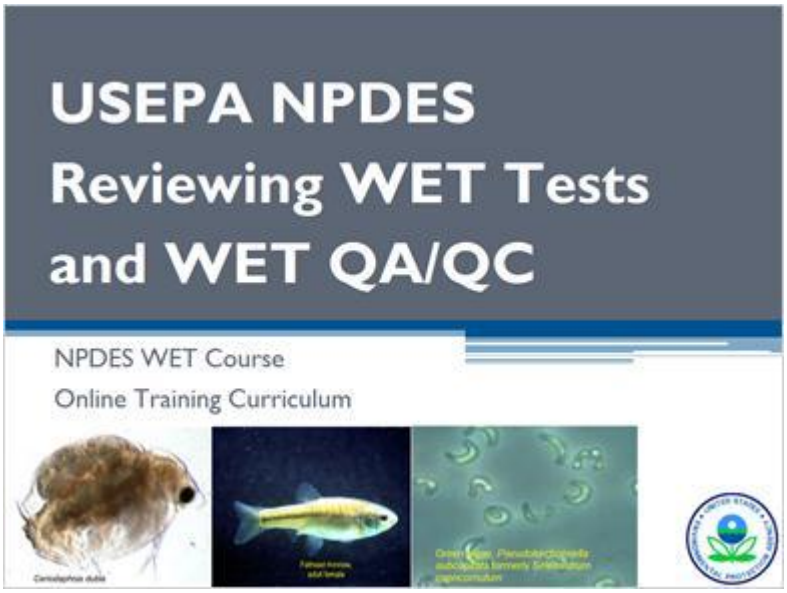
Module 3: USEPA NPDES Reviewing WET Tests and WET QA/QC



Notes:

Welcome to this presentation on the United States Environmental Protection Agency's, hereafter USEPA, National Pollutant Discharge Elimination System, or NPDES, Reviewing Whole Effluent Toxicity, or WET, Tests and WET Quality Assurance and Quality Control, QA/QC.

This presentation is part of a Web-based training series on Whole Effluent Toxicity 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 Whole Effluent Toxicity in the NPDES permits program.

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

Module 3: USEPA NPDES Reviewing WET Tests and WET QA/QC

Notes:

First, the introductions.

Your speakers for this presentation are, me, Laura Phillips, USEPA's National WET Coordinator with the Water Permits Division within the Office of Wastewater Management at the USEPA in Washington D.C., and Marcus Bowersox, USEPA HQs 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 permits program, which includes the use of Whole Effluent Toxicity 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 Whole Effluent Toxicity under the NPDES permits program. USEPA may revise and/or update this presentation in the future.

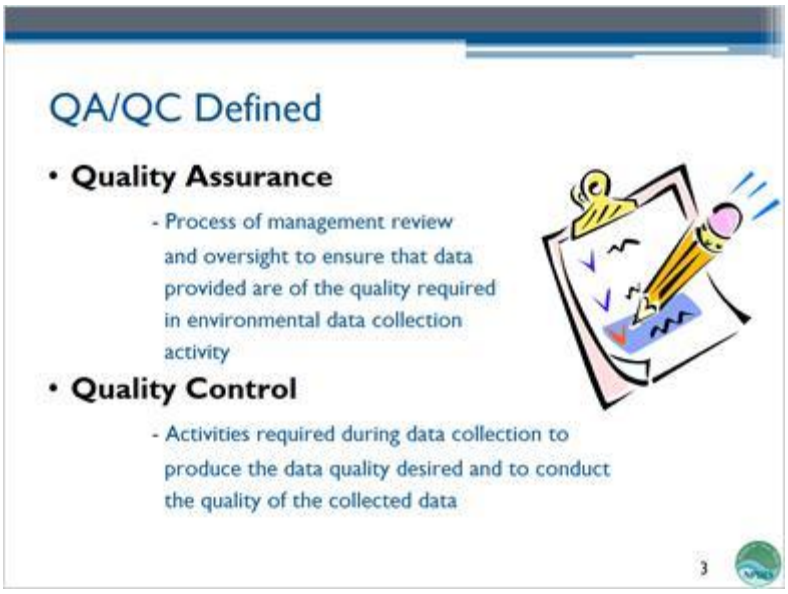
Also, this module was developed based on the live USEPA HQ NPDES WET

course that the Water Permits Division of the Office of Wastewater Management has been teaching to USEPA Regions and states for several years. This course, where possible, has been developed with both the non-scientist and scientist in mind, and while not necessary, it is recommended that a basic knowledge of biological principles and Whole Effluent Toxicity will be helpful to the viewer. Prior to this course, a review of the USEPA's Permit Writer's online course, which is also available at USEPA's NPDES website, is recommended.

When appropriate a blue button will appear on a slide. 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.

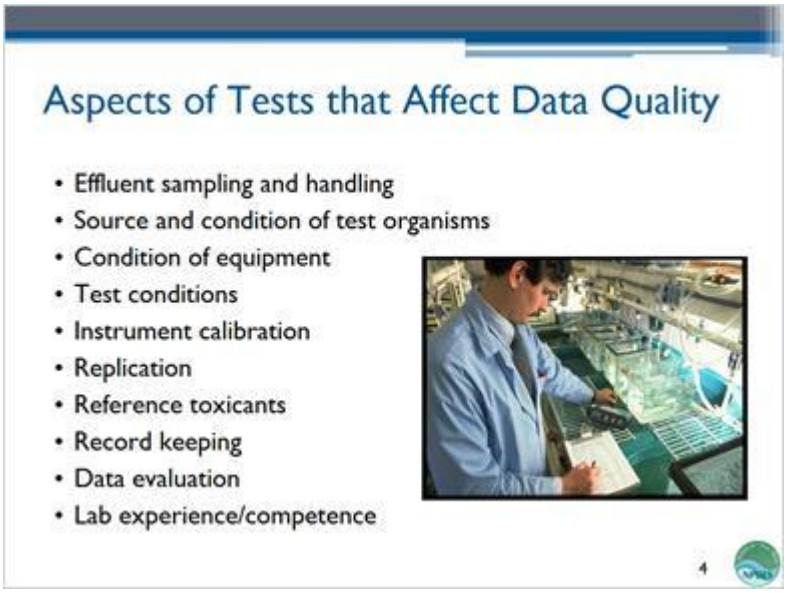
So now Marcus will guide you through reviewing USEPA WET tests and WET test Quality Assurance/Quality Control, referred to as QA/QC.

Module 3: USEPA NPDES Reviewing WET Tests and WET QA/QC



Notes:

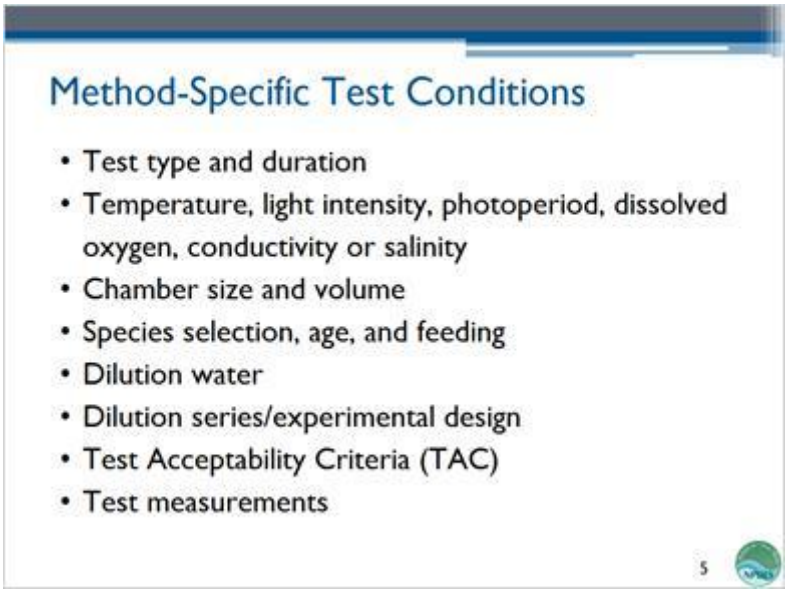
Thanks, Laura. In this presentation we are going to be discussing some of the aspects of Quality Assurance and Quality Control that are relevant to USEPA Whole Effluent Toxicity tests. First, we want to define Quality Assurance and Quality Control. Quality Assurance is the process of management review and oversight to ensure that data provided are of the quality required for the environmental data collection activity. In terms of Whole Effluent Toxicity QA, laboratories should have well defined standard operating procedures and a quality management plan that addresses all aspects of WET test laboratory operations. Quality Control is defined as the activities required during data collection to produce the quality of data desired. Quality Control for Whole Effluent Toxicity testing includes many types of activities, such as having an independent technician double check test organism response data recorded by another technician for a WET test; or checking that the proper number or test organisms are introduced into test chambers according to the respective USEPA WET test method. Using a laboratory that has a satisfactory Quality Assurance and Quality Control consistent performance can help ensure that valid WET test data are obtained.



Notes:

As noted in the previous slide, there are many aspects of Whole Effluent Toxicity tests that can play an important part in the quality of the data collected. Some of these include: effluent sampling and handling; source and condition of WET test organisms; condition of equipment; appropriate WET test conditions; instrument calibration; adequate treatment replication within the WET test; reference toxicants; record keeping; data evaluation; and an experienced laboratory with an appropriate level of proven competence. All of these aspects play an important role in the Quality Assurance and Quality Control of WET testing. Additional WET test conditions that are WET test method-specific are defined on the next slide.

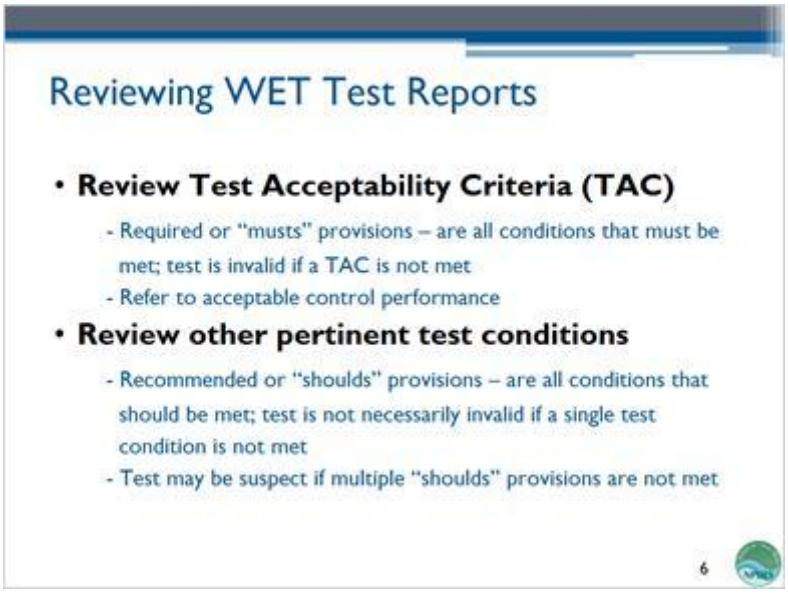
Module 3: USEPA NPDES Reviewing WET Tests and WET QA/QC



Notes:

The current USEPA WET testing methods include many prescribed WET test conditions that must or should be met to ensure valid WET test data. Many aspects of the WET test are defined in the USEPA WET test methods including: test type and duration; temperature, light intensity, photoperiod, dissolved oxygen, conductivity or salinity; chamber size and volume; test species selection, age of the test species, and feeding rate; dilution water, test dilution series or experimental design; Test Acceptability Criteria, or TACs; and other WET test measurements. This module will aid in defining some of the most important or required WET test conditions that you can use to help determine the validity of WET test data. Now, let's look at what should be reviewed when looking at a WET test report.

Module 3: USEPA NPDES Reviewing WET Tests and WET QA/QC



Notes:

When reviewing WET test data and test reports, there are two aspects of the data that need to be reviewed. The first priority is to review the Test Acceptability Criteria, or TACs. These are required, or "must," provisions that need to be met; unless TACs are met, the WET test is considered invalid, and a new WET test must be conducted. TACs refer to the acceptability of the performance of the WET test organisms in the control, and their evaluation will be discussed in detail in later slides of this module. Other pertinent test conditions also need to be reviewed, and these are the recommended, or "should," provisions in the WET test methods. These conditions should be met, but the WET test is not necessarily considered to be invalid if a single "should" test condition is not met. If upon review, multiple recommended WET test conditions were not met, then the WET test may be suspect, and a more detailed review may be needed to determine whether the WET test data are considered valid or not. Recommended WET test conditions will be further defined a little later in this module, but let's take a look at the USEPA WET test methods' Test Acceptability Criteria which are required to be met.

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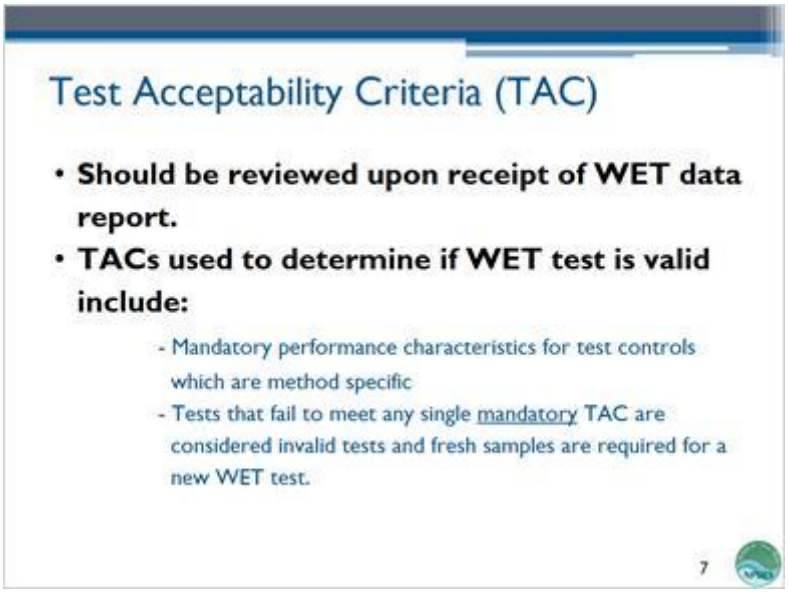

Notes:

Over the next couple of slides, we will be discussing the required USEPA WET test conditions and Test Acceptability Criteria. The TACs should be reviewed first upon receiving WET test reports to determine the validity of the WET test. TACs are mandatory performance characteristics for WET test controls which are specific to the type of WET test being conducted. WET tests that fail to meet any single TAC are considered invalid WET tests, and fresh effluent samples are required to be collected to begin a new WET test. TACs are specified for control performance in terms of the lethal and sublethal endpoints, as well as other WET test conditions, including the minimum number of replicates, the number of effluent test concentrations, and WET test water temperature.

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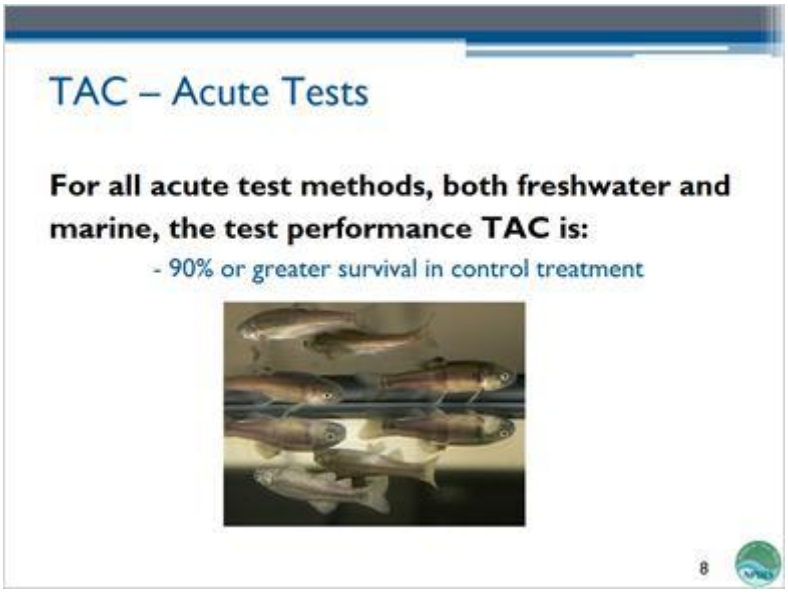

Notes:

As previously noted, the USEPA WET test method-specific Test Acceptability Criteria are required test conditions that must be met. For both freshwater and marine acute WET tests, the control performance TAC is that there cannot be more than 10% mortality of the control's test organisms, or 90% or greater survival of the control test organisms must be demonstrated in the test controls. Therefore, if an acute WET test indicates less than 90% survival in the controls, the WET test is considered invalid, and the WET test cannot be used for NPDES permit reasonable potential analysis and compliance purposes. Another new WET test must be initiated using a fresh effluent sample.

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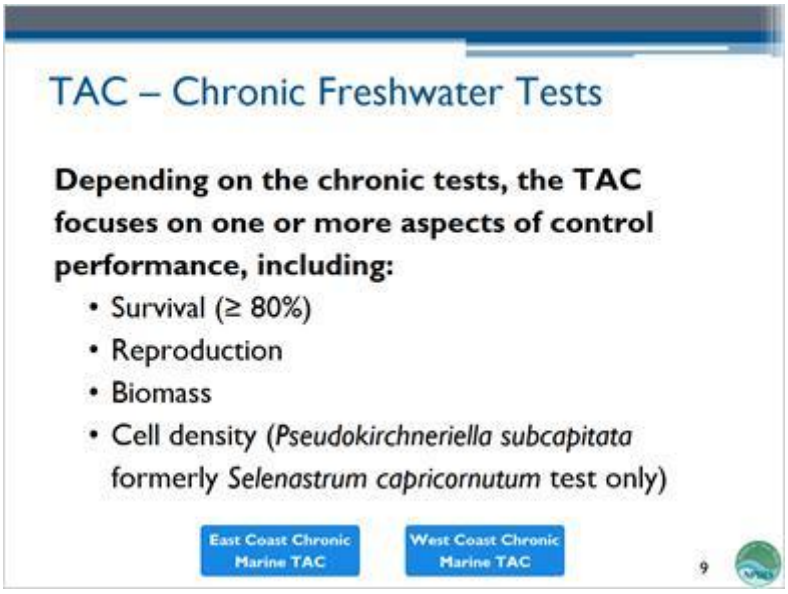
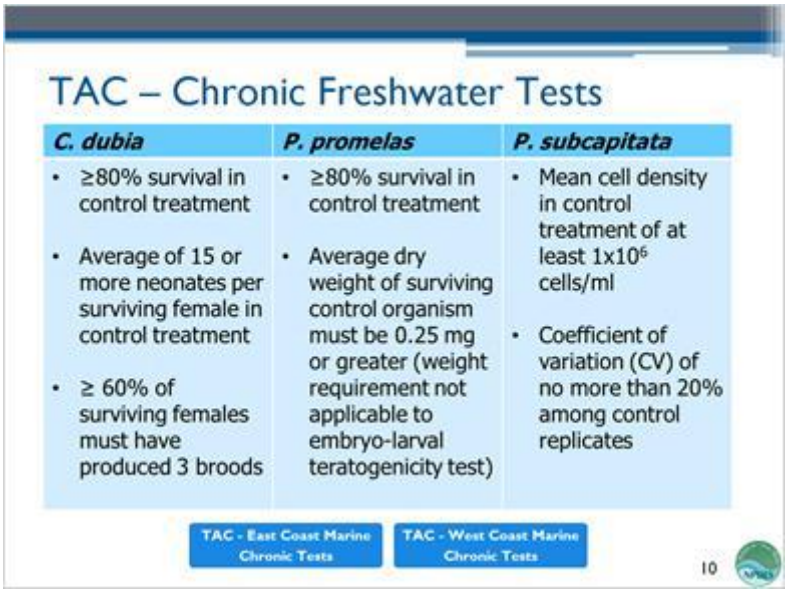


Notes:

For USEPA chronic WET tests, the Test Acceptability Criteria focus on more than one aspect of the control performance including both lethal and sublethal endpoints. Freshwater chronic WET tests have Test Acceptability Criteria for survival, reproduction, biomass, and/or cell density. The specific TACs for each chronic test are defined in the next slide.



Notes:

The USEPA control performance Test Acceptability Criteria for freshwater chronic tests are defined in the table presented here. There are currently three USEPA promulgated chronic freshwater WET test methods, and they include an invertebrate, *Ceriodaphnia dubia* or water flea, a fish, *Pimephales promelas* or fathead minnow, and an algae, *Pseudokirchneriella subcapitata* formerly *Selenastrum capricornutum*. The TACs for the water flea in the control treatment include greater than or equal to 80% survival, an average of 15 or more neonates per surviving female test organism, and greater than or equal to 60% of surviving females must have produced at least 3 broods of neonates. Chronic fathead minnow TACs include greater than or equal to 80% survival of the test organisms and an average dry weight of surviving fish in the control treatment must be greater than or equal to 0.25 mg. The green algae TAC includes a mean cell density in the control treatment of greater than or equal to one million (1,000,000) cells per milliliter and a coefficient of variation of less than or equal to 20% among the test control replicates.

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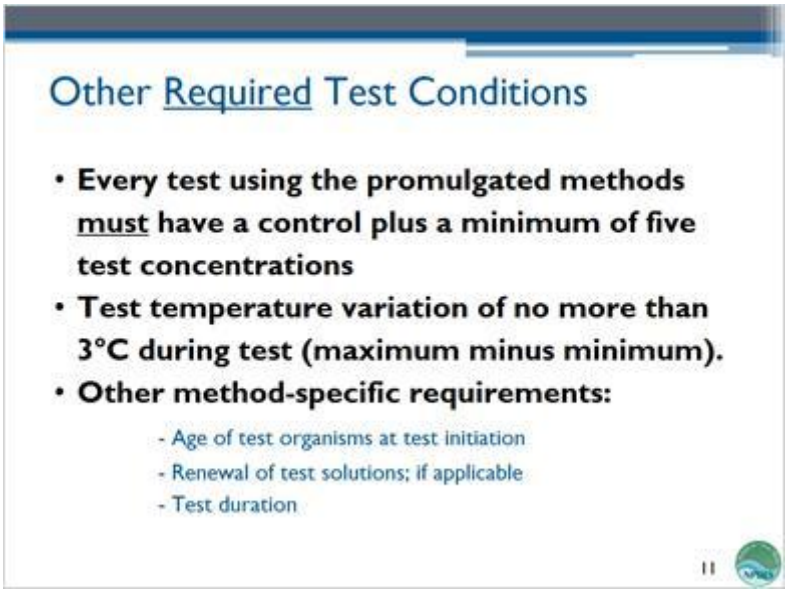
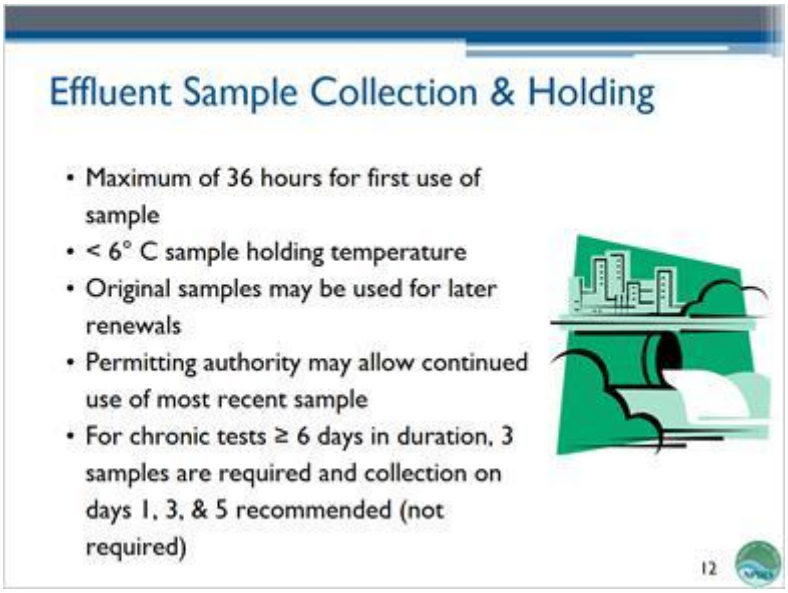


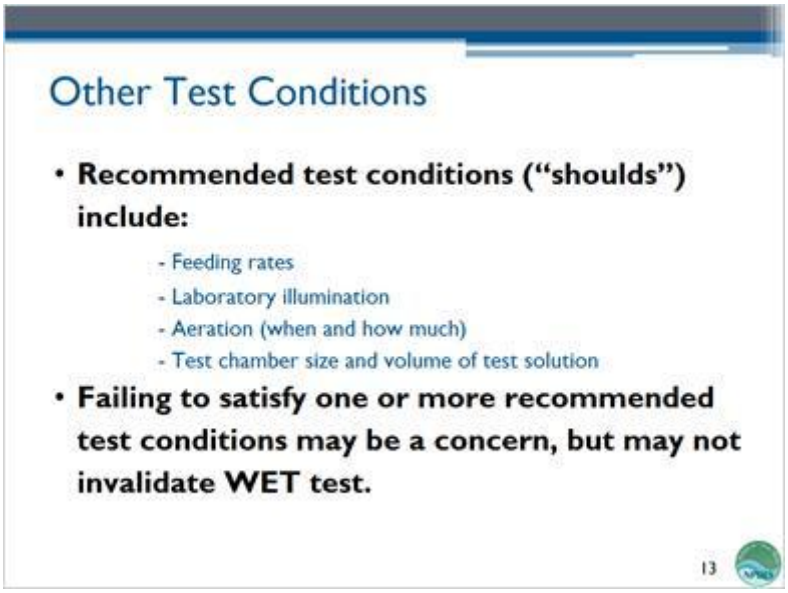
Notes:

Thus far, we have discussed required USEPA WET test conditions with respect to control performance during the WET test. Now, we will discuss some other required WET test conditions in addition to the performance of the controls. The test design for WET tests is prescribed in USEPA's WET test methods manuals, which require that every WET test have a control plus a minimum of five effluent test concentrations. There is no required maximum on the number of test concentrations, but at a minimum every WET test must have five effluent test concentrations plus a control. In addition, the temperature must not deviate by more than 3 degrees centigrade during the test, for example, the difference between the maximum and minimum temperatures observed in the test must be less than 3 degrees centigrade or the WET test is considered invalid. Other USEPA WET test method specific requirements include: the age of test organisms at test initiation, effluent sample holding times, renewal of WET test solutions, if applicable, and the duration of the WET test.



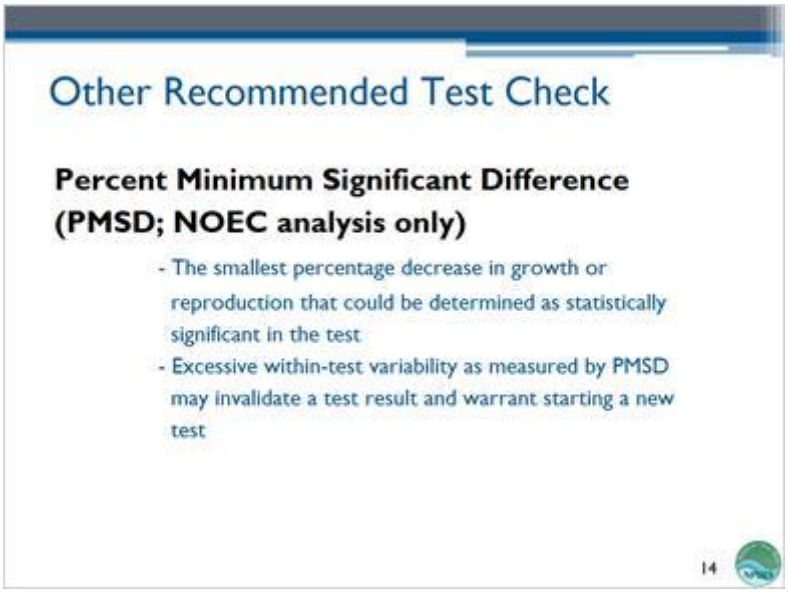
Notes:

There are many aspects of USEPA WET testing QA/QC that are required to be met by the laboratory, and for some QA/QC aspects, the permittee can play a vital role as to whether the effluent sample meets the required QA/QC. With respect to effluent sample collection and holding, the permittee and the laboratory both play a role in meeting QA/QC requirements. As prescribed by the USEPA WET test methods, effluent samples must be first used in testing within 36 hours of sample collection, but initial effluent samples may be used later for test renewals. Effluent samples must be stored at less than 6 degrees centigrade when being held prior to testing, including during transit from the facility to the laboratory. Typically, for chronic tests, multiple effluent samples are collected and used in WET testing. An example would be to collect a new effluent sample to be used in WET testing on days 1, 3 and 5 of a chronic *Ceriodaphnia* or fathead minnow WET test. This would ensure that no single effluent sample was used throughout the WET test and therefore provides a better representation of the effluent discharge in the WET test.



Notes:

We have reviewed many of the required USEPA WET test conditions that may invalidate a WET test if not met. But there are many recommended USEPA WET test conditions that if not met, may not necessarily invalidate a WET test. These include recommendations for feeding rates (if the test organisms are to be fed), laboratory illumination, when and how much aeration if appropriate to apply in a WET test, WET test chamber size and volume of the WET test solution. These are just a few of the recommended USEPA WET test conditions that can be reviewed and are identified in the USEPA WET test methods.



Notes:

For USEPA chronic WET tests, in which a No Observed Effect Concentration, or NOEC, is statistically derived from the WET test data, the Percent Minimum Significant Difference, or PMSD, also needs to be evaluated. PMSD is the smallest percentage decrease in an endpoint, for example, growth or reproduction, which can be identified as statistically significantly different in a WET test. A low PMSD indicates that the test had low variability, thus a smaller difference is considered significantly different. Excessively high PMSD in a WET test indicates that the WET test was highly variable such that statistically, one is not able to differentiate a toxic effect due to the effluent unless the toxic effect is very large. Excessive within-test variability, as measured by the PMSD, may invalidate a WET test and warrant starting a new WET test. USEPA has developed upper and lower bounds for the PMSD to verify that appropriate within-test variability is observed in the WET tests.

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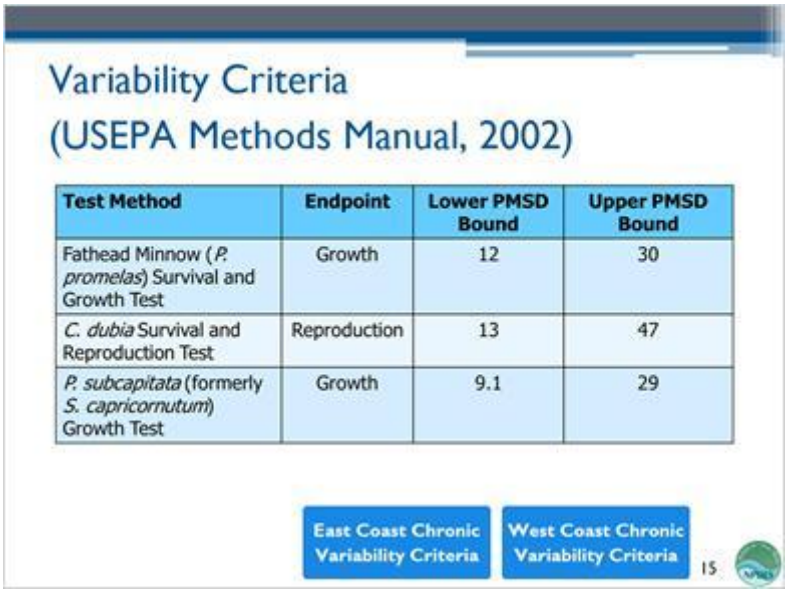
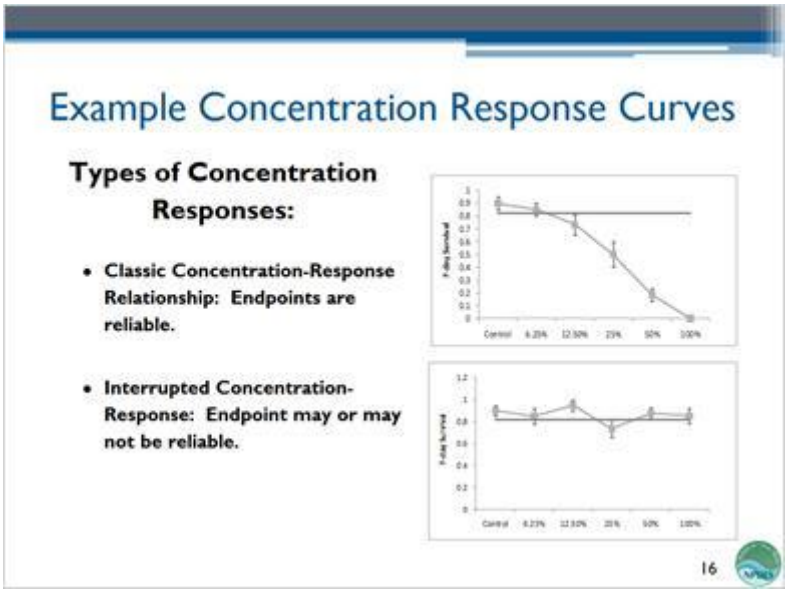


Notes:

Using previous WET test data, USEPA has developed appropriate lower and upper bounds for the PMSD observed in any chronic freshwater WET test. The lower and upper PMSD bounds for fathead minnow growth are 12% and 30%, respectively. The chronic *Ceriodaphnia dubia* freshwater WET test methods have lower and upper PMSD reproduction bounds of 13% and 47%, respectively. The chronic sublethal endpoint of cell density measured in the *Pseudokirchneriella subcapitata* chronic test has lower and upper PMSD bounds of 9.1% and 29%.



Notes:

All WET tests that are properly conducted should be able to be used to construct a concentration-response curve. The concentration-response curve is a relationship of the concentration of the effluent tested and the measured effect at the effluent concentration. WET test concentration responses include a classic concentration-response curve, in which the measured effect increases as the concentration of the effluent increases. Endpoints based on this type of concentration-response are reliable and should be used in determining the toxic effect of the effluent. Sometimes a concentration response becomes interrupted by an effect at one effluent concentration that is higher than the effects at the effluent concentrations above and below it. In those cases, it is unknown if the endpoint is reliable, and further review of the WET test data is warranted before determining whether the WET test endpoint is considered to be reliable for permitting decisions.

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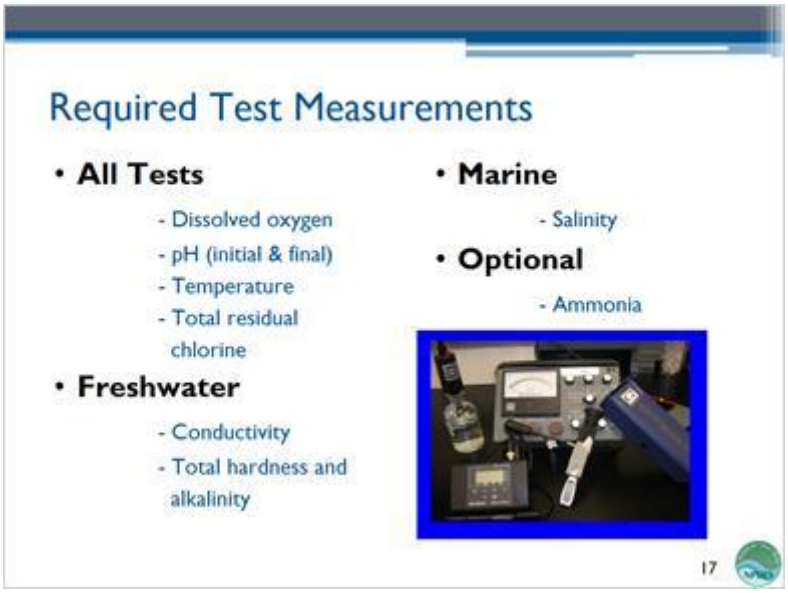

Notes:

As we have discussed, many of the USEPA required or recommended WET test conditions have to do with the response of the WET test organisms in the controls or the conducting of the WET test. Some other USEPA required WET test conditions deal with the water quality of the WET test solutions, including required measures of dissolved oxygen, pH, temperature, and total residual chlorine. These water quality measurements are required in all WET tests types, including acute or chronic and freshwater or marine WET tests. Some other water quality parameters are required depending on the WET test type. For freshwater tests, conductivity, total hardness, and total alkalinity are required, while for marine WET tests, salinity is required rather than conductivity, alkalinity, and hardness. One optional water quality parameter is total ammonia. Total ammonia is recommended for effluents from wastewater treatment facilities where ammonia may be present at toxic levels to aquatic life.

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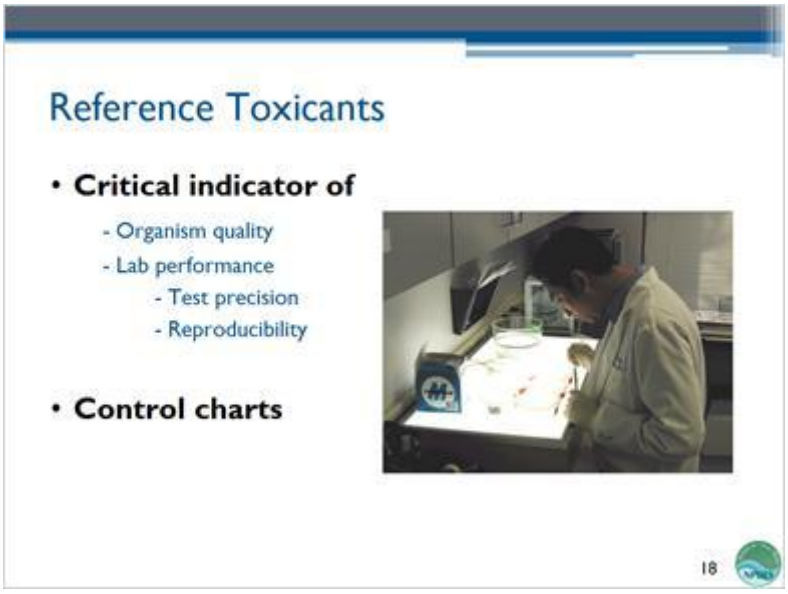

Notes:

Whole Effluent Toxicity test methods require testing laboratories to conduct positive control tests using known toxicants, also referred to as reference toxicants. Reference toxicants are a critical tool for documenting WET test organism health and lab performance in terms of WET test precision and reproducibility. Reference toxicant tests are conducted using the same USEPA WET test methods as effluent WET tests but utilize a concentration series of a toxicant that has been shown to elicit toxic effects at a certain concentration. For example, many laboratories use a salt such as sodium chloride or potassium chloride as a reference toxicant for evaluating the survival and reproduction of freshwater WET test species, such as *Ceriodaphnia dubia*. The results of the reference toxicant tests are compared within a laboratory control chart to demonstrate laboratory performance and WET test organism sensitivity over time.

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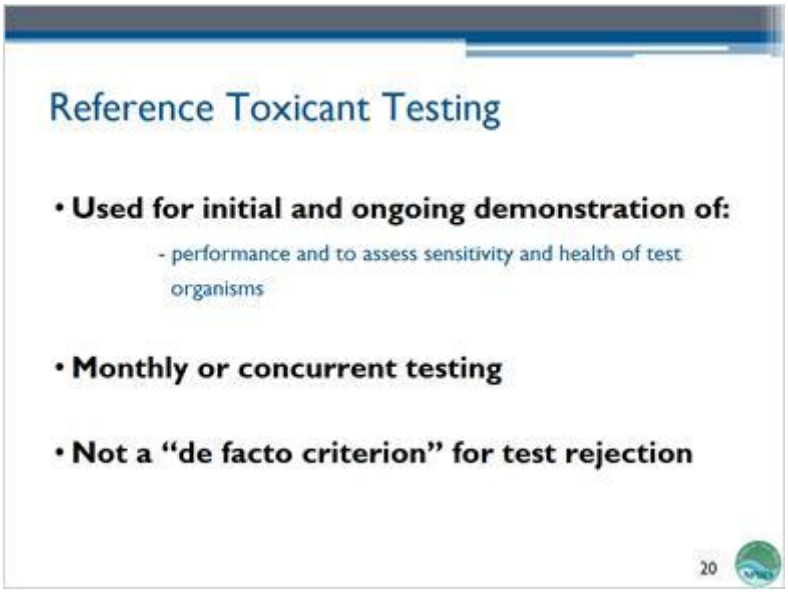
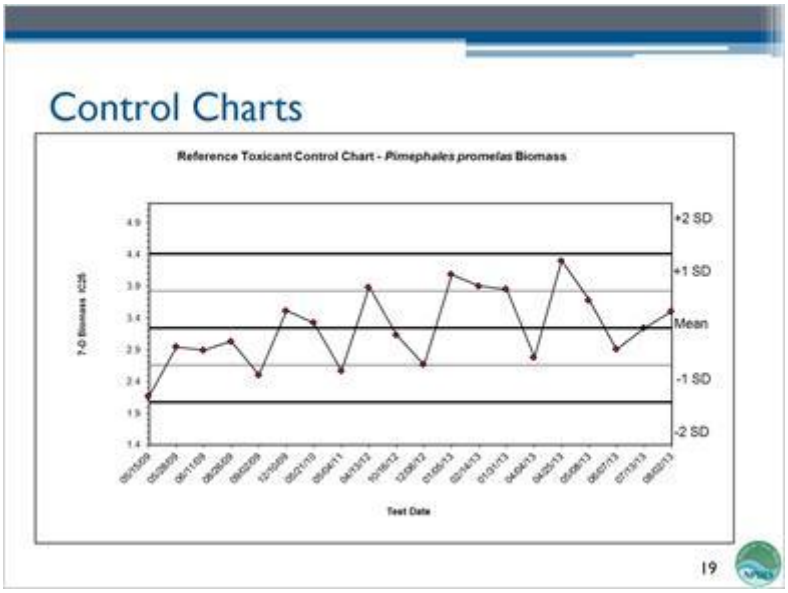


Notes:

The results of reference toxicant tests are used for initial and ongoing demonstrations of the laboratory's ability to perform the USEPA WET test methods, as well as to assess the health and sensitivity of the WET test organisms that the laboratory is using for effluent testing. Under the USEPA WET test methods, two testing scenarios are available for conducting reference toxicant tests; laboratories that culture their own WET test organisms, for instance *Ceriodaphnia dubia*, are only required to conduct monthly reference toxicant evaluations. For laboratories that purchase commercial WET test organisms, such as fathead minnows, concurrent reference toxicant testing is required to be conducted with each batch of WET test organisms used in effluent WET testing. A reference toxicant test result that is not within the bounds of the control chart is not an automatic disqualification of any WET tests that were conducted using those WET test organisms. Further investigation would need to be conducted to determine if the WET test results are still considered to be valid.



Notes:

The results of reference toxicant tests are tracked using control charts. Each USEPA WET test species and endpoint will have a unique control chart. The one presented in the figure on this slide is for the biomass endpoint of chronic fathead minnow reference toxicant tests (in this case the 7-day biomass IC_{25}). The control chart plots a minimum of the last 20 reference toxicant test data points. Using the 20 data points, the average along with the standard deviation is calculated. The average, plus or minus 1 or 2 standard deviations around that average, is plotted. Endpoints that fall outside 2 standard deviations of the running average for the WET test endpoint being measured should be investigated.

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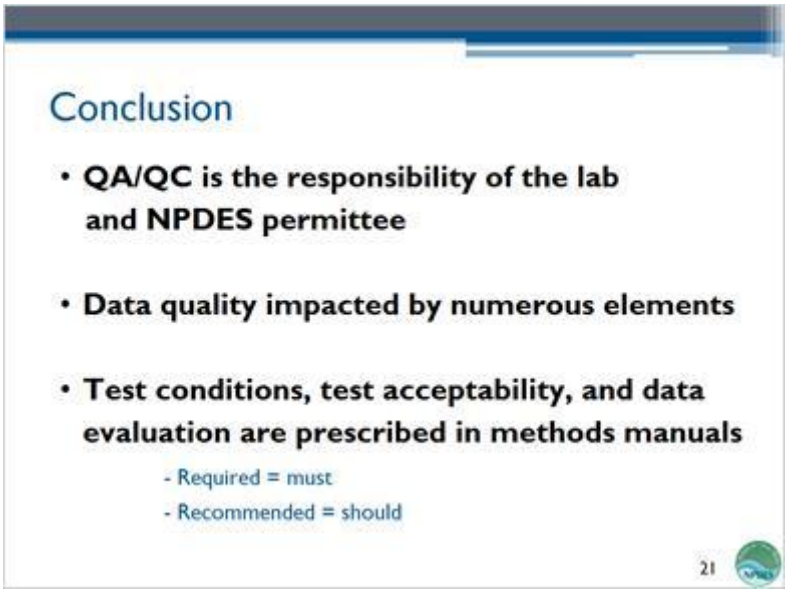

Notes:

So in summary, we have indicated that QA/QC is the responsibility of the laboratory and the NPDES permittee. Through proper implementation of operating procedures and WET test conditions, QA/QC issues can be minimized, ensuring that WET test data are likely to be considered valid. Hopefully, you have noticed that data quality can be impacted by numerous elements, but following the required and recommended USEPA WET test conditions can minimize questionable WET test data. The USEPA WET test methods prescribe the WET test conditions, Test Acceptability Criteria, and WET test data evaluations to be implemented as part of the NPDES WET permits program.

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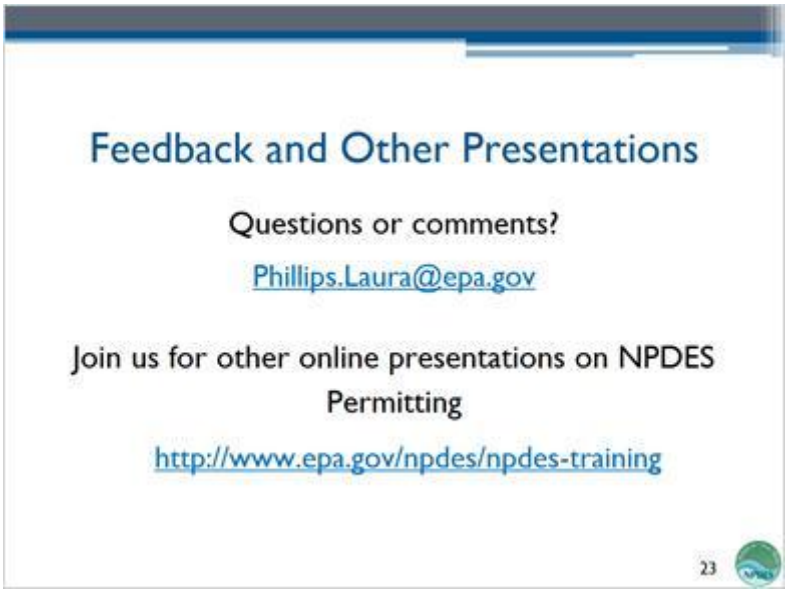

Notes:

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 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, USEPA HQ National WET Coordinator.

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!

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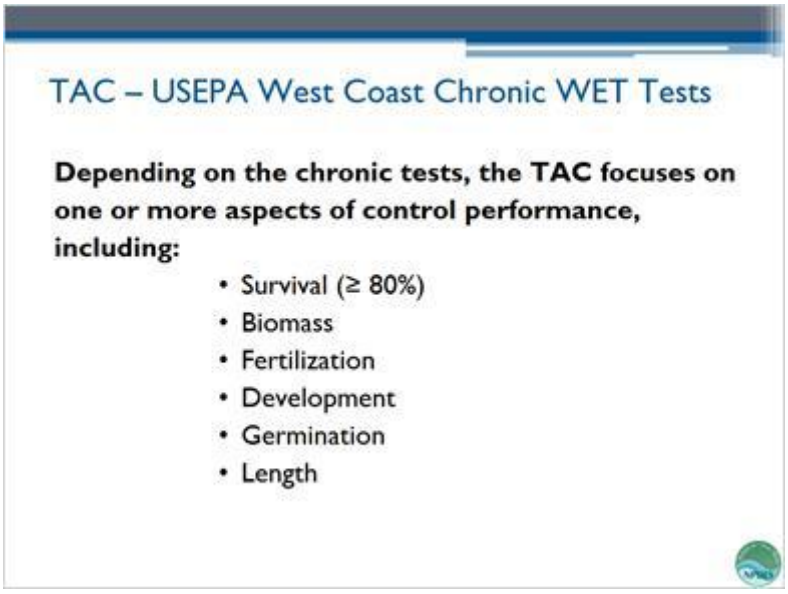
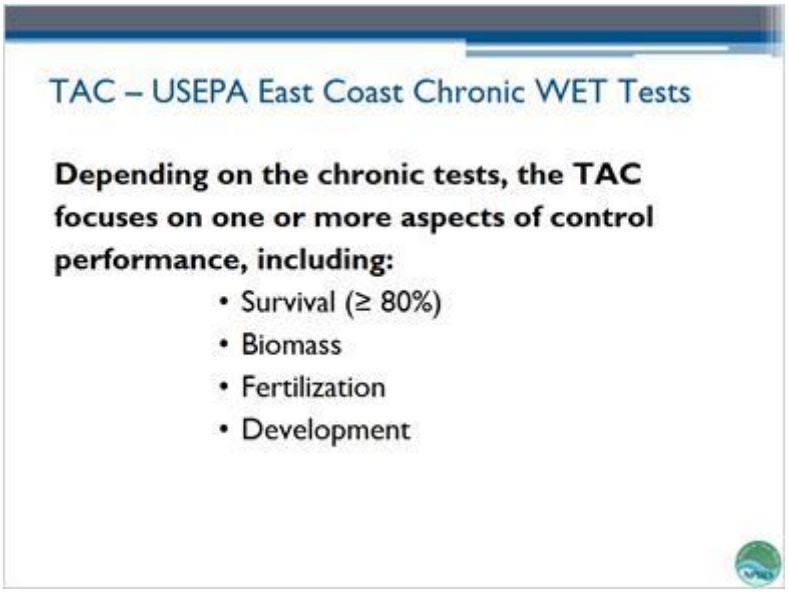


Notes:

USEPA West Coast marine chronic WET tests have Test Acceptability Criteria for survival, biomass, fertilization, development, germination and/or length. The specific USEPA TACs for each of the West Coast chronic marine WET tests are defined in the slides on the button of slide 10.



Notes:

USEPA East Coast marine chronic WET tests have Test Acceptability Criteria for survival, biomass, fertilization, and/or development. The specific USEPA TACs for each of the East Coast chronic marine WET tests are defined in the slide on the button of slide 10.

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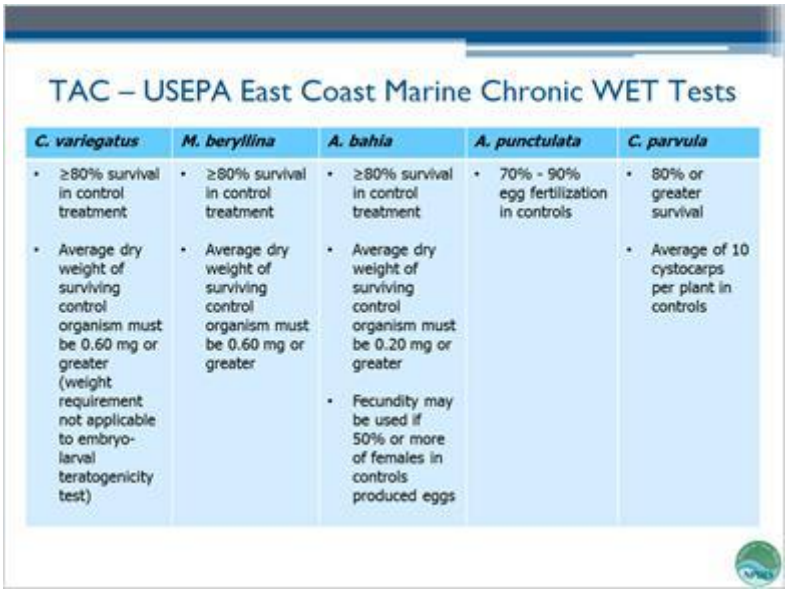
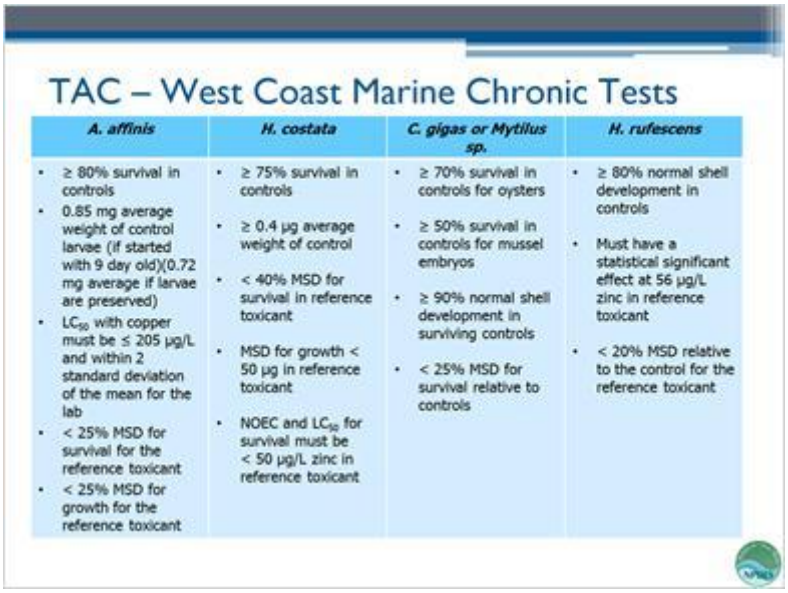


Notes:

The USEPA control performance Test Acceptability Criteria for East Coast marine chronic WET tests are defined in the table presented here. There are currently five USEPA promulgated East Coast marine chronic WET test methods, and they include two invertebrates, *Americamysis bahia* or mysid shrimp and *Arbacia punctulata* or sea urchin; two fish, *Cyprinodon variegatus* or sheepshead minnow and *Menidia beryllina* or inland silverside. Like the freshwater chronic testing, there is only one algae, *Champia parvula,* or red macroalga. The table identifies the relevant USEPA TACs for each of these WET test species.

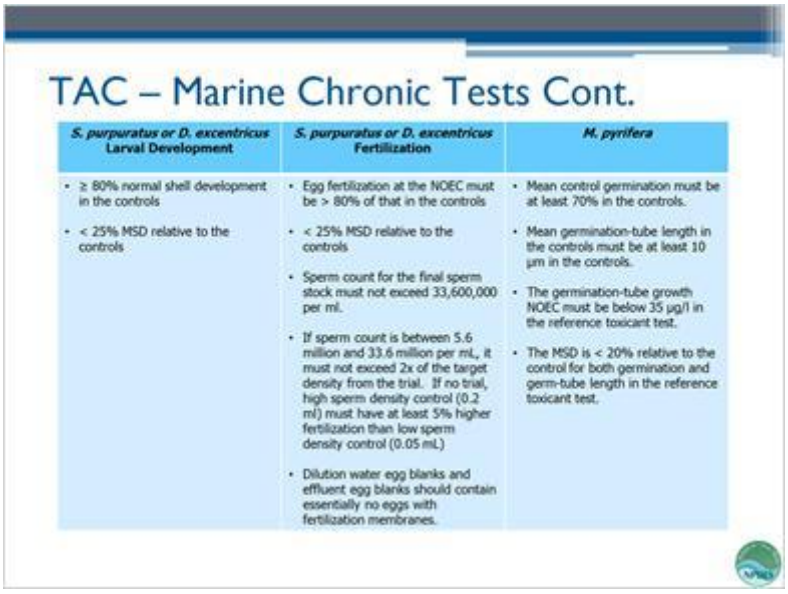


TAC – West Coast Marine Chronic Tests

A. affinis	*H. costata*	*C. gigas or Mytilus sp.*	*H. rufescens*
• ≥ 80% survival in controls • 0.85 mg average weight of control larvae (if started with 9 day old)(0.72 mg average if larvae are preserved) • LC_{50} with copper must be ≤ 205 µg/L and within 2 standard deviation of the mean for the lab • < 25% MSD for survival for the reference toxicant • < 25% MSD for growth for the reference toxicant	• ≥ 75% survival in controls • ≥ 0.4 µg average weight of control • < 40% MSD for survival in reference toxicant • MSD for growth < 50 µg in reference toxicant • NOEC and LC_{50} for survival must be < 50 µg/L zinc in reference toxicant	• ≥ 70% survival in controls for oysters • ≥ 50% survival in controls for mussel embryos • ≥ 90% normal shell development in surviving controls • < 25% MSD for survival relative to controls	• ≥ 80% normal shell development in controls • Must have a statistical significant effect at 56 µg/L zinc in reference toxicant • < 20% MSD relative to the control for the reference toxicant

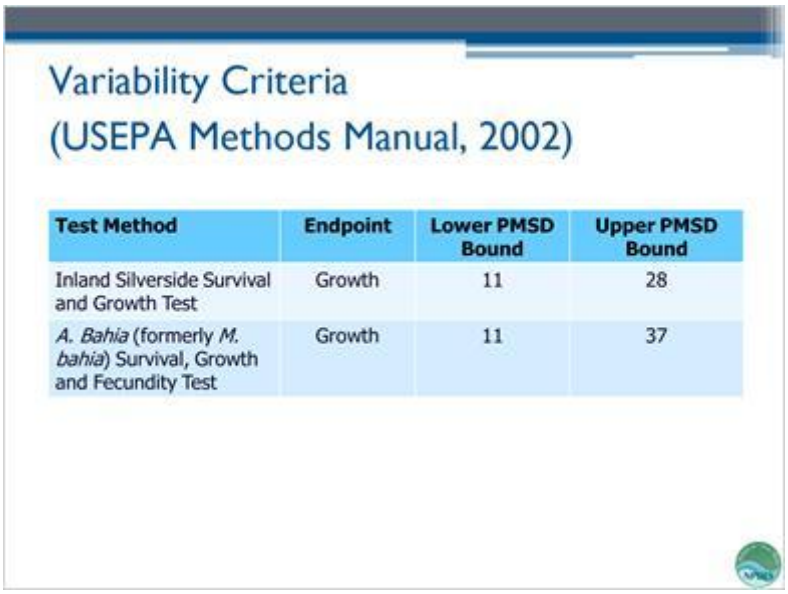
Notes:

The USEPA control performance Test Acceptability Criteria for West Coast marine chronic tests are defined in the table presented on the next two slides. There are currently seven USEPA West Coast marine chronic WET test methods, and they include eight different species or genera of WET test organisms. There is only one WET test species for fish, *Atherinops affinis* or topsmelt. There are five USEPA WET test methods for conducting West Coast chronic WET tests with seven invertebrate species or genera of WET test organisms, and these include: *Holmesimysis costata*, a mysid shrimp, *Crassostrea gigas*, the Pacific oyster, *Mytilus* sp., mussel species, *Stronglyocentrotus purpuratus*, the purple urchin, and *Dendraster excentricus*, the sand dollar. Just like the USEPA freshwater and East Coast marine WET test methods, there is only one plant, the Giant Kelp, *Macrocystis pyrifera*. The table identifies the relevant USEPA TACs for each of these WET test species.



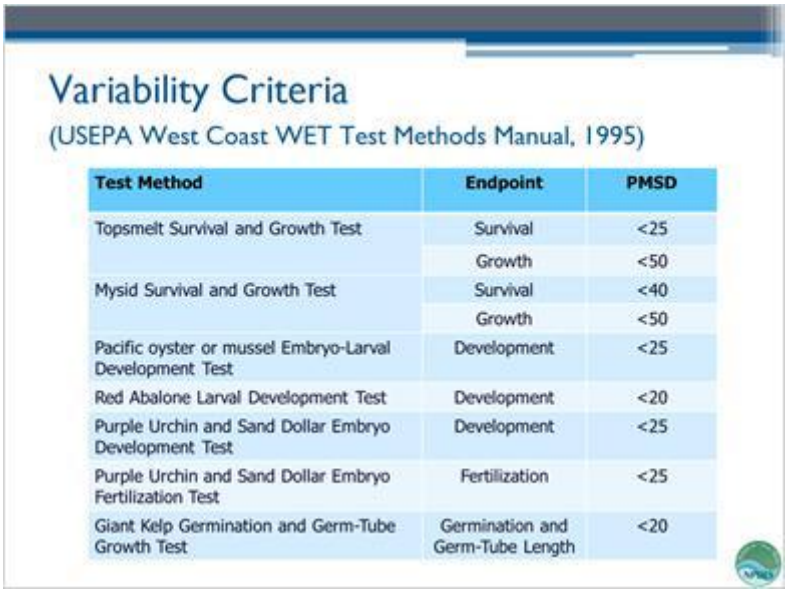
TAC – Marine Chronic Tests Cont.

S. purpuratus or D. excentricus Larval Development	*S. purpuratus or D. excentricus* Fertilization	*M. pyrifera*
• ≥ 80% normal shell development in the controls • < 25% MSD relative to the controls	• Egg fertilization at the NOEC must be > 80% of that in the controls • < 25% MSD relative to the controls • Sperm count for the final sperm stock must not exceed 33,600,000 per ml. • If sperm count is between 5.6 million and 33.6 million per mL, it must not exceed 2x of the target density from the trial. If no trial, high sperm density control (0.2 ml) must have at least 5% higher fertilization than low sperm density control (0.05 mL) • Dilution water egg blanks and effluent egg blanks should contain essentially no eggs with fertilization membranes.	• Mean control germination must be at least 70% in the controls. • Mean germination-tube length in the controls must be at least 10 µm in the controls. • The germination-tube growth NOEC must be below 35 µg/l in the reference toxicant test. • The MSD is < 20% relative to the control for both germination and germ-tube length in the reference toxicant test.



Notes:

The Percent Minimum Significant Difference, or PMSD, bounds for the USEPA East Coast marine WET test methods have only been calculated for the inland silverside and the mysid shrimp test. The lower and upper PMSD bounds for inland silverside growth are 11% and 28%, respectively. The chronic *Americamysis bahia* WET test method has lower and upper PMSD growth bounds of 11% and 37%, respectively.



Variability Criteria

(USEPA West Coast WET Test Methods Manual, 1995)

Test Method	Endpoint	PMSD
Topsmelt Survival and Growth Test	Survival	<25
	Growth	<50
Mysid Survival and Growth Test	Survival	<40
	Growth	<50
Pacific oyster or mussel Embryo-Larval Development Test	Development	<25
Red Abalone Larval Development Test	Development	<20
Purple Urchin and Sand Dollar Embryo Development Test	Development	<25
Purple Urchin and Sand Dollar Embryo Fertilization Test	Fertilization	<25
Giant Kelp Germination and Germ-Tube Growth Test	Germination and Germ-Tube Length	<20

Notes:

The Percent Minimum Significant Difference, or PMSD, bounds for the USEPA West Coast chronic marine WET test methods have only been calculated for the upper bound. The upper bound meaning that the PMSD must be less than this value. For the topsmelt survival and growth test, the PMSD for survival is 25% and the PMSD for growth is 50%. The mysid survival and growth PMSDs are 40% and 50%, respectively. The Pacific oyster and mussel embryo-larval development, as well as the purple sea urchin and sand dollar embryo development and fertilization PMSD is 25%. The Red Abalone larval development and the Giant Kelp germination and germ-tube length PMSD is 20%.