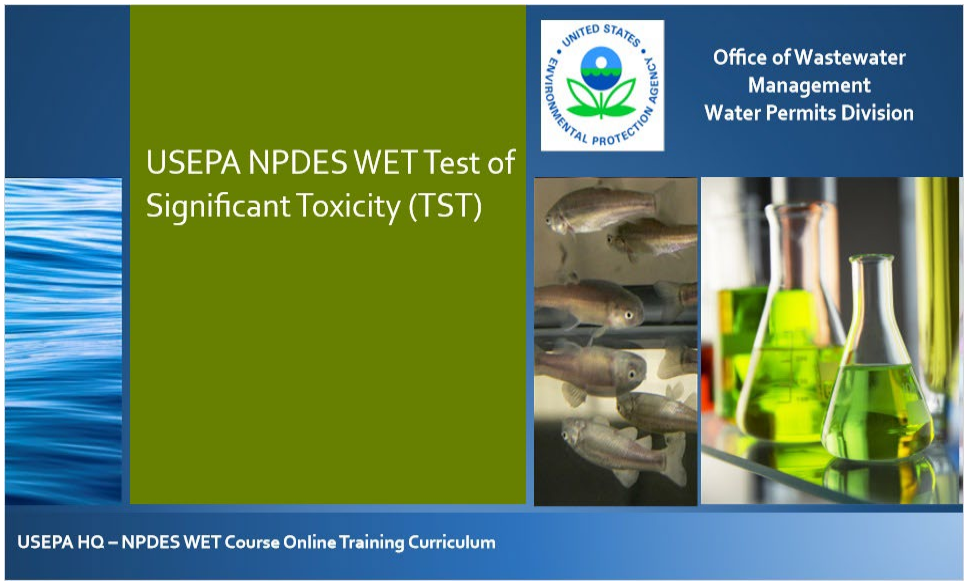
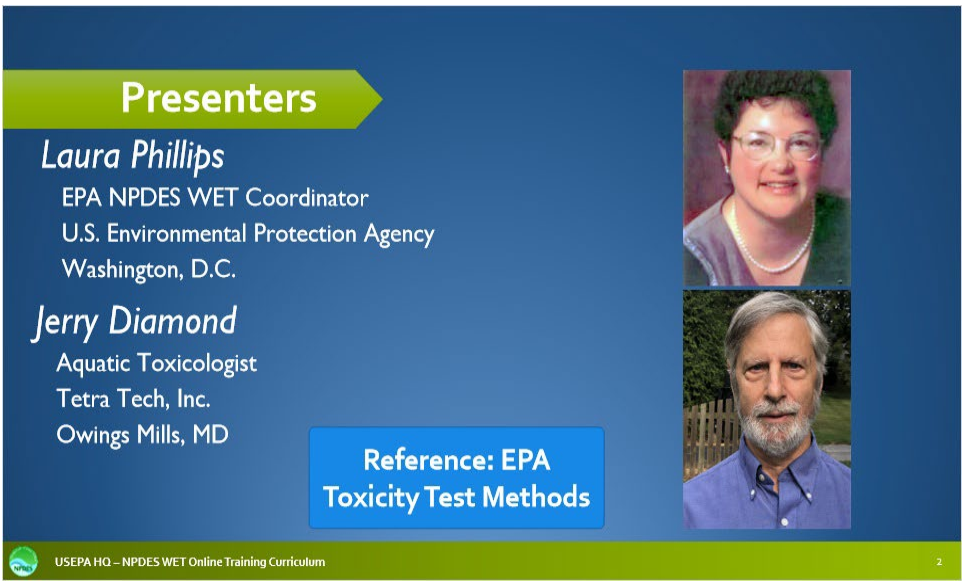
Module 6 - USEPA NPDES WET Test of Significant Toxicity



Notes:

Welcome to this presentation on the United States Environmental Protection Agency's, hereafter EPA, National Pollutant Discharge Elimination System, or NPDES, Whole Effluent Toxicity, or WET, Test of Significant Toxicity. This presentation is part of a web-based training series on WET, sponsored by EPA's 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 three important housekeeping items.



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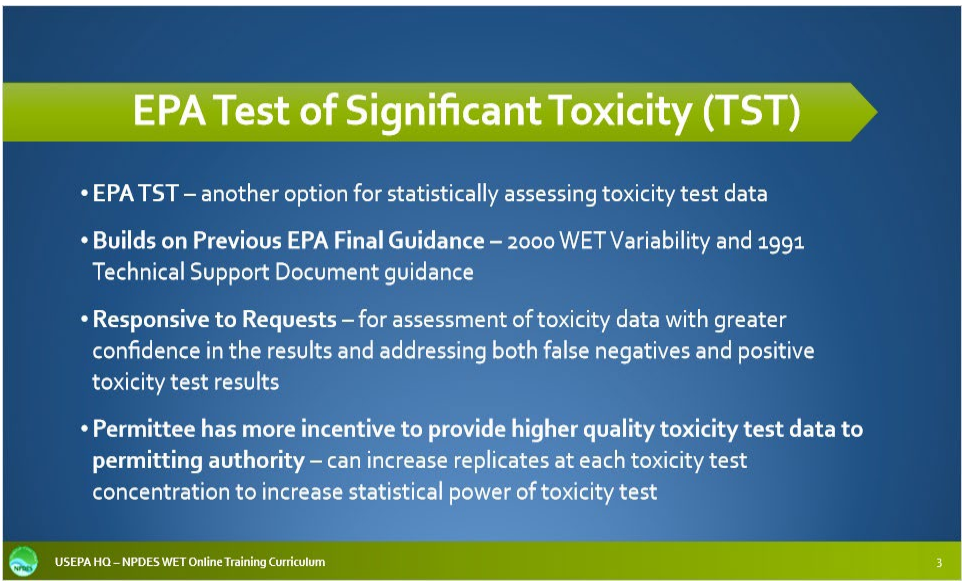
First, the introductions.
Your speakers for this presentation are, me, Laura Phillips, and I am the EPA's NPDES WET Coordinator with the Water Permits Division within the Office of Wastewater Management at EPA Headquarters in Washington, D.C., and Jerry Diamond, EPA Headquarters' contractor and an aquatic toxicologist with Tetra Tech, Incorporated in Owings Mills, Maryland. Second, now for the housekeeping items. You should be aware that all the materials used in this presentation have been reviewed by EPA staff for technical and programmatic accuracy; however, the views of the speakers are their own and do not necessarily reflect those of EPA. The NPDES permit program, which includes the use of toxicity testing, is governed by the existing requirements of the Clean Water Act and EPA'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 EPA policy and guidance on WET in the NPDES permit program. EPA may revise and/or update the contents of this presentation in the future.
Throughout this module, the term "state" means a state, the District of Columbia, the territories including the Commonwealth of Puerto Rico, the United States Virgin Islands, Guam, American Samoa, the Commonwealth of the Northern Mariana Islands, and the Trust Territory of the Pacific Islands and Tribes (40 CFR Part 122.2). The term "authorized Tribe" means those federally recognized Indian Tribes with authority to administer Clean Water Act water quality standards, WQS, program. In

some instance we may use the term “permitting authority” to include EPA, states, territories, and Tribes that have been authorized to administer the NPDES permit program.

This module was developed based on the live EPA Headquarters’ NPDES WET course that the Water Permits Division of the Office of Wastewater Management has been teaching to EPA regions, states, territories and authorized Tribes. This course, where possible, has been developed with both the non-scientist and scientist in mind. Also, while not necessary, a basic knowledge of biological principles and WET will be helpful to the viewer. Prior to this course, a review of the EPA's NPDES Permit Writers’ online course, which is available at EPA's NPDES training website, is recommended. See the “Resources” tab for a link to the NPDES training website.

When appropriate a blue button will appear on a slide to provide more information. By clicking this button, additional slides will present information regarding either freshwater or marine EPA toxicity 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 EPA’s toxicity test methods that will be presented throughout this module.

Now that you know who we are and we have covered the housekeeping items, let me turn this over to Jerry to go over EPA’s NPDES WET Test of Significant Toxicity.



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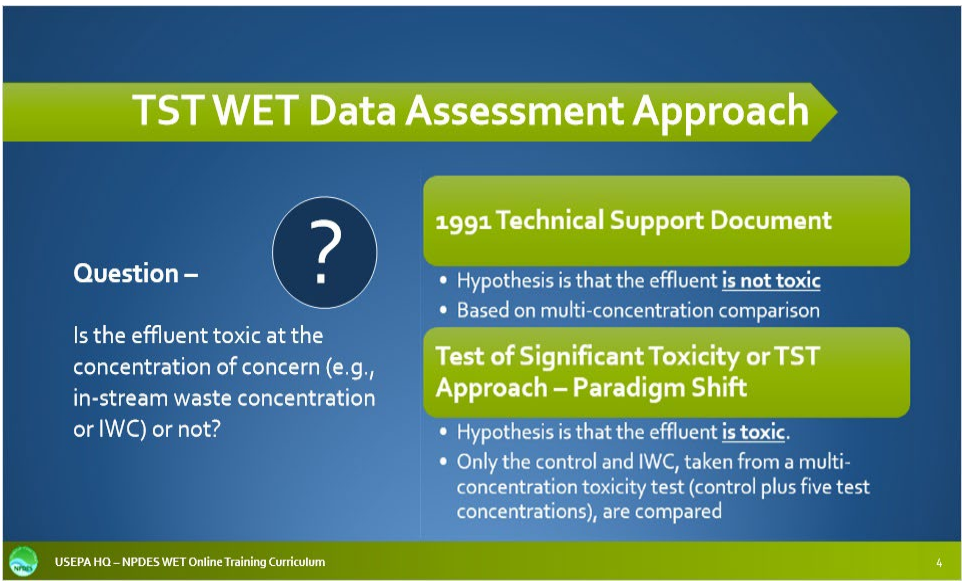
Thanks Laura. In 2010, EPA published both a technical document outlining the analytical basis for the Test of Significant Toxicity, or TST, statistical approach and an NPDES implementation document for using the TST. EPA developed the TST as another option for statistically analyzing valid toxicity test data, which builds on EPA's 2000 *Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System*, hereafter EPA's WET Variability Guidance, and EPA's 1991 *Technical Support Document for Water Quality-based Toxics Control*, hereafter EPA's 1991 TSD.
TST was developed by EPA in response to requests by the scientific community, regulatory agencies, and permittees for a statistical procedure that streamlines the analysis of valid toxicity test data and provides improvement in the interpretation of toxicity test data by addressing both false positive and false negative error rates. The TST explicitly addresses both false positive and false negative rates using a transparent statistical analysis framework. This framework provides incentives for a permittee to produce high quality toxicity test data through adequate laboratory performance of EPA toxicity test methods. High quality valid toxicity test data means having high statistical power in the toxicity test, which translates to higher statistical confidence in the interpretation of the toxicity test results.



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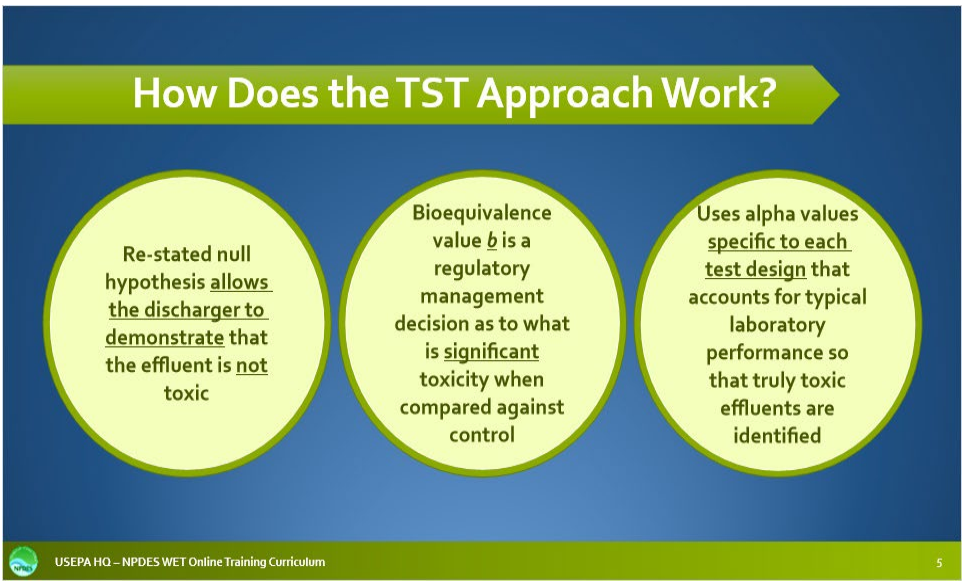
The TST statistical approach starts with the question: is the effluent toxic at the permitted in-stream waste concentration, or IWC? This is the question frequently asked by the permit writer and the permittee because the answer to this question indicates whether the permittee is in compliance with their NPDES WET permit conditions. The answer to this question should be either yes or no, which from a statistical perspective, is addressed using a hypothesis statistical approach. The hypothesis statistical approach, discussed in EPA's 1991 TSD and in the statistical guidance section of EPA's 2002 toxicity test methods manuals, relies on a null hypothesis, or the proposed theory, that the effluent is not toxic. The statistical approach then determines if the null hypothesis should be rejected; that is, whether the organisms' response in the effluent at the IWC is significantly worse than the control organism response and, therefore, the effluent should be declared toxic. For the short-term chronic toxicity test data analysis, the no observed effect concentration, or NOEC endpoint, is one of the EPA-recommended statistical approach endpoints and is based on the null hypothesis that the effluent is not toxic. The NOEC approach requires the analysis of multiple effluent test concentrations and a control as stipulated in the EPA 2002 toxicity test methods. The TST is a different hypothesis statistical approach from what is used in the NOEC analysis. Although the toxicity test is conducted using at least five effluent test concentrations and a control as required by the EPA 2002 toxicity test methods for NPDES compliance monitoring, the TST compares the organisms' response only in the IWC effluent test concentration to the organisms' response in the control. In the

TST approach, the null hypothesis is that the effluent is toxic, and the statistical analysis is used to determine whether this null hypothesis can be rejected. Therefore, using the TST approach the permittee may be able to demonstrate that their effluent is not toxic.



Notes:

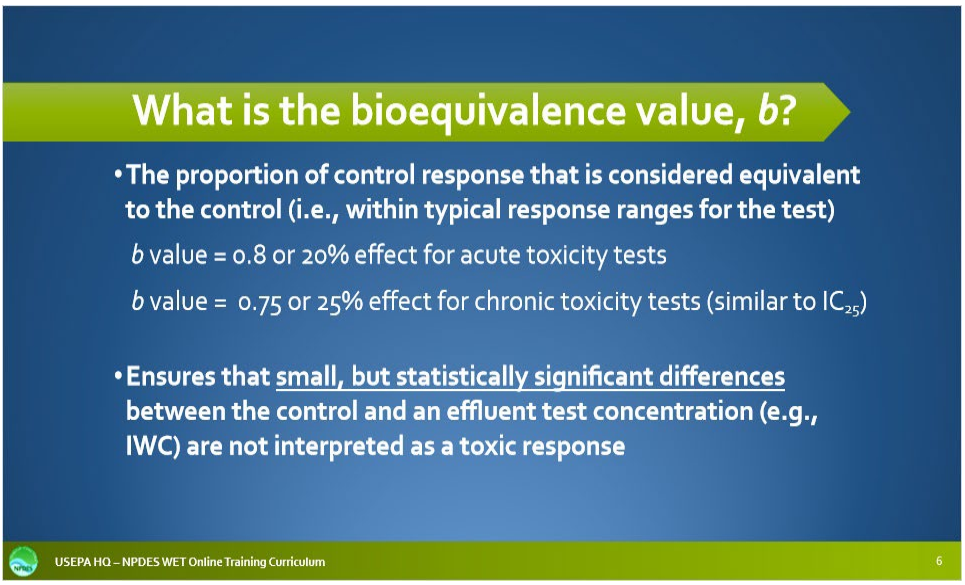
As noted in the previous slide, the TST null hypothesis is that the effluent is toxic, which means that toxicity test results must demonstrate that the effluent is not toxic. This is a different hypothesis statistical approach from the NOEC that is discussed in EPA's 1991 TSD, which relies on a null hypothesis that the effluent is not toxic, and the statistical approach determines if the null hypothesis should be rejected and therefore the effluent should be declared toxic. The restated null hypothesis allows the discharger to demonstrate that the effluent is not toxic.

In developing the TST statistical approach, the EPA builds upon an extensive statistical literature search that used a similar re-stated null hypothesis as the TST. The literature discusses the benefits of using what is called a bioequivalence statistical approach along with the re-stated null hypothesis of the TST. The bioequivalence approach states that the organism response in the effluent can be within certain bounds of the control organisms' response and still be "biologically equivalent," thus, not considered a toxic response. In the null hypothesis formula using TST, this bioequivalence bound is denoted as "*b*." The value for "*b*" is based on a regulatory management decision regarding the maximum observed percent difference from the effluent test concentrations to the control that is considered non-toxic. The *b* value is different for acute and chronic toxicity methods, as discussed later in this module.

In EPA's NOEC hypothesis approach there is a Type I error rate, or alpha value, used for all EPA toxicity test methods, which is 0.05 or five percent. This is commonly referred to as the false positive rate using the EPA hypothesis statistical approach.

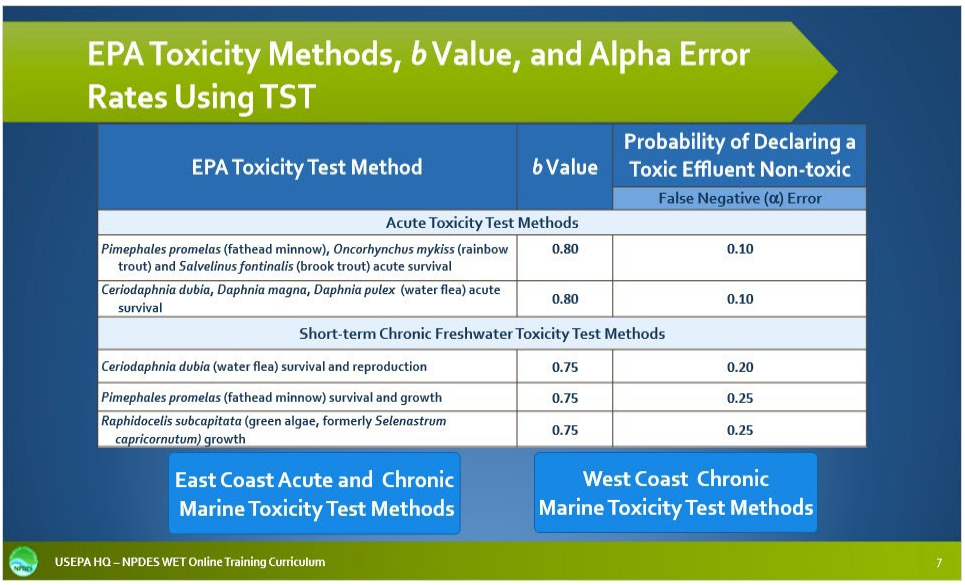
The Type II error rate (denoted beta), or false negative rate, using the NOEC approach was not formally established by EPA for the different EPA toxicity test methods.

In EPA's TST approach, the alpha value is equivalent to the false negative rate in EPA's NOEC approach. The alpha value when using the TST is different depending on the EPA toxicity test method design and was derived by EPA considering routine laboratory performance with each EPA toxicity test method. Using actual laboratory performance information ensures that the test-specific alpha values are appropriate, and that the actual effluent toxicity is identified. The beta value, or Type II error rate, using the TST approach is equivalent to the Type I or false positive rate using the traditional hypothesis, for example NOEC, statistical approach and is set at 0.05, or five percent.



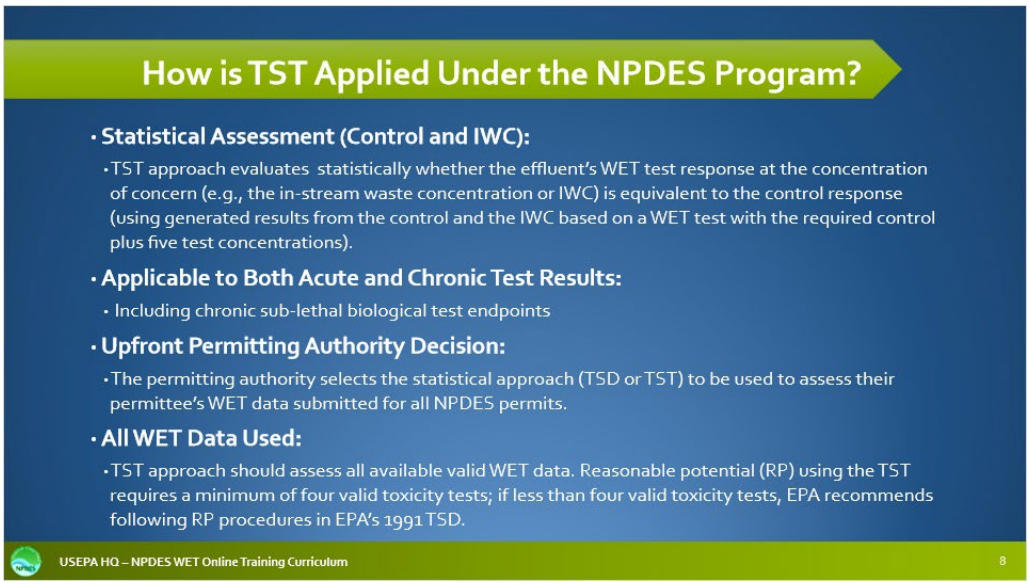
Notes:

The TST approach uses a bioequivalence value in the analysis called "*b,*" which represents the proportion of the control response observed in the IWC that is considered equivalent to the control. For acute toxicity tests, the *b* value is equal to 0.8, which means 80 percent of the control response. For chronic toxicity tests, the *b* value is equal to 0.75, which means 75 percent of the control response, or a 25 percent effect, similar to the 25 percent inhibition concentration, or IC_{25}. Using a bioequivalence value in the TST approach helps ensure that small differences between the control and the IWC organism responses are not interpreted as a toxic effect under routine laboratory performance of the toxicity test method.



Notes:

This table summarizes the *b* value and alpha or false negative error rate values, used in the TST statistical approach for freshwater EPA 2002 toxicity test methods. Use the buttons to see *b* values and false negative rates for EPA 2002 East Coast marine toxicity test methods and for EPA 1995 West Coast marine toxicity test methods.



Notes:

As previously noted, the TST statistical approach analyzes the organisms' responses measured in the control and the IWC treatment from valid toxicity test data generated using EPA toxicity test methods. Even though the TST approach analyzes the control and the IWC test concentrations, EPA toxicity tests require a control plus at least five effluent test concentrations unless an EPA-approved Alternative Test Procedure is in place.

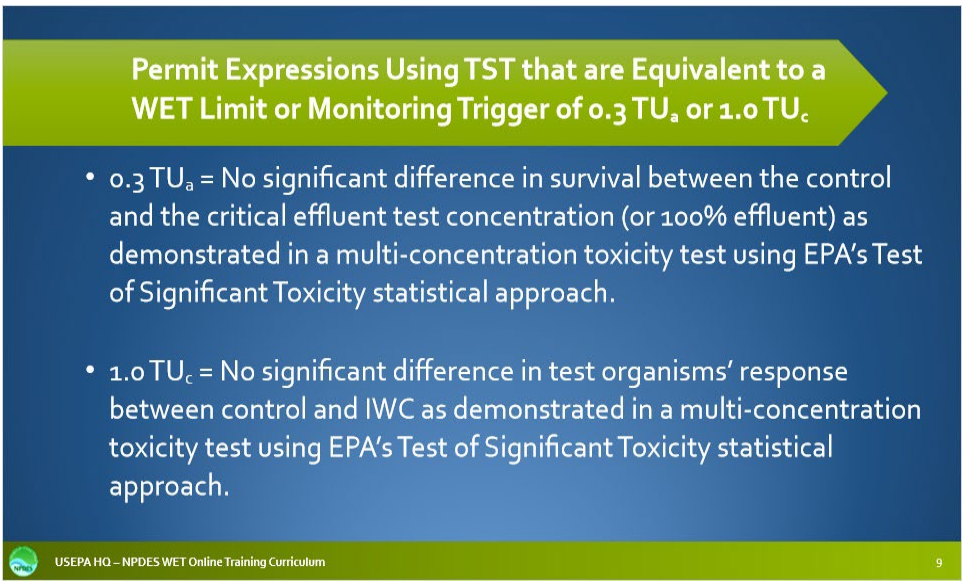
The TST approach is used to analyze any toxicity test biological endpoint, for both acute and chronic, including chronic sub-lethal biological test endpoints such as reproduction or growth.

As discussed in the NPDES WET Permit Conditions, Permit Language and Technical Considerations module, the permitting authority should identify in the permit the type of statistical analysis approach the permittee must use to analyze valid toxicity test data. Permit writers and permittees should not choose which statistical approach, for example TST, IC_{25}, or NOEC, to use after toxicity testing has been initiated.

As with any statistical approach, in using the TST approach, all valid data from a given toxicity test should be considered, as noted in EPA's 2000 *Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136)*.

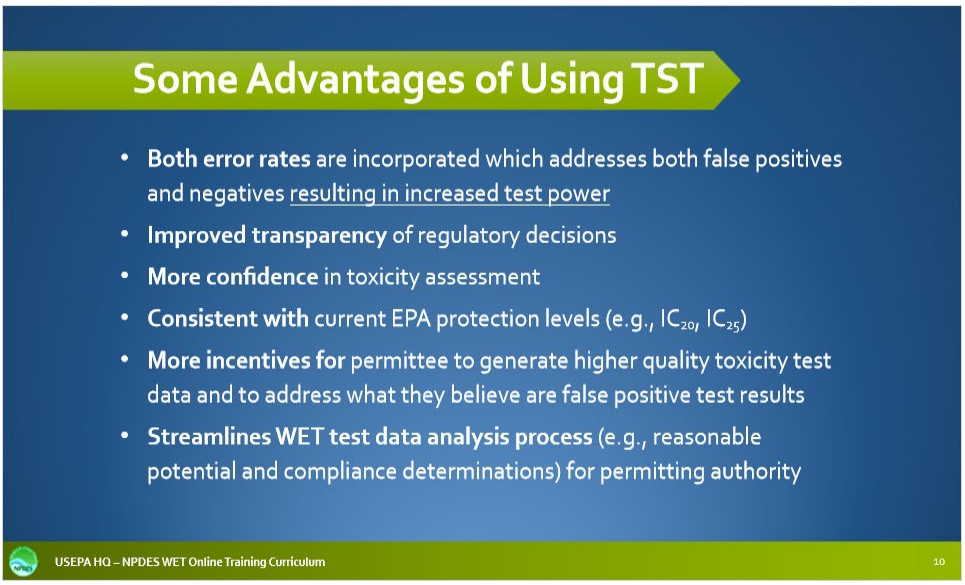
Reasonable potential analysis or RPA when using the TST is conducted differently from the RPA procedures described in EPA's 1991 TSD. The TST reasonable potential approach will be described in more detail later in this module. Data from a minimum of four valid toxicity tests are used in reasonable potential analysis

when using the TST approach. If there are less than four valid toxicity tests for a given effluent, the statistical approach described in EPA's 1991 TSD should be used to determine reasonable potential.



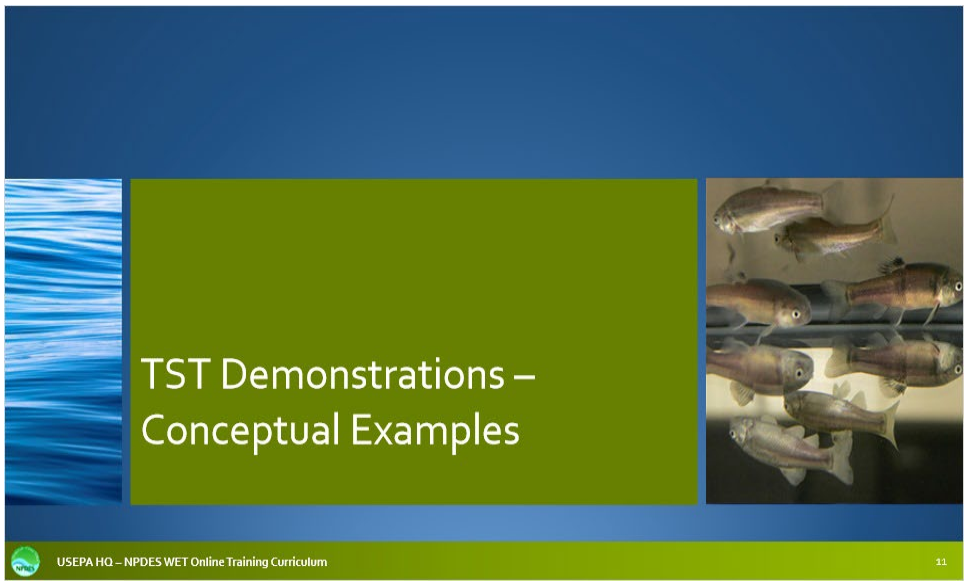
Notes:

The Overview of EPA's NPDES WET Permit Program module discusses EPA's recommended acute and chronic toxicity criteria. The acute toxicity criterion is 0.3 acute toxicity units, or TU_a. The chronic toxicity criterion is 1.0 chronic toxicity units, or TU_c. The TST statistical approach is directly applicable to evaluating NPDES permit compliance with these EPA-recommended toxicity criteria. For EPA acute toxicity tests, compliance with a toxicity limit derived from the acute toxicity criterion of 0.3 TU_a can be interpreted as having no significant difference in organism survival between the control and the critical effluent test concentration under acute mixing conditions, as stipulated by the permitting authority. The TST statistical approach for analyzing acute toxicity tests directly compares whether organism survival in the critical effluent concentration, which for many states, is 100 percent effluent, is equivalent to the survival observed in the controls.
For chronic EPA toxicity tests, compliance with a toxicity limit derived from the EPA-recommended chronic toxicity criterion of 1.0 TU_c can be interpreted as having no significant difference in the organisms response, including chronic sub-lethal biological responses, between the control and the critical effluent test concentration under chronic mixing conditions, as stipulated by the permitting authority, typically the chronic IWC. The TST statistical approach for analyzing chronic toxicity tests directly compares organism responses in the control to the critical effluent test concentration as defined in the permit.



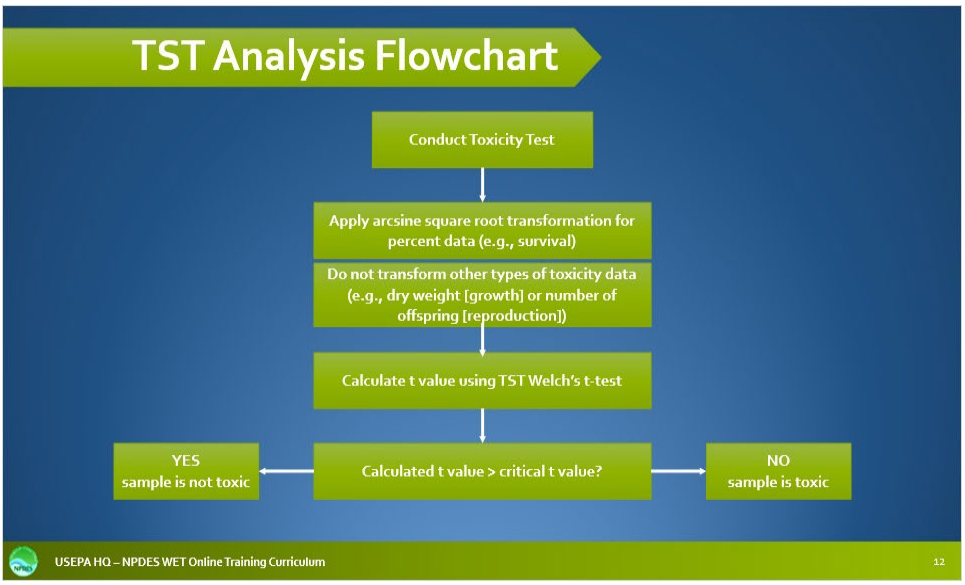
Notes:

The TST statistical approach has several advantages over other types of statistical approaches for analyzing valid toxicity test data. First, under the TST approach, unlike the NOEC and IC_{25} approaches, both false positive and false negative error rates are explicitly identified. Second, because both error rates are quantified, the mathematics of analyzing toxicity test data when using the TST is very transparent. Given the within-test variability observed and the magnitude of the effect observed in the IWC as compared to the controls, the permittee can readily determine whether their effluent should be declared toxic using the TST. Third, because regulatory decisions based on the TST approach are transparent, there is more confidence in and improved interpretation of toxicity test data. Fourth, the TST approach was designed based on regulatory management decisions to be consistent with toxicity protection levels used by permitting authorities, as measured by other statistical approaches, for example IC_{20}, IC_{25}. Fifth, because the TST approach uses a re-stated null hypothesis that is opposite the traditional null hypothesis, the permittee has incentives to generate high quality data and prove they are not toxic. If the effluent is truly not toxic, higher quality data can only help demonstrate that the organisms' responses at the IWC are bioequivalent to the control. Finally, the TST statistical approach is relatively simpler and more straightforward. Thus, the analysis of toxicity test data as well as reasonable potential determinations, can be streamlined.



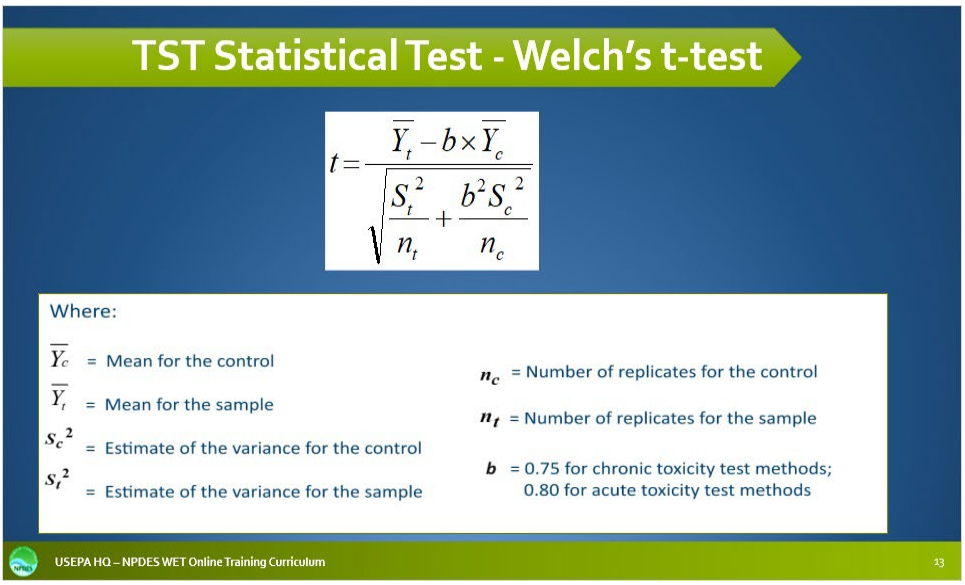
Notes:

We will now present the details of the TST statistical approach, including the equations used, followed by a few examples illustrating the TST approach.



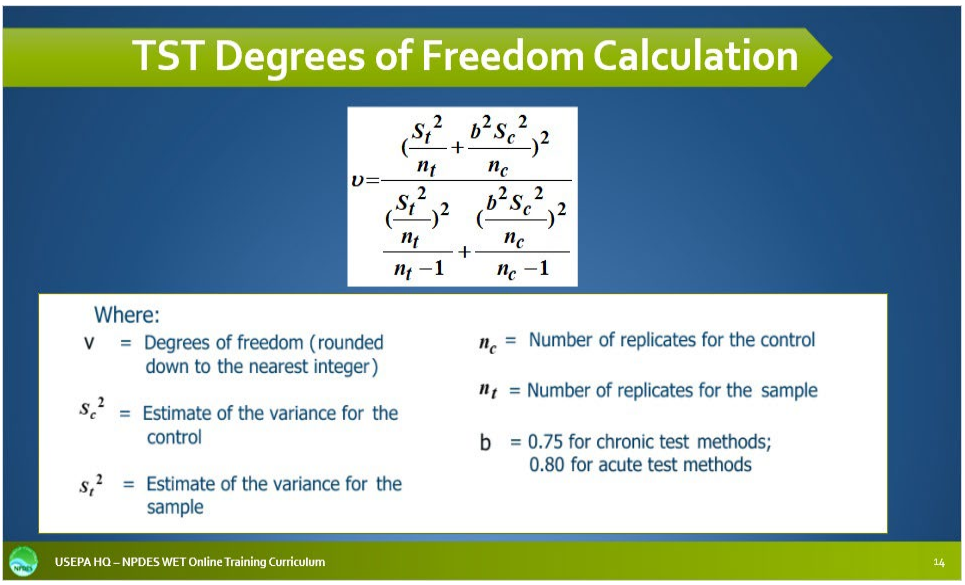
Notes:

The analysis flowchart using the TST approach is straightforward. After a valid toxicity test has been conducted using approved EPA toxicity test methods, data are used to analyze the organism response in the control and IWC from the multi-concentration toxicity test. If the response being measured is expressed as percent data, for example, percent organism survival or percent normal larval development, the data should be arcsine square root transformed prior to the analysis, which is consistent with the statistical guidance given in EPA's 2002 toxicity test methods manuals. Other non-percentage toxicity data, for example dry weight or number of offspring, are not transformed prior to the analysis. The data are then used to calculate a t-value using the Welch's t-test. If the calculated t is greater than the table t-value, the null hypothesis is rejected which means that the effluent is not declared toxic. If the calculated t-value is less than or equal to the table t-value, one cannot reject the null hypothesis and therefore, the effluent is declared toxic.



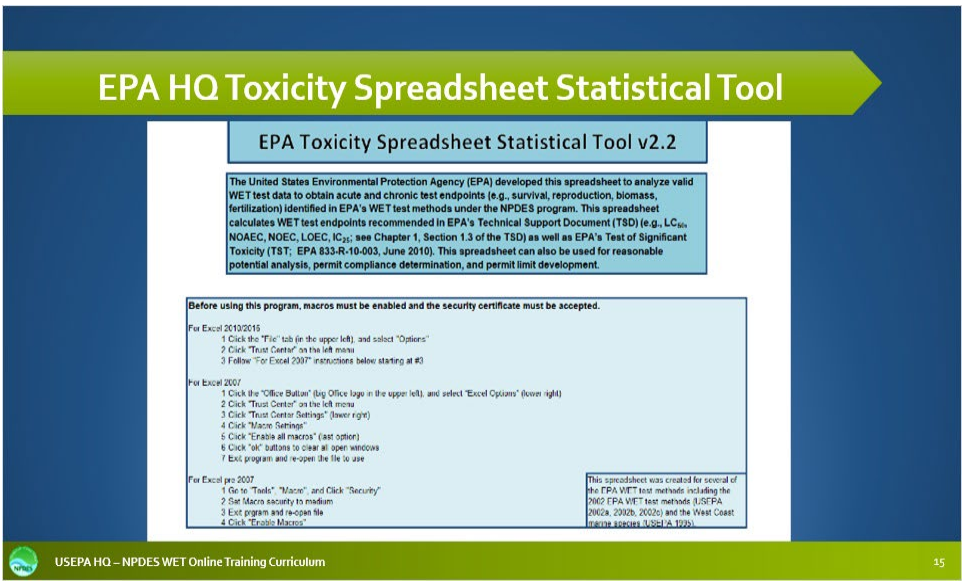
Notes:

This slide shows the formula used to calculate the *t*-value, or t, based on valid toxicity test data from the control and the IWC which would be one of the test concentrations in a toxicity test. The numerator of the formula calculates the difference between the IWC mean, or Y_t, and the control mean, or Y_c, multiplied by the *b* value (0.8 for an acute test and 0.75 for a chronic test). The denominator is the square root of the sum of the variances, or s, observed among replicates for the IWC, or s_t, and the control, or s_c, each divided by their respective number of replicates, or n. The control variance is multiplied by the square of the bioequivalence *b* value.



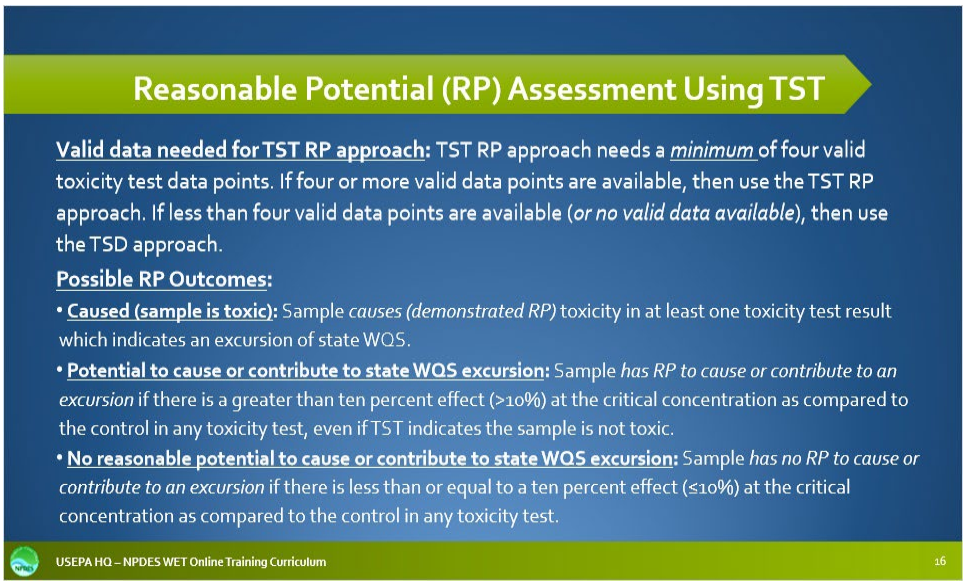
Notes:

The table t-value, to which the Welch's t-value from the previous slide is compared, is determined by calculating the degrees of freedom. For Welch's t-test, the degrees of freedom, or V, is calculated using the formula on this slide. The Welch's formula is a generalized version of the standard simplified formula used in t-tests where the number of replicates and/or the variance may not be equal between the control and IWC treatments.



Notes:

EPA's Headquarters toxicity spreadsheet statistical tool, which includes the ability to perform TST statistical analysis, is found on EPA's NPDES website. The toxicity statistical analysis spreadsheet can also be used to analyze acute and chronic EPA toxicity test data for both hypothesis, for example, NOEC, and point estimate statistical approach endpoints, such as, LC_{50} and IC_{25}, using the statistical approaches included in the EPA 2002 toxicity test method manuals.



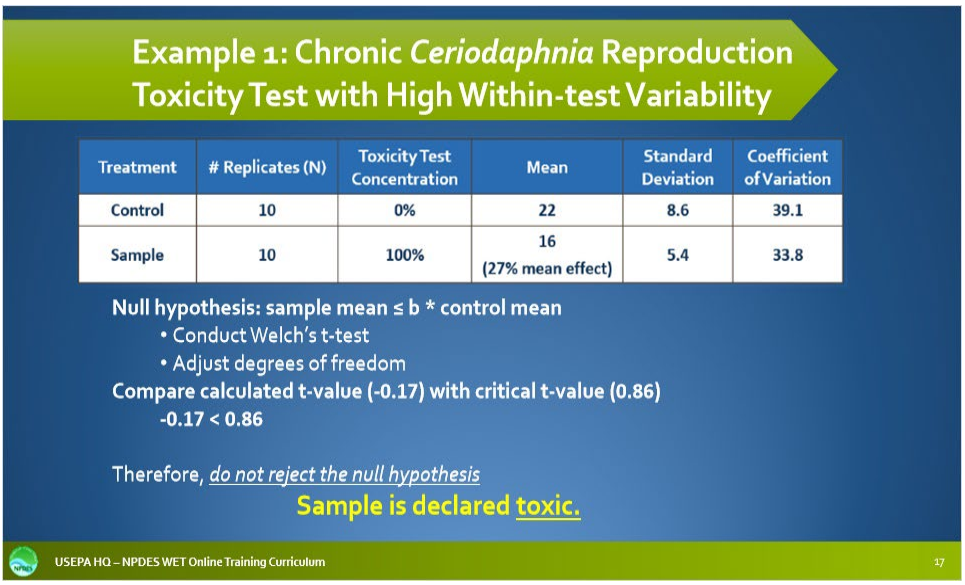
Notes:

Reasonable potential, or RP, analysis using the TST statistical approach requires valid toxicity test data from a minimum of four toxicity tests. If valid toxicity test data for fewer than four toxicity tests are available, the TST cannot be used for RP analysis. In that case, the EPA TSD statistical approach should be used for RP analysis.

The three possible outcomes of a toxicity test RP determination using the TST approach are: 1) the effluent causes an excursion of state water quality standards as measured in at least one valid toxicity test, therefore RP has been demonstrated, the sample is considered toxic; 2) the effluent has the potential to cause or contribute to an excursion of water quality standards as measured by a toxicity test, when the effluent has a greater than 10 percent difference in one or more valid toxicity tests between the IWC and the control test organisms' response and therefore RP has been demonstrated; and 3) the effluent does not cause an excursion of the water quality standards as measured by a toxicity test, when there is less than a 10 percent difference between the IWC and the control test organisms' response and therefore RP has not been demonstrated so the effluent is not considered toxic.

Using the TST approach, cause is demonstrated by at least one valid toxicity test where the effluent is declared toxic. Potential to cause or contribute is determined by examining the percent effect observed at the critical concentration in each valid toxicity test. If one or more toxicity tests exhibit more than a 10 percent effect in the critical concentration as compared to the control organism response, the

sample has the potential to cause or contribute to an excursion of the state's toxicity water quality standards. If the valid toxicity tests exhibit less than or equal to 10 percent effect at the critical concentration when compared against the control organism response, then the TST would indicate that the effluent from each toxicity test is not considered toxic, then the sample does not have RP.

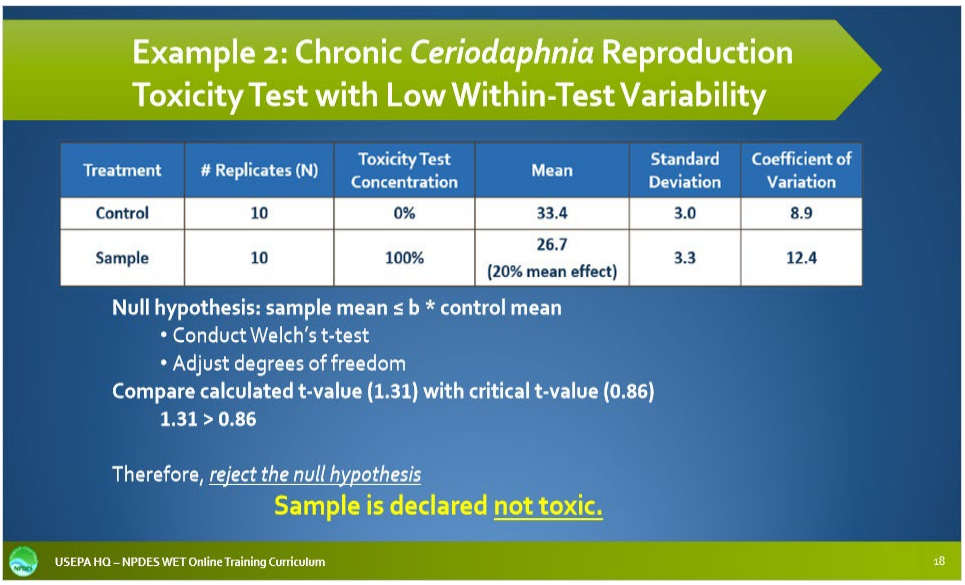


Notes:

In this slide and the next, we will demonstrate the application of the TST approach using two examples of toxicity test data. Both examples use toxicity test data from EPA's chronic *Ceriodaphnia* survival and reproduction tests. We will provide toxicity test data for the control and the sample in each example, which was taken from toxicity tests having a control and five test concentrations, as required in the EPA 2002 toxicity test methods.

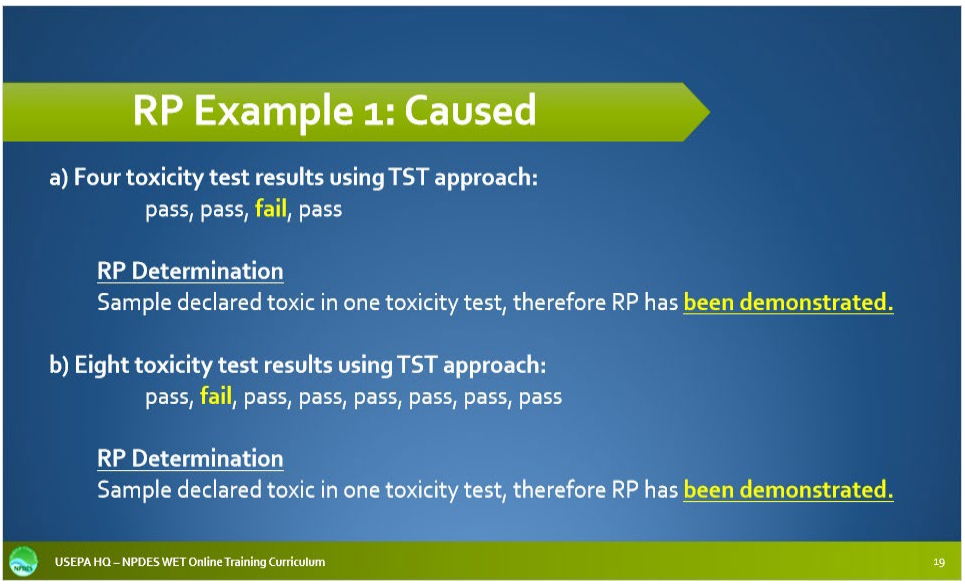
In the first example, the sample had 27 percent fewer *Ceriodaphnia* offspring as compared to the control, as shown by the mean reproduction for the control, 22 offspring, and the sample, 16 offspring. The standard deviation of the number of offspring for the control treatment is 8.6. This represents a relatively high degree of variability among the control replicates as compared to what has been observed by EPA for toxicity laboratories in the U.S. for this EPA 2002 freshwater invertebrate toxicity test method. The coefficient of variation, CV, for the controls is equal to the standard deviation, 8.6, divided by the control mean, 22, times 100, which equals 39 percent. In examining over 700 valid chronic *Ceriodaphnia* toxicity tests conducted by toxicity laboratories across the U.S., EPA found that 80 percent of those tests achieved a control CV for *Ceriodaphnia* reproduction that was less than 33 percent. So, the control CV of 39 percent is relatively high compared to what is typically achieved for this EPA toxicity test method biological test endpoint. This indicates that these toxicity test results have low precision, suggesting that the quality of the data in this toxicity test example is not as high as would be expected for this toxicity test method. The TST statistical analysis of this example data indicates that the null

hypothesis, that the sample has more than a 25 percent effect as compared to the control, cannot be rejected and, therefore, the sample is declared toxic. The TST approach was developed such that an effect in the sample greater than or equal to a 25 percent effect would nearly always be identified as toxic. We note that if this test data were analyzed using the TSD-based hypothesis approach, for example NOEC or a standard t-test, the sample, due to the variability, would not be declared toxic, even though there was a 27 percent decrease in reproduction in the sample.



Notes:

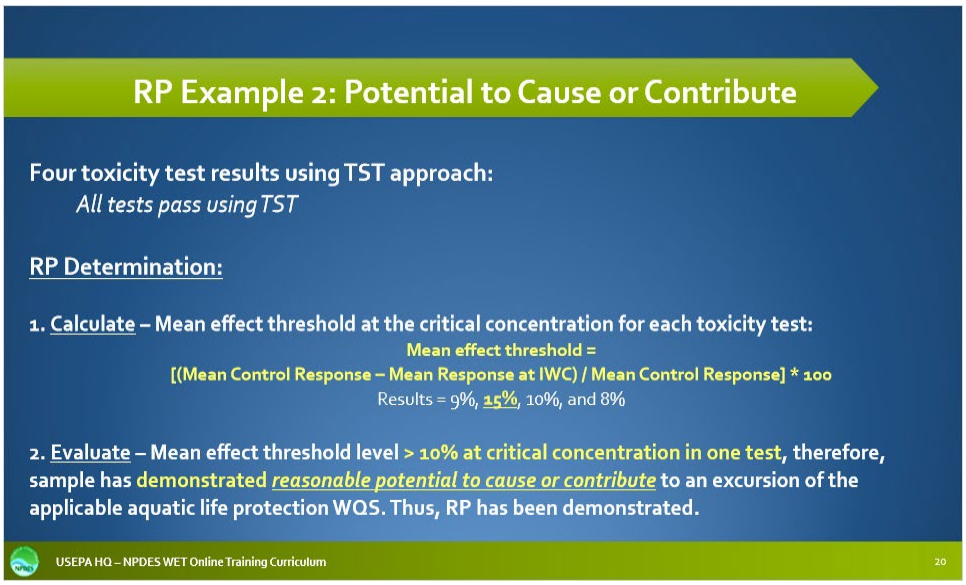
In this second example, we have reproduction data for a chronic *Ceriodaphnia* toxicity test. The mean effect in the sample is less than 25 percent, it is 20 percent mean effect in this example, and the reproduction data for both the control and sample treatments are relatively precise, with low standard deviations. In fact, if we calculate the CV for the controls, like we did in the previous example, we see that the CV is equal to the standard deviation, 3.0, divided by the control mean, 33.4, times 100, or approximately nine percent. EPA previously determined that a CV of nine percent represents the 10th percentile of CVs for this toxicity test method biological test endpoint. In other words, only about 10 percent of the more than 700 valid toxicity tests examined by EPA for this toxicity test method and biological test endpoint exhibited a CV less than or equal to nine percent. The analysis of these toxicity test data using the TST approach indicated that the null hypothesis should be rejected; that is, these toxicity test data indicate that this sample had less than a 25 percent effect on *Ceriodaphnia* reproduction as compared to the controls. Therefore, the sample is declared not toxic based on these toxicity test results. We note that the TSD-based hypothesis approach, NOEC or standard t-test, would have declared the effluent toxic based on these test results because of the high precision or low variability observed in the controls and the sample.



Notes:

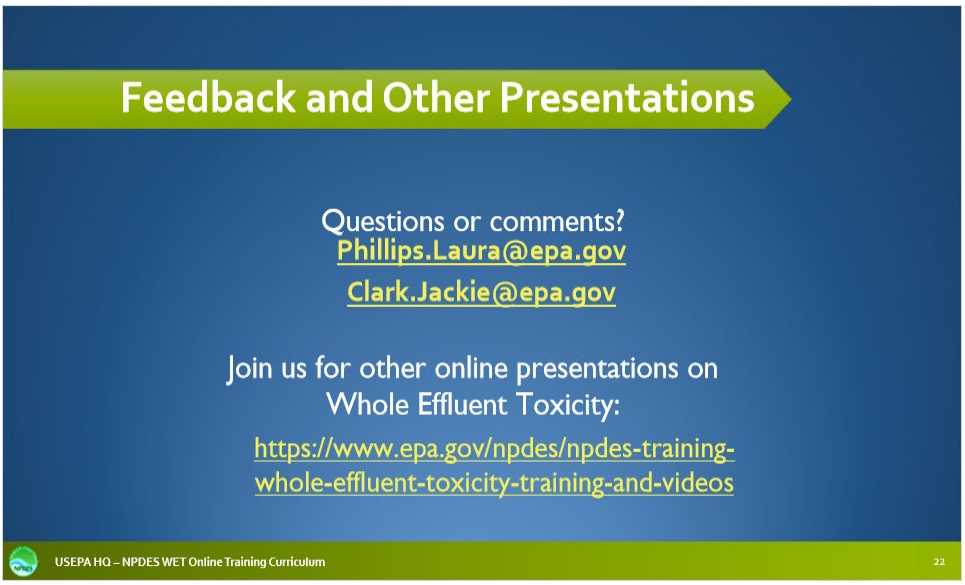
In this slide we give two examples demonstrating the reasonable potential analysis approach using TST. In the first example, four valid toxicity tests were conducted, one of which was a "fail," meaning that the sample was declared toxic using the TST approach. Because at least one toxicity test was declared toxic for this sample, the sample has demonstrated *cause* in terms of an excursion of state water quality standards and, therefore, the sample has demonstrated reasonable potential and should have a limit in their NPDES permit.

In the second half of this slide, a facility completed eight valid toxicity tests, one of which was declared toxic "fail." The RP determination is the same using the TST approach as in the example above. So long as one toxicity test is declared toxic, the sample has demonstrated RP, regardless of the number of toxicity tests conducted and should have a limit in their NPDES permit.



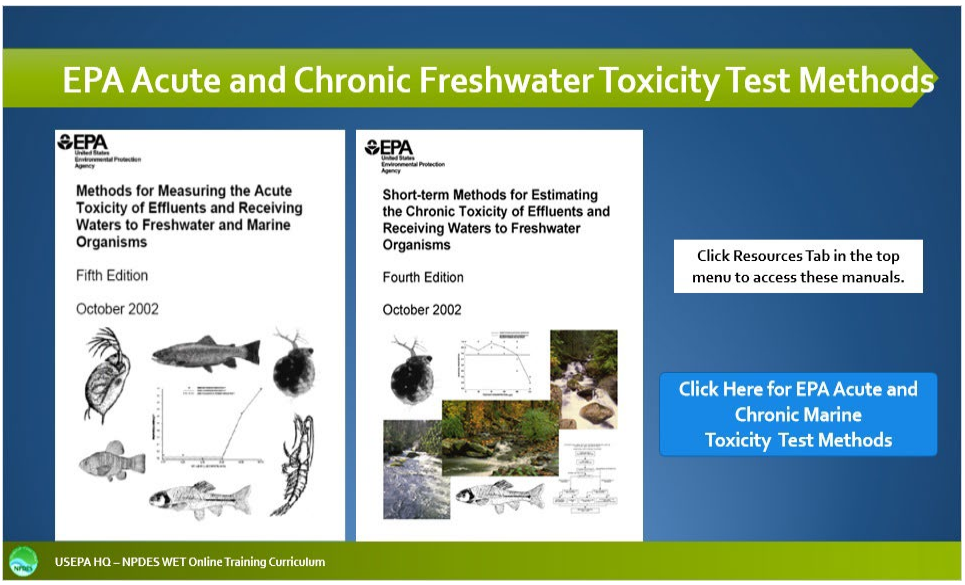
Notes:

In this example, four valid toxicity tests were completed and all of them were declared not toxic at the critical concentration, that is all tests "passed," using the TST approach. The next step in the RP process using the TST approach is to calculate the mean effect observed at the critical concentration in each valid toxicity test. In this example, the mean effect observed at the critical concentration in each valid toxicity test was 9, 15, 10, and 8 percent. Using the TST approach, if any one toxicity test exhibits more than a 10 percent effect at the IWC, the sample is considered to have the *potential to cause or contribute* to an excursion of the applicable toxicity water quality standards. Since one toxicity test exhibited more than a 10 percent effect, the sample has demonstrated RP and should have a toxicity limit in their NPDES permit. We note that if a facility passed all their toxicity tests and they had less than or equal to a 10 percent effect at their critical concentration in all their toxicity tests, the sample would not have demonstrated RP, even if the sample had little or no dilution at their critical concentration.



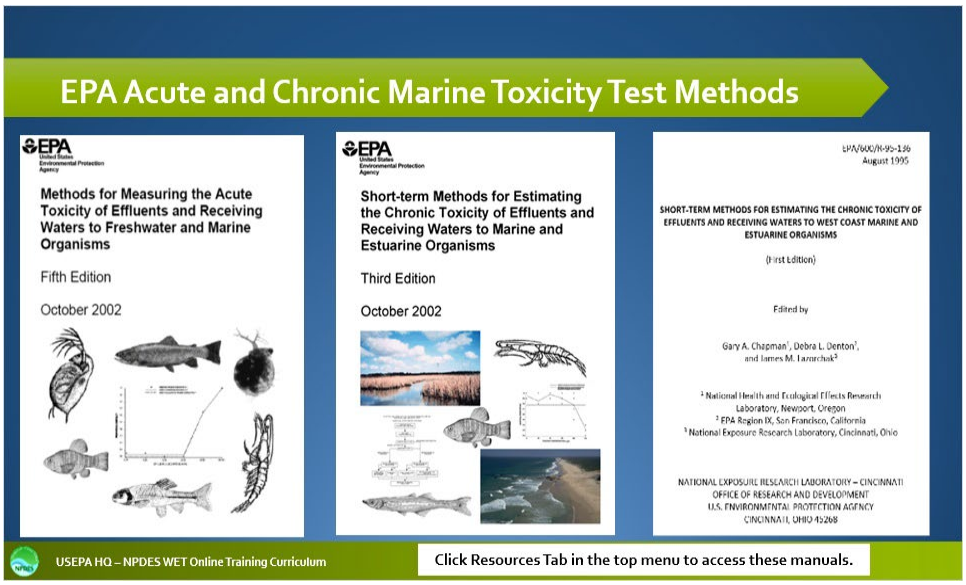
Notes:

Thank you for joining us for this EPA'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 EPA'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, EPA Headquarters NPDES WET Coordinators.
Remember, you will find all of the EPA's NPDES WET online training presentations, under the EPA's NPDES training section found on the Office of Wastewater Management's NPDES website.
See you next time!



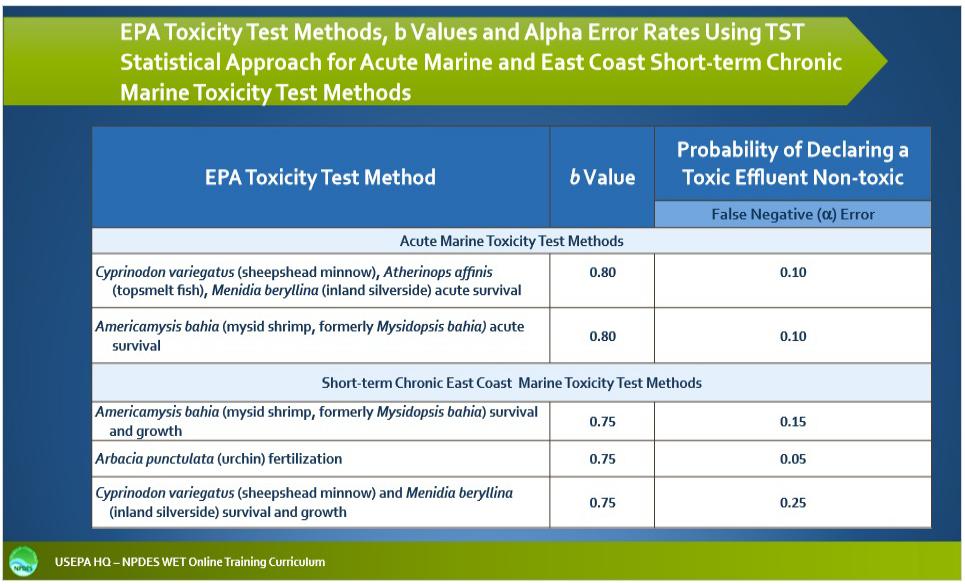
Notes:

The module presented here examines EPA's freshwater acute toxicity 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 EPA's short-term chronic freshwater toxicity 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.



Notes:

This course also provides an opportunity to view EPA's acute marine toxicity 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; short-term chronic marine toxicity 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 chronic toxicity test methods; or short-term chronic marine toxicity 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 chronic toxicity test methods.



EPA Toxicity Test Methods, b Values and Alpha Error Rates Using TST Statistical Approach for Acute Marine and East Coast Short-term Chronic Marine Toxicity Test Methods

EPA Toxicity Test Method	*b* Value	Probability of Declaring a Toxic Effluent Non-toxic
		False Negative (α) Error
Acute Marine Toxicity Test Methods		
Cyprinodon variegatus (sheepshead minnow), *Atherinops affinis* (topsmelt fish), *Menidia beryllina* (inland silverside) acute survival	0.80	0.10
Americamysis bahia (mysid shrimp, formerly *Mysidopsis bahia)* acute survival	0.80	0.10
Short-term Chronic East Coast Marine Toxicity Test Methods		
Americamysis bahia (mysid shrimp, formerly *Mysidopsis bahia*) survival and growth	0.75	0.15
Arbacia punctulata (urchin) fertilization	0.75	0.05
Cyprinodon variegatus (sheepshead minnow) and *Menidia beryllina* (inland silverside) survival and growth	0.75	0.25

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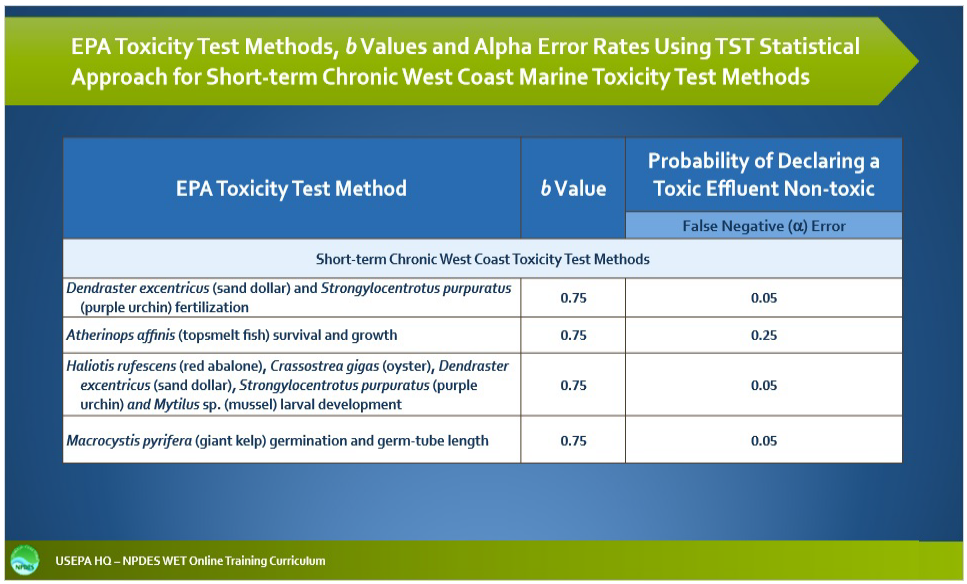
Notes:

This table summarizes the *b* value and alpha, or false negative error rate values, used in the TST statistical approach for the EPA 2002 acute marine toxicity tests and the EPA 2002 East Coast short-term chronic marine toxicity test methods.



EPA Toxicity Test Methods, *b* Values and Alpha Error Rates Using TST Statistical Approach for Short-term Chronic West Coast Marine Toxicity Test Methods

EPA Toxicity Test Method	*b* Value	Probability of Declaring a Toxic Effluent Non-toxic
		False Negative (α) Error
Short-term Chronic West Coast Toxicity Test Methods		
Dendraster excentricus (sand dollar) and *Strongylocentrotus purpuratus* (purple urchin) fertilization	0.75	0.05
Atherinops affinis (topsmelt fish) survival and growth	0.75	0.25
Haliotis rufescens (red abalone), *Crassostrea gigas* (oyster), *Dendraster excentricus* (sand dollar), *Strongylocentrotus purpuratus* (purple urchin) *and Mytilus* sp. (mussel) larval development	0.75	0.05
Macrocystis pyrifera (giant kelp) germination and germ-tube length	0.75	0.05

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Notes:

This table summarizes the *b* value and alpha, or false negative error rate values, used in the TST statistical approach for EPA 1995 West Coast short-term chronic marine toxicity test methods.