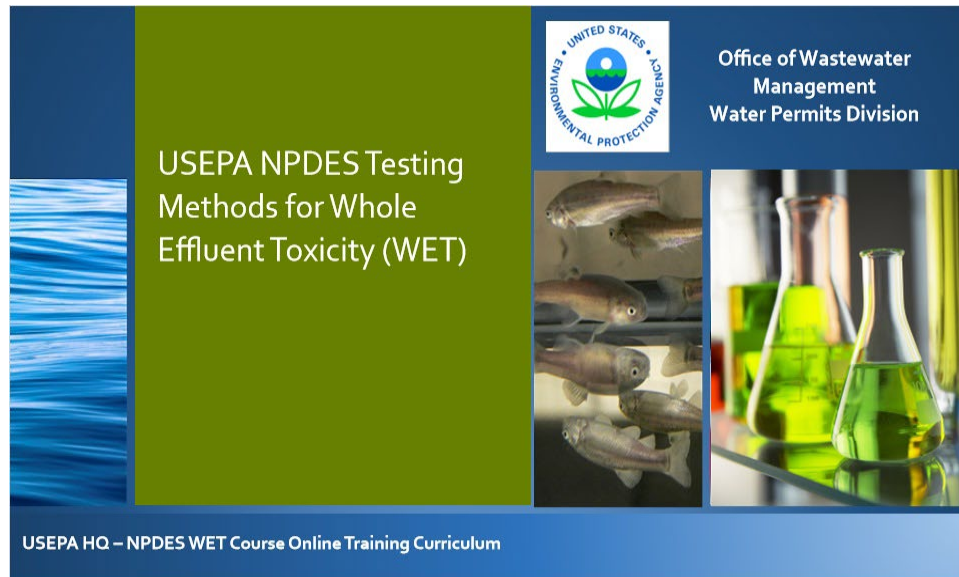


# Module 2 - USEPA NPDES Testing Methods for Whole Effluent Toxicity (WET)



## Notes:

Welcome to this presentation on the United States Environmental Protection Agency's, hereafter USEPA, Testing Methods for Whole Effluent Toxicity, or WET. This presentation is part of a web-based training series on WET sponsored by the USEPA Office of Wastewater Management's Water Permits Division.

You can review this stand-alone presentation, or, if you have not already done so, you might also be interested in viewing the other presentations in the series, which cover the use of WET in the NPDES permit program.

Before we get started with this presentation, I'll make some introductions and cover two important housekeeping items.

## Module 2 - USEPA NPDES Testing Methods for Whole Effluent Toxicity (WET)

**Presenters**

*Laura Phillips*  
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U.S. Environmental Protection Agency  
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Tetra Tech, Inc.  
Owings Mills, MD

**Reference: EPA  
Toxicity Test Methods**

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### Notes:

First, the introductions.

Your speakers for this presentation are, me, Laura Phillips, USEPA's NPDES WET Coordinator with the Water Permits Division within the Office of Wastewater Management at USEPA Headquarters in Washington D.C., and Marcus Bowersox, USEPA Headquarters' contractor and an aquatic toxicologist with Tetra Tech, Incorporated in Owings Mills, Maryland. Second, now for those housekeeping items.

You should be aware that all the materials used in this presentation have been reviewed by USEPA staff for technical and programmatic accuracy; however, the views of the speakers are their own and do not necessarily reflect those of USEPA. The NPDES permit program, which includes the use of WET testing, is governed by the existing requirements of the Clean Water Act and USEPA's NPDES permit implementation regulations. These statutory and regulatory provisions contain legally binding requirements. However, the information in this presentation is not binding. Furthermore, it supplements, and does not modify, existing USEPA policy and guidance on WET under the NPDES permit program. USEPA may revise and/or update this presentation in the future.

Also, this module was developed based on the live USEPA Headquarters NPDES WET course that the Water Permits Division of the Office of Wastewater Management has been teaching to USEPA regions and states for several years. This

## Module 2 - USEPA NPDES Testing Methods for Whole Effluent Toxicity (WET)

course, where possible, has been developed with both the non-scientist and scientist in mind. Also, while not necessary, basic knowledge of biological principles and Whole Effluent Toxicity will be helpful to the viewer. Prior to this course, a review of USEPA's NPDES Permit Writer's online course, which is available at USEPA's NPDES website, is recommended.

When appropriate a blue button will appear on a slide to provide access to more information. By clicking this button, additional slides will present information regarding either freshwater or marine USEPA WET test methods. When these additional slides are finished, you will be automatically returned to the module slide where you left off. The blue button on this slide provides the references for USEPA's WET test methods that will be presented throughout this module.

Alright. Marcus will guide us through the USEPA WET test methods used to conduct WET testing for the NPDES permit program.

## What is a WET Test?

WET = Whole Effluent Toxicity

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graph LR; A((A controlled laboratory experiment)) --> B((Uses standardized procedures and analyses)); B --> C((Measures effects of an effluent sample on live organisms));
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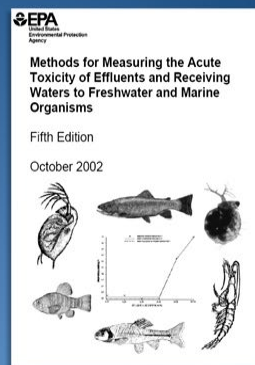
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### Notes:

First, let's define WET, or Whole Effluent Toxicity. WET tests are controlled laboratory experiments using standardized procedures and analyses to measure effects of a permitted effluent sample on live WET test aquatic organisms. Depending on the test endpoint being measured (lethality versus sub-lethal effects), WET tests are considered to be acute or chronic. In acute tests, the exposure periods are considered short-term and therefore are no longer than 96 hours measuring only lethality, defined as the ability to survive after exposure to an effluent. Chronic tests assess possible impacts from effluent exposure to aquatic test organisms for critical lifecycle measurements and therefore may be longer than 96 hours and are usually conducted for 7 days, or in some cases up to 8 days maximum. Chronic tests measure both lethality and immobility and sub-lethal test endpoints such as growth, development, and reproduction.

## EPA Toxicity Test Method Manuals

- Health and safety
- Quality assurance
- Facilities, equipment, supplies
- Test organisms
- Dilution water
- Effluent sampling and handling
- Test methods
- Report preparation



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### Notes:

The USEPA has standardized WET test methods for freshwater and marine test organisms. The current USEPA WET test methods manuals were released in 2002 for freshwater and East Coast marine species, whereas the current USEPA West Coast marine WET test manual was released in 1995. These documents define many important aspects of WET test methods for each USEPA approved test species, including, but not limited to, test organism culturing and handling, health and safety, quality assurance, facilities, equipment and supplies, dilution water, effluent sampling and handling, and report preparation. Many of these subjects are discussed in more detail later in this module.

## EPA WET Test Design

- Replicates
- Randomization
- Static or static renewals



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### Notes:

The test set-up or test design for each of the WET test methods is specific to the test organisms and may include different requirements, such as: the number of replicates, randomization of test chambers, and whether to conduct tests using either a static, static-renewal, or flow-through mode. A static test is one in which the test solution that organisms are exposed to is the same throughout the exposure, whereas in a static-renewal test, the solution in the test chambers is replaced with fresh solutions after some predetermined amount of time, typically 24 or 48-hours. In many instances, a static-renewal test will incorporate more than one effluent sample to account for possible effluent variability over time. For the promulgated USEPA WET test methods, all WET tests used for NPDES permitting must consist of a control and five serial dilutions of the effluent. Dilutions of effluent should be made with one of two types of water, as described in the next slide.

## Selection of Dilution Water

- Laboratory water or receiving water
- Choice of water is dependent on the objectives of the test
  - Absolute toxicity: use standard lab dilution water
  - Estimate of toxicity in uncontaminated receiving water: use receiving water
  - Contaminated receiving water: use lab water



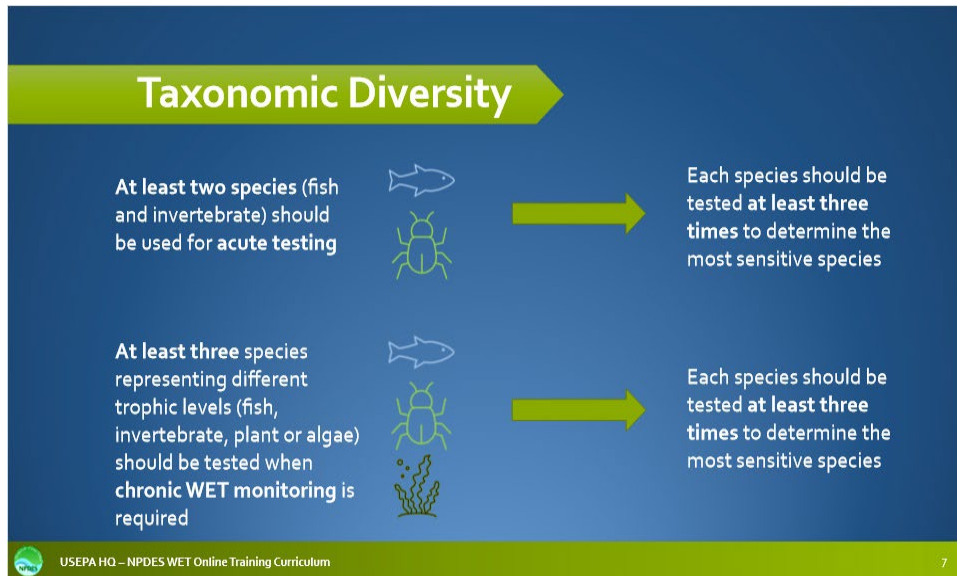
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### Notes:

USEPA's WET testing methods indicate that WET tests should be conducted using one of two choices of dilution water: receiving water or laboratory water. The choice of dilution water is dependent on the objectives of the WET test. If the objective is to determine the absolute toxicity of the effluent alone, then standard laboratory water would be used. If an estimate of the toxicity in receiving water is the objective, then receiving water that is known to be non-toxic to the WET test species would be used. Generally, receiving water upstream or outside of the area affected by an effluent discharge should be used in this case. If the receiving water is known or suspected to be contaminated, then standard laboratory water should be used.






**Notes:**

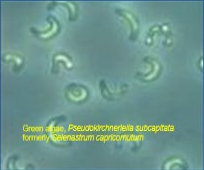
According to USEPA's 1991 Technical Support Document for Water Quality-based Toxics Control, or TSD, it is recommended that at least two USEPA approved WET test species, representing different trophic levels, such as a fish and an invertebrate, should be used in acute WET tests. For chronic WET tests, USEPA recommends testing with at least three USEPA approved WET test species, including a fish, an invertebrate, and a plant or algae. Under both acute and chronic WET testing, each test species should be assessed against the permitted effluent at least 3 times to determine the most sensitive test species to be used for determining whether there is or may be an excursion of a state's water quality standard and/or for monitoring to determine compliance with NPDES WET permit limits. USEPA has developed test methods for acute and chronic WET testing using standardized or surrogate WET test species for each of the three trophic levels.



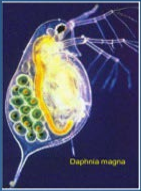
## Freshwater Organisms used in Toxicity Testing




*Ceriodaphnia dubia*




Green alga, *Pseudokirchneriella subcapitata*  
formerly *Selenastrum capricornutum*



*Daphnia magna*




Fathead minnow,  
adult female



*Oncorhynchus mykiss*

**East Coast Marine Species**

**West Coast Marine Species**



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### Notes:

USEPA has standard WET test species for both acute and chronic tests. The pictures presented here represent some of the freshwater test species, including *Ceriodaphnia dubia* (an invertebrate - water flea), *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum* - an algae), *Daphnia magna* (another invertebrate - water flea), *Pimephales promelas* (vertebrate - fathead minnow), and *Oncorhynchus mykiss* (vertebrate - rainbow trout).

## Acute Freshwater Toxicity Test Methods

	Species	Test Type	Test Endpoint
Fish	<ul style="list-style-type: none"> <li>Fathead Minnow - <i>Pimephales promelas</i></li> <li>Rainbow Trout - <i>Oncorhynchus mykiss</i></li> <li>Brook Trout - <i>Salvelinus fontinalis</i></li> </ul>	24-, 48-, or 96-h static, renewal, or flow-through	Survival
Invertebrate	<ul style="list-style-type: none"> <li>Water Flea - <i>Ceriodaphnia dubia</i>, <i>Daphnia magna</i>, <i>Daphnia pulex</i></li> </ul>	24-, 48-, or 96-h static, renewal, or flow-through	Survival



Acute Marine Toxicity  
Test Methods



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### Notes:

Standard acute fish WET test methods include those for fathead minnows, rainbow trout, and brook trout. Fathead minnows are the standard WET test species for warm-water receiving waters, while rainbow trout or brook trout can be used as test species in those instances where the receiving water may be classified for cold water aquatic life. Standard acute invertebrate WET tests methods include those for the water fleas: *Ceriodaphnia dubia*, *Daphnia magna*, and *Daphnia pulex*. Acute WET tests are typically less than 96-hours long and can be as short as 24-hours. Acute WET tests are typically conducted in static- or static-renewal mode but could be conducted as a flow-through test if the potential toxicants are volatile. The only test endpoint measured in an acute WET test is lethality.

## Short-Term Chronic Freshwater Toxicity Test Methods

	Species	Test Type	Test Endpoint
Fish	Fathead Minnow (Method 1000.0) <i>Pimephales promelas</i>	7-day renewal	Growth Survival
Invertebrate	Water Flea (Method 1002) <i>Ceriodaphnia dubia</i>	3 brood tests that are 6–8-day renewal	Reproduction Survival
Plant	Green Algae (Method 1003.0) <i>Pseudokirchneriella subcapitata</i> (formerly <i>Selenastrum capricornutum</i> )	96-hour non-renewal	Growth



East Coast Chronic  
Toxicity Test Methods

West Coast Chronic  
Toxicity Test Methods



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### Notes:

USEPA has three standard species for conducting chronic freshwater WET tests, including: *Pimephales promelas* (the fathead minnow), *Ceriodaphnia dubia* (a water flea), and *Pseudokirchneriella subcapitata* (the green algae). Fathead minnow chronic tests are seven (7) days in length, and the test endpoints include mortality/immobilization and growth. *Ceriodaphnia dubia* chronic WET tests are three brood tests which are six (6) to eight (8) days in length depending on the control response, and the test endpoints are mortality/immobilization and reproduction. The green algae WET test using *Pseudokirchneriella subcapitata* is ninety-six (96) hours in length, and the test endpoint measured is growth in terms of cell density.

**Acute or Chronic Toxicity Tests**

**Acute Toxicity**

- **Test Endpoint:** Mortality
- **Test Duration:** Either 24, 48, or 96 hours

**Chronic Toxicity**

- **Test Endpoints:** mortality/immobility, growth/biomass, and reproduction
- **Test Duration:** 8 days or less

East Coast Marine Chronic Toxicity Test Endpoints

West Coast Marine Chronic Toxicity Test Endpoints

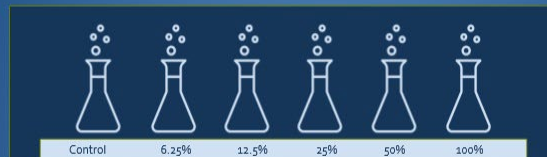
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**Notes:**

This slide summarizes the test endpoints and durations of acute freshwater and marine and chronic freshwater WET tests. Acute WET tests can be 24-, 48-, or 96-hours in length. Typically, acute tests that are over 48-hours in length are conducted in static-renewal mode to prevent overall water quality, especially dissolved oxygen, from changing during the test. For chronic toxicity, the test endpoints include both lethal endpoints, measured as lethality or immobility, as well as sub-lethal endpoints, measured as growth or reproduction. For freshwater chronic tests, test duration is no longer than 8 days.

## EPA WET Test Solutions

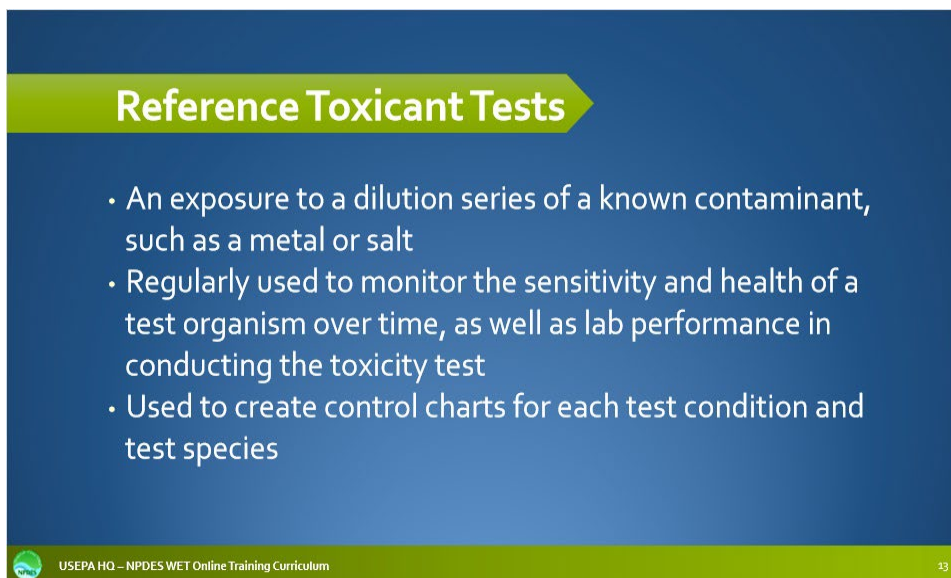
- Dilution Water - Negative Control
- Effluent Concentrations - 5 treatments
- Reference Toxicant - Positive Control



### Notes:

Two test controls are typically employed for WET testing, a negative control and a positive control. The negative control consists of 100% dilution water. The dilution water could be either receiving water or laboratory water as noted previously, but the responses of the organisms in the negative control must meet USEPA's WET test methods' Test Acceptability Criteria, or TACs, for each WET test species in order for the WET test to produce valid data for NPDES permit compliance purposes. USEPA's TACs for different WET test methods are discussed later in this module.

WET tests used in the NPDES permits program must consist of five effluent dilutions plus a control in order to determine the effects of the effluent at different effluent test concentrations. These effluent test concentrations should include the In-stream Waste Concentration, or IWC, and other effluent test concentrations that bracket the IWC (i.e., some less and some greater) to allow for an effective evaluation of the concentration-response pattern observed in the test. In addition to the negative control, a positive control is generally conducted using a separate reference toxicant test that may or may not be run concurrently with the effluent test. Reference toxicant testing will be discussed more in the next slide.



### Reference Toxicant Tests

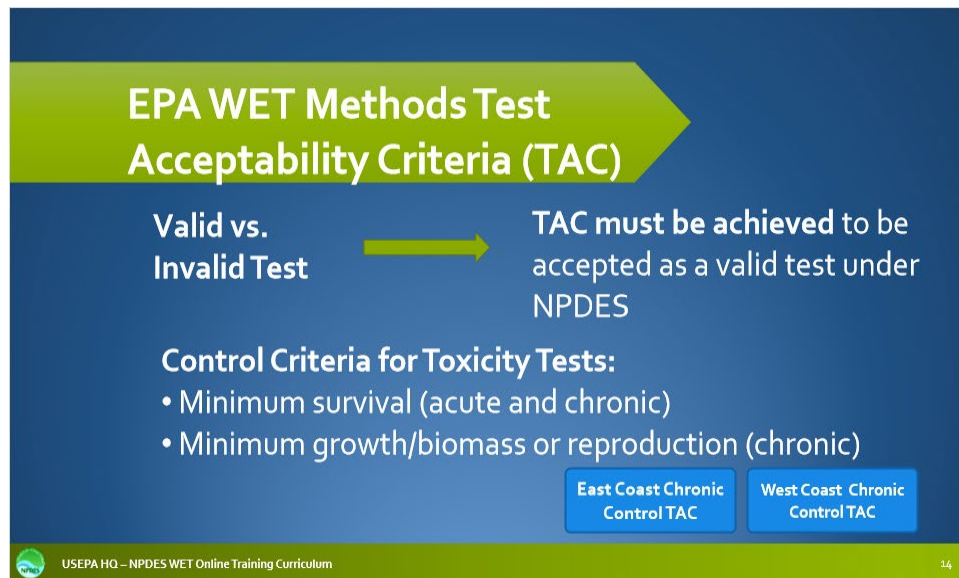
- An exposure to a dilution series of a known contaminant, such as a metal or salt
- Regularly used to monitor the sensitivity and health of a test organism over time, as well as lab performance in conducting the toxicity test
- Used to create control charts for each test condition and test species

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#### Notes:

The 2002 USEPA WET test methods define a reference toxicant tests as an exposure to a dilution series of a known contaminant, such as a metal or salt for which the WET test organism response is well established. The choice of reference toxicants is typically lab and species specific, however, they should be fairly stable chemicals that are soluble in water at concentrations that are toxic to the WET test species. Potassium chloride, sodium chloride, and copper sulfate, for example, are all suitable reference toxicants. A reference toxicant testing program is used as part of a laboratory Quality Assurance (QA) program to demonstrate the sensitivity and health of test organisms used in WET tests over time, as well as an approach for determining a lab's performance in conducting the WET tests. Since the test organism's response to a reference toxicant should be similar every time it is conducted, a control chart for each test condition and test species can be created. USEPA recommends that the most recent twenty (20) test endpoints be displayed and evaluated in the control chart. Each test endpoint needs to be within plus or minus 2 standard deviations of the running average endpoint value (e.g.,  $IC_{25}$ ). Reference toxicant tests, control charts, and other Quality Assurance/Quality Control aspects of WET testing are evaluated in detail in the Reviewing WET Tests and WET QA/QC module.





### Notes:

All USEPA WET tests must meet method-specific Test Acceptability Criteria, or TACs, in order for the WET tests to be considered valid. The TACs for freshwater tests consist of minimum survival, growth and reproduction that is considered acceptable in the controls of the test. For acute WET testing, the TAC is that the controls are to have no more than 10% mortality (or greater than or equal to 90% survival) at the end of the test. Chronic tests include TACs for both survival and biomass or reproduction (sub-lethal test endpoints) depending on the WET test method.

## EPA Toxicity Test Methods

- EPA toxicity test methods must be followed as they are written in EPA toxicity test manuals
- New permits and permit re-issuance incorporate the toxicity test methods into the permit
  - Incorporate by reference the EPA toxicity test methods in general permit conditions
  - Direct reference by citing current EPA toxicity test methods



### Notes:

The USEPA WET test methods must be followed as written. NPDES permit writers should incorporate the WET test methods into new permits and permit re-issuance by incorporation by reference to the USEPA WET test methods in the permit's general permit conditions section or as a direct reference by citing the current USEPA WET test methods.

## 2002 Freshwater Toxicity Test Method Requirements

### *Ceriodaphnia dubia* survival & reproduction toxicity test

- Blocking by known parentage
- Exclude counting 4<sup>th</sup> brood neonates

### Fathead minnow chronic toxicity test

- Pathogen control/sterilization techniques if pathogen possible issue
- Biomass endpoint
- Four replicates per test concentration

### *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) growth test

- Use ethylenediaminetetraacetic acid (EDTA) in micronutrient stock solution

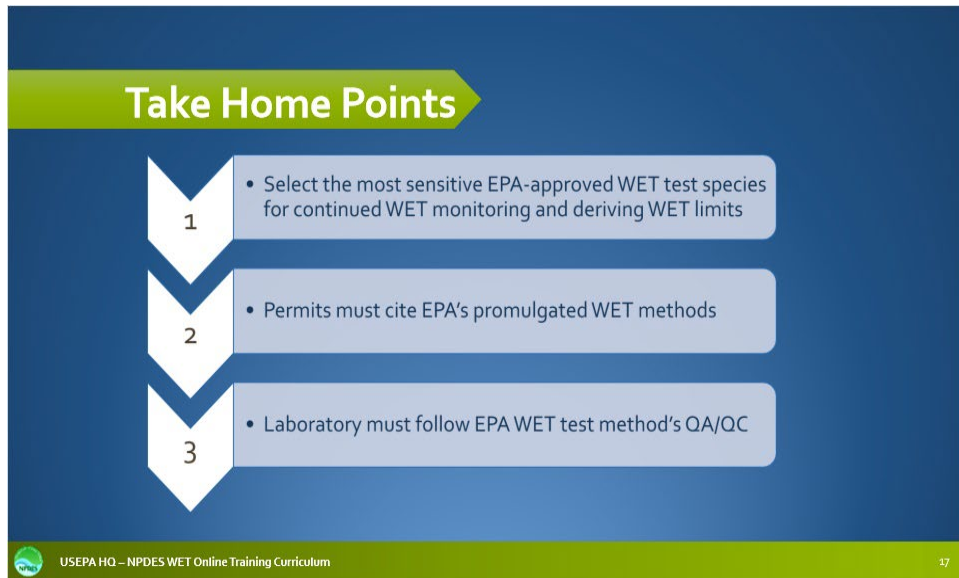


### Notes:

Some of the requirements in the USEPA WET test methods as published in the 2002 USEPA WET test methods include those to the *Ceriodaphnia* survival and reproduction toxicity test. These requirements included the use of blocking by known parentage and the exclusion of fourth broods to determine the reproduction test endpoint result.

Requirements included in the fathead minnow chronic toxicity test include the ability to control for potential pathogen (microorganism) interference using sterilization techniques, the use of the biomass test endpoint, and the use of a minimum of four replicates per test concentration.

The green alga test requires the use of EDTA in micronutrient stock solutions.

A presentation slide titled "Take Home Points" in a green arrow-shaped header. Below the header, three numbered points are listed in light blue boxes, each preceded by a white downward-pointing arrow containing the number. The background is dark blue. At the bottom, there is a green footer bar with the USEPA logo, the text "USEPA HQ – NPDES WET Online Training Curriculum", and the page number "17".

## Take Home Points

- 1 • Select the most sensitive EPA-approved WET test species for continued WET monitoring and deriving WET limits
- 2 • Permits must cite EPA's promulgated WET methods
- 3 • Laboratory must follow EPA WET test method's QA/QC

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**Notes:**

In conclusion, some of the points that we hope you learned in this module were that the most sensitive USEPA-approved WET test species should be used for continued WET monitoring and for the potential development of NPDES WET permit limits. New NPDES permits and re-issued permits need to cite the most recent USEPA WET test methods either by a direct cite or by incorporation by reference. WET testing laboratories must follow USEPA WET test methods Quality Assurance/Quality Control (QA/QC), which includes the use of negative controls (dilution water) and reference toxicant tests.

## Feedback and Other Presentations

Questions or comments?

[Phillips.Laura@epa.gov](mailto:Phillips.Laura@epa.gov)

[Clark.Jackie@epa.gov](mailto:Clark.Jackie@epa.gov)

Join us for other online presentations on  
Whole Effluent Toxicity:

<https://www.epa.gov/npdes/npdes-training-whole-effluent-toxicity-training-and-videos>



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### Notes:

Thank you for joining us for this USEPA's NPDES Whole Effluent Toxicity training presentation. We hope that you have enjoyed it!

If you have any questions or comments on this or any part of the USEPA's NPDES WET online training curriculum, click on the email address given on this slide to send a message to Laura Phillips or Jackie Clark, USEPA HQ NPDES WET Coordinators.

Remember, you will find all of the USEPA's NPDES WET online training presentations, under the USEPA's NPDES training section found on the Office of Wastewater Management's NPDES website.

See you next time!



#### Notes:

The pictures presented here represent some of the USEPA WET test species used in acute and chronic marine WET testing on the East Coast, including *Arbacia punctulata* (a sea urchin), *Menidia beryllina* (the inland silverside), *Cyprinodon variegatus* (the sheepshead minnow), *Americamysis bahia* (formerly *Mysidopsis bahia*, a mysid shrimp), and *Champia parvula* (the red macro-algae).



## Acute Marine Toxicity Test Methods

	Species	Test Type	Test Endpoint
Fish	Topsmelt – <i>Atherinops affinis</i>	24-, 48-, or 96-h static, renewal, or flowthrough	Survival
	Sheepshead Minnow – <i>Cyprinodon variegatus</i>		
	Silversides – <i>Menidia beryllina</i> , <i>Menidia menidia</i> , <i>Menidia peninsulae</i>		
Invertebrate	Mysid – <i>Americamysis bahia</i> (formerly <i>Mysidopsis bahia</i> )	24-, 48-, or 96-h static, renewal, or flowthrough	Survival



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### Notes:

Standard acute marine WET test species include sheepshead minnow and three different species of silversides, as well as the mysid shrimp, *Americamysis bahia* (formerly *Mysidopsis bahia*). As indicated for freshwater acute tests, test length can be 24-, 48-, or 96-hours and can be conducted in a static, static-renewal, or flow-through mode. The only test endpoint measured in an acute WET test is lethality.

## Short-term Chronic East Coast Marine Toxicity Test Methods

	Species	Test Type	Test Endpoint
Fish	Sheepshead Minnow – <i>Cyprinodon variegatus</i>	7-day or 9-day renewal	Growth, Survival, Teratogenicity
	Inland Silverside – <i>Menidia beryllina</i>	7-day renewal	Growth, Survival
Invertebrate	Mysid shrimp – <i>Americamysis bahia</i> formerly <i>Mysidopsis bahia</i>	7-day renewal	Growth, Survival, Egg Development
	Sea Urchin – <i>Arbacia punctulata</i>	1-hour and 20-minute static	Fertilization
Plant	Red Macroalga – <i>Champia parvula</i>	7-day to 9-day static non-renewal	Reduction in cystocarp production



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### Notes:

USEPA has multiple fish and invertebrates for conducting chronic East Coast marine WET tests, including the sheepshead minnow and inland silverside, the mysid shrimp, and the sea urchin. The plant or algae WET test method is limited to the red macroalga. Durations for most chronic marine East Coast tests are 7 days, but the sea urchin fertilization test is a 1 hour and 20 minute exposure. WET test endpoints for chronic marine East Coast WET tests include survival, growth, teratogenicity, egg development, fertilization, and a reduction in cystocarp production (algae reproduction).

## Short-term Chronic West Coast Marine Toxicity Test Methods

	Species	Test Type	Test Endpoint
Fish	Topsmelt – <i>Atherinops affinis</i>	7-day renewal	Growth Survival
Invertebrate	Mysid shrimp – <i>Holmesmysis costata</i>	7-day renewal	Growth Survival
	Pacific oyster – <i>Crassostrea gigas</i> or Mussel – <i>Mytilus</i> sp.	48-hour static	Shell Development Survival
	Red Abalone – <i>Haliotis rufescens</i>	48-hour static	Shell Development
	Purple Urchin – <i>Strongylocentrotus purpuratus</i> or Sand Dollar – <i>Dendraster excentricus</i>	72-hour static	Larval Development Survival
		40-minute static	Egg Fertilization
Plant	Giant Kelp – <i>Macrocystis pyrifera</i>	48-hour static	Germination Length



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### Notes:

USEPA has standard WET test methods for conducting short-term chronic marine test with species that are more indicative of those on the West Coast, including Topsmelt, mysid shrimp, Pacific oyster, mussel, red abalone, purple urchin, sand dollar, and giant kelp. Durations for chronic marine USEPA West Coast WET tests methods vary from 40-minutes for the purple urchin or sand dollar fertilization test, to 48-hours for the Pacific oyster and mussel shell development test, to 7 days for the mysid and Topsmelt survival and growth test. WET test endpoints for the USEPA West Coast chronic marine WET test methods include growth, survival, shell development, larval development, egg fertilization, length and germination.

## EPA East Coast Marine Chronic Toxicity Tests

- Test endpoints are mortality/immobility, growth, reproduction, fertilization, or development
- Test duration is 9 days or less



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### Notes:

For USEPA East Coast chronic WET tests, the test endpoints include lethal endpoints measured as mortality or immobility, as well as sub-lethal endpoints measured as growth, reproduction, fertilization, or development. For USEPA East Coast marine chronic WET tests, the test duration is no longer than 9 days.

## EPA West Coast Marine Chronic Toxicity Tests

- Test endpoints are
  - Lethal: mortality/immobility, and,
  - Sub-lethal: growth, length, germination, fertilization, or larval or shell development
- Test duration 7 days or less



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### Notes:

For USEPA West Coast chronic tests, the WET test endpoints include lethal endpoints measured as mortality or immobility, as well as sub-lethal endpoints measured as growth, length, germination, fertilization, or larval or shell development. For USEPA West Coast marine chronic WET tests, the test duration is no longer than 7 days.

## West Coast Marine Organisms Used in Toxicity Testing



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### Notes:

The pictures presented here represent some of the USEPA WET test species used in chronic marine WET tests on the West Coast of the United States, including *Atherinops affinis* (the topsmelt), *Holmesimysis costata* (a mysid shrimp), *Crassostrea gigas* (the Pacific oyster), *Mytilus sp.* (a blue mussel), *Haliotis rufescens* (red abalone), *Strongylocentrotus purpuratus* (the purple sea urchin), *Macrocystis pyrifera* (the giant kelp), and *Dendraster excentricus* (a sand-dollar).



## EPA Toxicity Test Methods Test Acceptability Criteria (TAC) – East Coast Chronic Marine

- **Valid vs. Invalid test**

- TAC must be achieved to be accepted as a valid test under NPDES

- **Control Criteria for East Coast Marine Tests:**

- minimum survival (chronic)
- minimum growth, reproduction, fertilization, or development (chronic)



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### Notes:

For acute USEPA East Coast marine WET testing, the TAC is that the test controls have no more than 10% mortality or no less than 90% survival at the end of the WET test. Chronic tests include TACs on both survival (lethal endpoints) and on growth, reproduction, fertilization, or development (sub-lethal endpoints) depending on the WET test method.

## EPA Toxicity Test Methods Test Acceptability Criteria (TAC) – West Coast Chronic Marine

- **Valid vs. Invalid test**

- TAC must be achieved to be accepted as a valid test under NPDES

- **Control Criteria for West Coast Marine Tests:**

- minimum survival (chronic)
- minimum growth, length, germination, fertilization, or development (chronic)
- minimum LC<sub>50</sub>, NOEC, and MSD in reference toxicant testing depending on the test species

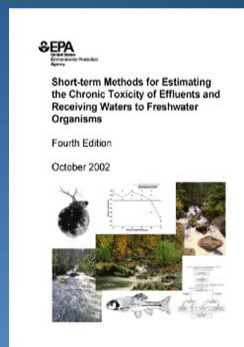
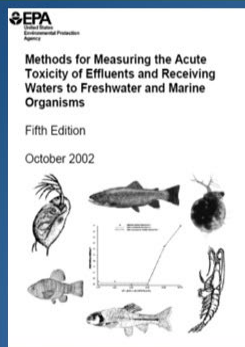


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### Notes:

Chronic USEPA West Coast marine WET tests include TACs for both survival (lethal endpoints) and for growth, length, germination, fertilization, or development (sub-lethal endpoints) depending on the WET test method.

## EPA Freshwater Toxicity Test Methods



USEPA Marine Toxicity Test Methods

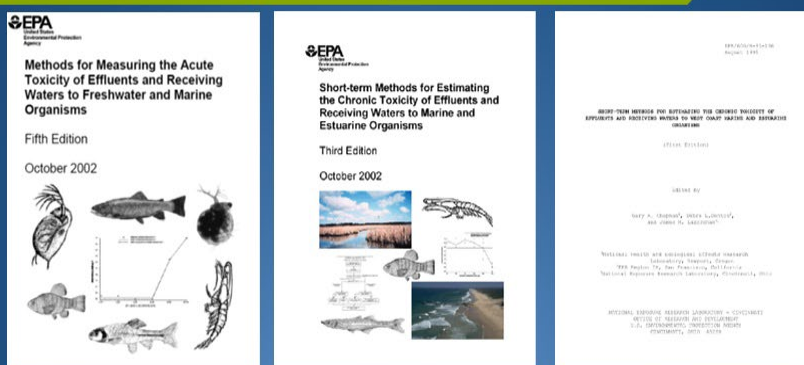


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### Notes:

The base module presented here examines USEPA's freshwater acute WET test methods entitled "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms", Fifth Edition, EPA-821-R-02-012, hereafter acute toxicity test methods. In addition, this module provides USEPA's short-term chronic freshwater WET test methods entitled "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms", Fourth Edition, EPA-821-R-02-013, hereafter chronic toxicity test methods.

## EPA Marine Toxicity Test Methods



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### Notes:

This course also provides an opportunity to view USEPA's acute marine WET test methods entitled "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms," Fifth Edition, EPA-821-R-02-012; short-term chronic marine WET test methods used by states on the Atlantic Ocean or Gulf of Mexico entitled "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms," Third Edition, EPA-821-R-02-014, hereafter East Coast test methods; or short-term chronic marine WET test methods used by states on the Pacific Ocean entitled "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms," First Edition, EPA-600-R-95-136, hereafter West Coast test methods.