyond their ability to control.

**Ceasing a TRE**
Permittees may cease TRE activities if they demonstrate to the executive director
that the effluent no longer causes significant mortality to the test organisms. The
permit defines a cessation of significant mortality as no test failures for a period
of 12 consecutive weeks with at least weekly testing. This permit language
accommodates situations where operational errors and upsets, spills, or sampling
errors triggered the TRE, in contrast to a situation where a single toxicant or
group of toxicants cause lethality.

When a permittee ceases TRE activities under the cessation of significant
mortality provision, that permittee continues WET testing as required in the
permit. This provision is not applicable if the significant mortality ceases for 12
consecutive weeks as a result of the permittee taking corrective action. Corrective
actions include source reduction or elimination, process changes, housekeeping
improvements, changes in chemical use, and/or modification to wastewater
treatment.
Toxicity Control Measures

After the TRE, the TCEQ may amend the permit to include a chemical-specific (CS) limit, a best management practice (BMP), or a whole effluent toxicity (WET) limit.

If appropriate, the permittee may apply for a permit amendment to remove the WET limit by replacing it with a chemical-specific limit or a BMP prior to the end of the compliance period (see below). If there are no further demonstrations of mortality during the compliance period, the WET limit does not become effective. If the WET limit does become effective, the permittee may, after three years of compliance, submit a major amendment application to request removal of the WET limit and resumption of routine WET testing.

Chemical-Specific Limit

In order to be eligible for a chemical-specific limit in lieu of a WET limit, the permittee has to demonstrate that one or more known pollutants caused the mortality and should attempt to determine a specific concentration of the pollutant that will not cause mortality. A chemical-specific limit may be inadequate to address mortality in the following situations:

• failure to identify the toxicant or toxicants;
• presence of multiple toxicants; or
• lack of a routine test method capable of detecting a pollutant at levels causing persistent significant mortality.

BMP

In terms of WET testing, BMPs are defined as a practice or combination of practices that remove toxicity from the effluent by eliminating the source of toxicity. In order to be eligible for a BMP in lieu of a WET limit, the permittee has to demonstrate that such a provision can adequately address mortality. If successful, the BMP becomes an enforceable part of the permit. A BMP does not include making changes to operations or housekeeping practices to reduce toxicity. In these cases, the source of toxicity still remains.

WET Limit

Failure to identify the toxicant or toxicants, presence of multiple toxicants, or lack of a routine test method capable of detecting a pollutant at levels causing toxicity, are examples of cases where a CS limit or BMP may be inadequate to address toxicity. In such cases, where no other appropriate toxicity control measure has been identified, the permit will be amended to add a WET limit with a compliance period, if appropriate.
**WET Limit Violations**

If the permittee fails a WET test while the limit is in effect, the testing frequency for the species increases to monthly until the permittee passes (does not demonstrate significant mortality) three consecutive tests, after which the permittee may resume the specified testing frequency.

**Test Substitution**

The TCEQ normally requires permittees to conduct the chronic or 48-hour acute WET tests and the 24-hour acute (100% end-of-pipe) WET tests as separate permit requirements. If the chronic or 48-hour acute WET test includes a test of 100% effluent in the dilution series, the permit allows the results from that test (after 24 hours of exposure) to fulfill the requirements in the 24-hour acute tests. The permittees then report the survival of organisms in the 100% effluent concentrations after 24 hours.

The permit stipulates that the 24-hour acute WET testing provision applies whether the test results submitted are for this requirement, the 48-hour acute requirements, or the chronic requirements. The permittee may add a 100% effluent dilution to chronic or 48-hour acute tests and submit the results after 24 hours to fulfill the 24-hour acute testing requirements.

**Toxicity Attributable to Dissolved Salts**

Permittees may be exempt from compliance with the total toxicity provisions in the Standards if they demonstrate that dissolved salts are causing the effluent to be toxic. This exemption is allowed under the definition of toxicity in the Standards and under the 24-hour, 100% end-of-pipe acute toxicity provisions (See § 307.6(e)(2)(B) of the Standards).

The definition of toxicity in the Standards excludes adverse effects caused by concentrations of dissolved salts when the salts originate in a permittee’s source water. This exemption would affect compliance with the chronic and 48-hour acute WET testing provisions.

According to § 307.3(a)(65) of the Standards, “Source water is defined as surface water or groundwater that is used as a public water supply or industrial water supply (including cooling water supply). Source water does not include brine water that is produced during the extraction of oil and gas, or other sources of brine water that are substantially uncharacteristic of surface waters in the area of the discharge.”
Also, dischargers that exhibit 24-hour acute toxicity caused by: (1) concentrations of dissolved salts that originate from the source water or (2) an excess, deficiency, or imbalance of dissolved salts in the effluent are exempted from compliance with the 24-hour, 100% end-of-pipe acute toxicity provision. These exemptions, which are specified in § 307.6(e)(2)(B) of the Standards, do not include instances where individually toxic components (for example, the pollutants listed in Table 1 of the Standards) have formed a salt compound that is causing the effluent to be toxic.

The following two sections further explain the exemptions for dissolved salts.
Figure 6. Procedure for Exemption from Total Toxicity Requirements because of Dissolved Salts
TDS Exemption—24-Hour Acute (100% End-of-Pipe) Tests

When a permittee believes failure of the 24-hour acute tests occurred because of dissolved salts and seeks an exemption for that demonstration of toxicity, the permittee will have to demonstrate that dissolved salts are a cause of toxicity in the effluent. Because the effluent may have multiple toxicants, the permittee then has to prove that dissolved salts are the primary cause of toxicity. The following paragraphs describe the process in more detail.

Are Dissolved Salts a Cause of Toxicity?

To confirm that dissolved salts are a cause of toxicity in the effluent, the permittee is required to conduct at least one set of toxicity identification evaluation (TIE) characterization tests including an ion-exchange procedure.

- If the TIE tests fail to prove that dissolved salts are a cause of toxicity, the permittee should continue with the TRE to identify the toxicant or toxicants and to reduce or eliminate the acute toxicity.

- If the TIE tests show that dissolved salts are a cause of toxicity in the effluent, the permittee then has to prove that they are the primary cause of acute toxicity.

Are Dissolved Salts the Primary Cause of Toxicity?

The permittee should use a combination of the following techniques to show that dissolved salts are the primary cause of acute toxicity:

- conduct WET tests using an alternate species that is more tolerant of dissolved salts.

- conduct side-by-side WET tests using the toxic effluent as well as a mock effluent formulated to mimic the ionic composition of the effluent.

- perform measurements of high levels of dissolved salts in the effluent.

- perform an analysis of the ionic components of the dissolved salts.

- use computer models that predict the acute toxicity of saline waters.

- perform WET tests using sea salts that are formulated to correct ionic imbalances.
The permittee may suggest other methods to demonstrate that dissolved salts are the primary cause of toxicity for the TCEQ’s review and consideration.

- If these techniques show that dissolved salts are not the primary cause of acute toxicity, the permittee will continue with the TRE to address the toxicity.

- If the techniques prove that dissolved salts are the primary cause of toxicity, the TRE requirements cease.

**Evaluating the Use of an Alternative Test Species**

When the TRE ceases because dissolved salts are the primary source of acute toxicity, the TCEQ evaluates or requires the permittee to evaluate the use of an alternative test species or modified test protocol.

The permittee may be required to continue conducting the 24-hour acute tests if an alternate test protocol successfully resolves the acute toxicity caused by the dissolved salts in the effluent. The TCEQ may then initiate an amendment of the permit to include these measures or include it during the next permit application.

If an alternate species is unavailable, or if test protocol modifications such as ionic adjustments are unsuccessful, the permittee will most likely be required to continue testing with the standard test species that is unaffected by the dissolved salts.

**TDS Exemption—Chronic and 48-Hour Acute Tests**

When a permittee believes effluent toxicity evidenced by a chronic or 48-hour acute WET test is caused by dissolved salts and seeks an exemption for that demonstration of toxicity, the permittee should follow an approach similar to that described in the previous subsection. EPA will review any protocol that could affect permits or other regulatory actions that are subject to EPA approval.

First, permittees have to show that dissolved salts are a cause of toxicity in the effluent. Since the effluent may contain multiple toxicants, permittees have to prove that dissolved salts are the primary source of toxicity. Next, permittees have to show that the dissolved salts are coming from their source water. Permittees need to complete each step in this process to receive the exemption for dissolved salts. The following paragraphs describe this process in more detail.
**Are Dissolved Salts a Cause of Toxicity?**

To confirm that dissolved salts are a cause of effluent toxicity, the permittee will conduct at least one set of TIE characterization tests including an ion-exchange procedure. If the TIE tests show that dissolved salts are not a cause of effluent toxicity, the permittee should continue with the TRE to identify the toxicant or toxicants and to reduce or eliminate the toxicity.

If the TIE tests show that dissolved salts are a cause of effluent toxicity, the permittee then has to prove that they are the primary cause of toxicity.

**Are Dissolved Salts the Primary Cause of Toxicity?**

The permittee may use the techniques described in the previous section “TDS Exemption—24-Hour Acute (100% End-of-Pipe) Tests” on page 26 to prove that dissolved salts are the primary cause of toxicity. If these techniques fail to do so, the permittee should continue with the TRE to address the toxicity. If the techniques prove that dissolved salts are the primary cause of toxicity, the permittee then has to prove that the dissolved salts are coming from the source water.

**Are Dissolved Salts Coming from Source Water?**

To help prove that dissolved salts originate from the source water, the permittee should sample the facility’s intake water and/or raw water source and compare its dissolved salt concentration and ionic composition with those of the effluent. Increases in the dissolved salt content of the effluent due to process evaporation should also be evaluated where appropriate. In any case, if the effluent’s TDS concentration is greater than that of the source water or if the effluent’s ionic composition varies significantly from that of the source water, effluent limits or control measures may be included in the permit.

- If the dissolved salts are not from the source water, the permittee has to comply with the total toxicity provisions of the Standards. If a protocol for an instream biological survey is approved by EPA, it may be possible for the permittee to attempt to demonstrate that aquatic life in the receiving water is not adversely affected by the TDS levels in the proposed permit.

- If the dissolved salts are from the source water, the permittee may cease the TRE. Upon cessation of the TRE, TCEQ staff will, in conjunction with the permittee, evaluate the use of an alternative test species or a modified test protocol. The permittee may be required to continue testing if modifying the test protocol or using an alternate species resolves the toxic effect of the dissolved salts in the effluent. The TCEQ will then amend the permit to include these measures.
If an alternate species is unavailable or tests using a modified test protocol still demonstrate toxicity due to dissolved salts, the permittee will most likely be required to continue testing with the standard test species that is unaffected by the dissolved salts.

Discharges to marine waters are reviewed on a case-by-case basis and are subject to EPA review and approval in accordance with the MOA between the TCEQ and EPA concerning the TPDES program.

**Ammonia Toxicity**

**Controlling Potential Ammonia Toxicity**

Ammonia, a common component of domestic wastewater, has been shown to be toxic to aquatic organisms. Models used to determine effluent limits for oxygen-demanding constituents do not account for the toxicity that ammonia can exert. Therefore, to preclude receiving water toxicity, permits for certain types of facilities that have either

- ammonia limits to maintain instream dissolved oxygen criteria; or
- categorical ammonia limits that exceed 4 mg/L at the edge of the mixing zone will now include either modified limits for total ammonia or a chronic WET limit for the more sensitive species with a WET testing frequency of six times a year.

The modified ammonia limit or WET limit applies to the following types of facilities that discharge to perennial waters or within 3 miles of perennial waters:

- major domestic facilities (design flow ≥ 1 MGD)
- minor domestic facilities (design flow < 1 MGD) that discharge to a water body that:
  - contains a threatened or endangered species or
  - is listed for ammonia on an EPA-approved 303(d) list
- industrial facilities that have WET testing requirements
- industrial facilities that discharge to a water body that:
  - contains a threatened or endangered species or
  - is listed for ammonia on an EPA-approved 303(d) list
By following these guidelines, the TCEQ will ensure that it is not authorizing the discharge of toxic amounts of ammonia.

**Toxicity Attributable to Ammonia**

TCEQ recognizes that the technology-based daily average ammonia-nitrogen limit of 3.0 mg/L included in most major domestic discharge permits generally precludes chronic toxicity to test species. Therefore, the TCEQ will implement this limit to address chronic toxicity attributable to ammonia in domestic discharge permits. The ammonia limit will be implemented in domestic discharge permits as follows:

- For those facilities whose permits contain interim or final effluent phases that include a daily average ammonia-nitrogen limit of 3.0 mg/L, the persistent toxicity requirements are suspended until the effective date of the limit.

- For those facilities whose permits do not contain interim or final effluent phase that include a daily average ammonia-nitrogen limit of 3.0 mg/L, TCEQ staff will amend the permits to include this limit.

The 3.0 mg/L ammonia-nitrogen limit is normally implemented in lieu of a chronic WET limit. However, should this limit prove ineffective in precluding toxicity, TCEQ staff will amend the permit to include an alternative limit and/or corrective measures protective of the receiving waters.

For those domestic facilities with seasonal ammonia limits and for industrial facilities with ammonia limits, such limits will not exceed 4.0 mg/L at the edge of the mixing zone (or 10 mg/L at the edge of the ZID for those permittees with 48-hour acute testing) unless the permittee agrees to a WET limit for the more sensitive species and a testing frequency for that species of six times per year (November, December, January, February, March, and July).

**Toxicity Attributable to Diazinon**

The Standards previously contained a special provision (§ 307.6(e)(2)(E)) for those domestic wastewater facilities entering TREs due to Diazinon toxicity. However, since Diazinon can no longer be sold to the public, the previous conditions granting the TRE exemption (primary cause of toxicity and ubiquitous within the wastewater collection system) can no longer be met, so the special provision is no longer included in the Standards. Diazinon will now be treated as any other toxicant and will be subject to effluent limits.