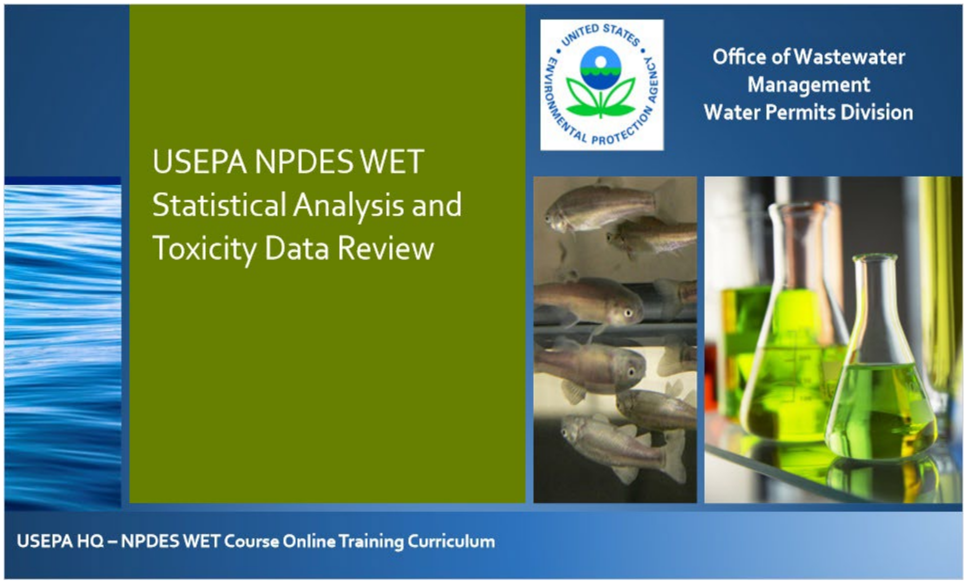
Module 4 - USEPA NPDES WET Statistical Analysis and Toxicity Data Review



Notes:

Welcome to this presentation on the United States Environmental Protection Agency's, hereafter USEPA, National Pollutant Discharge Elimination System, or NPDES, Whole Effluent Toxicity, or WET, Statistical Analysis and Toxicity Data Review. 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.

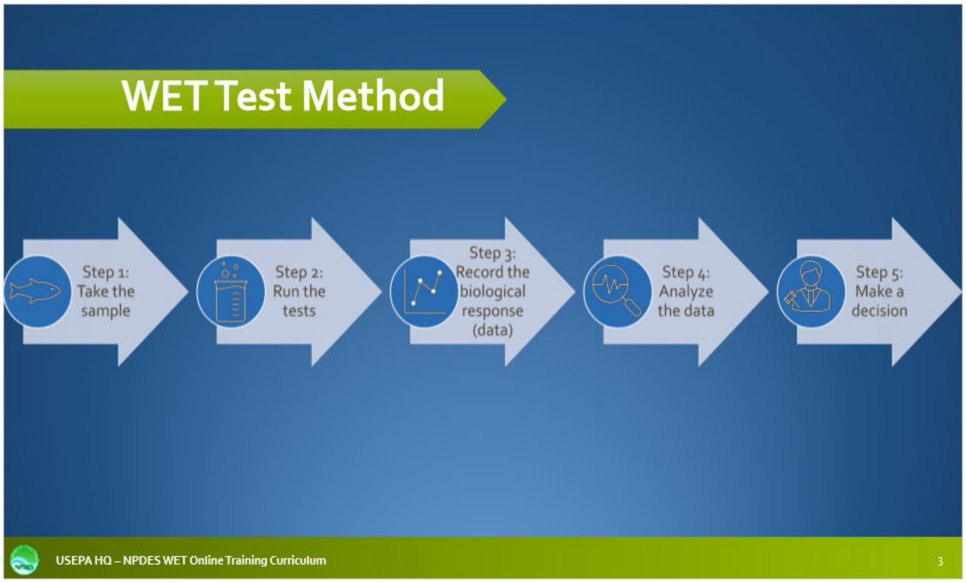
Notes:

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 the USEPA Headquarters in Washington, D.C., and Jerry Diamond, USEPA Headquarters' contractor and an aquatic toxicologist with Tetra Tech, Incorporated in Owings Mills, Maryland. Second, now for that housekeeping item. 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 in the NPDES permits program. USEPA may revise and/or update the contents of 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 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 WET will be helpful to the viewer. Prior to this course, a review of the USEPA's

NPDES Permit Writers' online course, which is available at USEPA's NPDES website, is recommended.

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 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.

Let me turn this over to Jerry and we will look at USEPA WET statistical analysis and toxicity data review.



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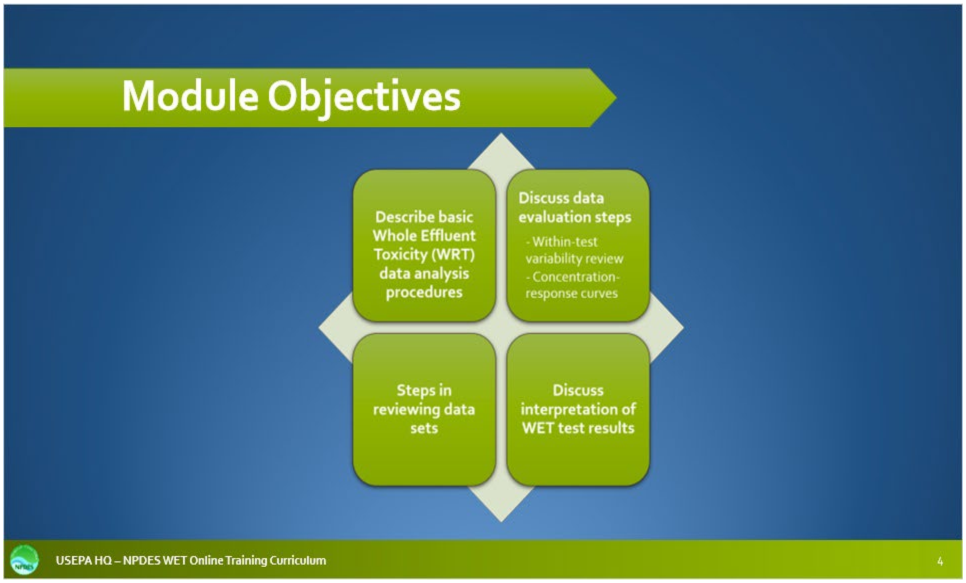
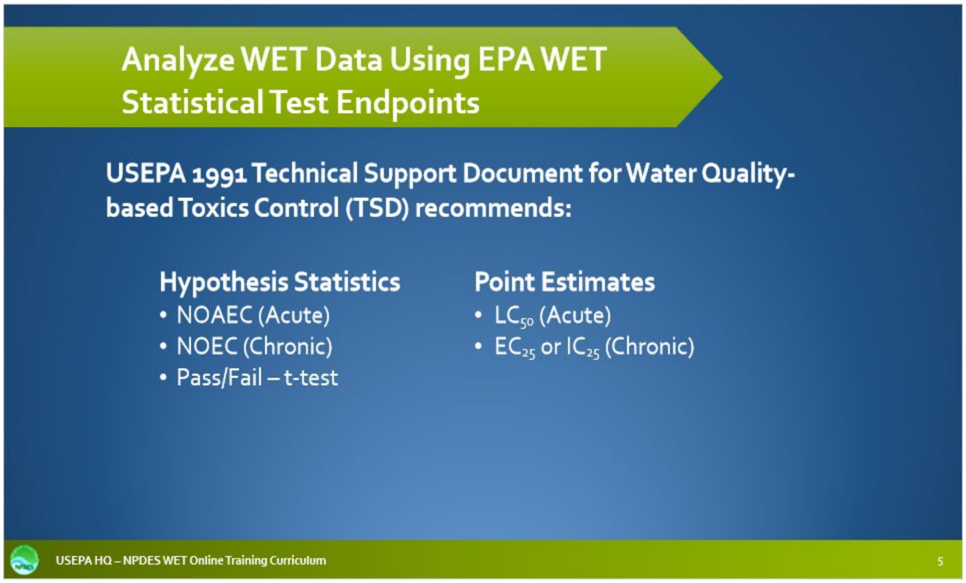
Thanks Laura. The first step during the process of conducting WET testing is to collect an effluent sample according to the sample collection procedures provided in the USEPA WET test methods. Step two is to run the tests according to the prescribed USEPA test methods. Third, the organism responses are recorded, including mortality, and chronic sub-lethal test endpoints according to each test method. Fourth, valid WET test data are analyzed using recommended statistical approaches that are used for the fifth, or final, step determining whether the permitted effluent is in compliance with an NPDES permit's WET triggers or limits. This module will discuss Step 4, the statistical analysis of WET test data. In addition, the review of WET test data for Quality Assurance and Quality Control, or QA/QC, will be covered later in this module.



Notes:

The overall objective of this module is to describe the USEPA recommended statistical approaches, which are included as recommendations in the appendices of the USEPA 2002 promulgated WET test methods as guidance for interpreting data. The recommended statistical approaches are used to determine whether observed test organism responses to various effluent concentrations indicate that the effluent is toxic based on test endpoints.



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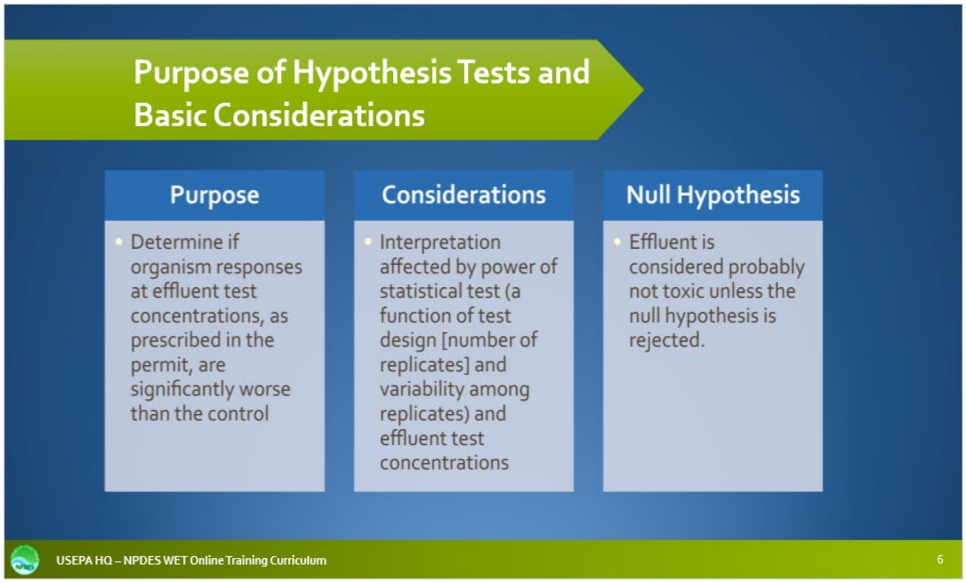
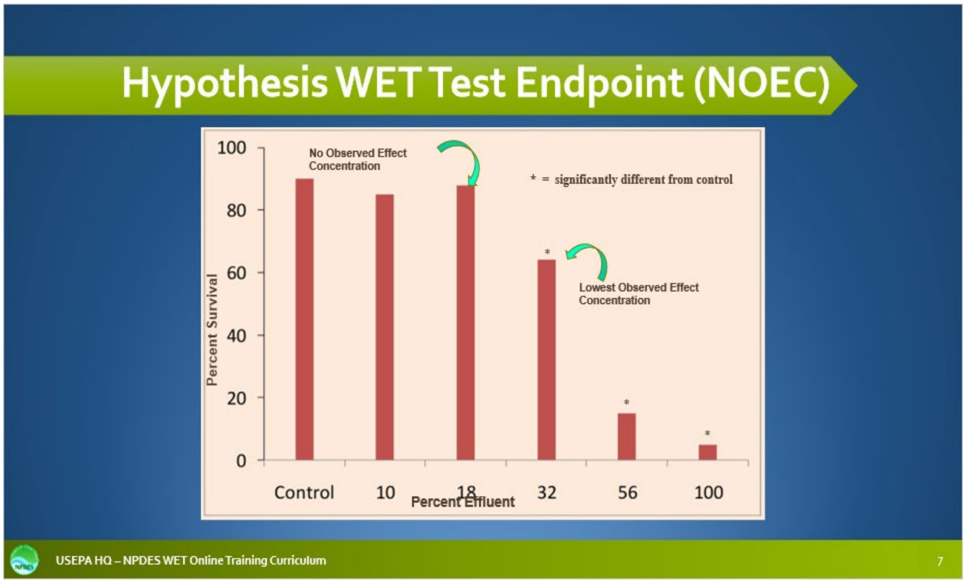
Two different statistical approaches for analyzing valid WET test data are recommended in USEPA's 1991 Technical Support Document for Water Quality-based Toxics Control, commonly referred to as the USEPA TSD. These recommendations are also provided as additional guidance in the appendices of USEPA's WET test methods. Both data interpretation approaches involve the evaluation of the concentration-response pattern observed using valid test data. The two approaches are: hypothesis tests and point estimation. The analysis of WET data using point estimation determines the effluent concentration at which a certain effect occurs, such as a 50% effect on aquatic organism survival. The statistical test endpoints derived to evaluate data using point estimation include the lethal concentration to 50% of the test organisms or LC_{50} for acute WET data; and the EC_{25}, or the 25% effect concentration, or IC_{25}, the 25% inhibition concentration, typically used when evaluating chronic WET test data. In contrast, hypothesis tests evaluate whether the test organism response in a given effluent test concentration is significantly different than in the control treatment. The statistical test endpoints derived from the hypothesis statistical evaluation of data include the no observed adverse effect concentration, or NOAEC, which is the highest effluent test concentration at which there is no adverse effect. The no observed effect concentration, or NOEC, is the highest effluent test concentration at which there is no chronic effect observed. Additionally, when testing ambient or stormwater samples with just a control and a 100% test concentration, a pass/fail result from a t-test may be used.



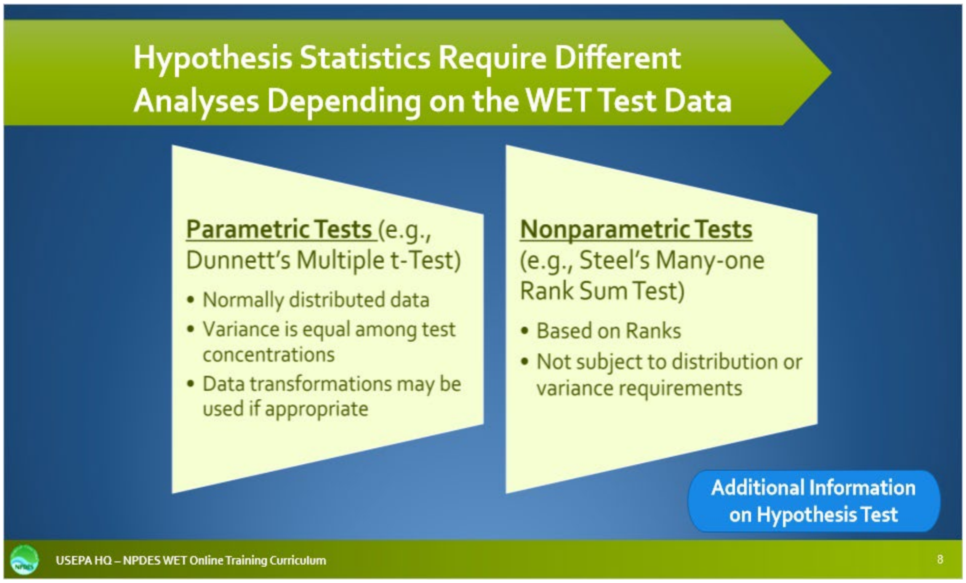
Notes:

When determining a statistically significant test organism response from WET test data using a hypothesis approach, whether it is survival, reproduction, or any other biological endpoint, interpretation is affected by the power of the statistical analysis. The power of the statistical analysis relates to the details of the WET test design, such as the number of test replicates, the number of test organisms in each test replicate, and variability in the test organism response being measured among replicates within a test. The confidence of the result when using a hypothesis approach to analyze data relies on the level of precision among replicates within each effluent test concentration. The more variability that exists among replicates within a given test concentration, the less able you are to tell if the test organism response in that concentration is significantly different from the control treatment. The null hypothesis commonly used when evaluating WET test data using the hypothesis approach is that the effluent is considered not toxic unless the data demonstrates otherwise. With a hypothesis approach, one cannot confirm the null hypothesis, one can only reject or not reject the null hypothesis. This is an important and often misunderstood aspect of hypothesis statistical approaches. If, for example, one uses the NOEC approach to interpret data, and cannot reject the null hypothesis, that there is no difference in organism response between each effluent WET test concentration and the control treatment, then the statistically correct answer in this case is we do not know whether the effluent is toxic or not. We will discuss how this point is addressed later in this module.



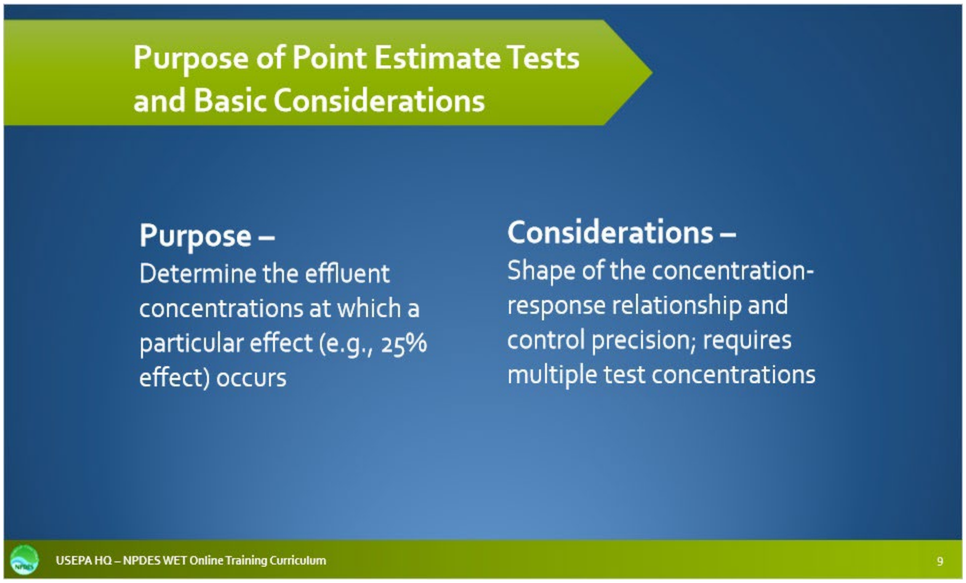
Notes:

In this example, we examine the observed survival response in a WET test. The y-axis shows percent survival, and the x-axis shows effluent test concentrations. Using the hypothesis approach to evaluate these test data, the organism response observed in each effluent test concentration is compared statistically to the organism response observed in the control treatment. The lowest effluent test concentration in which there is a statistically significant difference relative to the control treatment in this example is 32%. As a result, 32% is identified as the lowest observed effect concentration, or LOEC. As can be seen in the graph, all effluent test concentrations from 32% up to 100% indicate a statistically significant difference relative to the control treatment. Note that there is no statistically significant difference relative to the controls in the 10% or 18% effluent test concentrations. The NOEC is the highest effluent test concentration in which the organism response is not statistically different from the control treatment. Therefore, in this example, 18% effluent is identified as the NOEC.



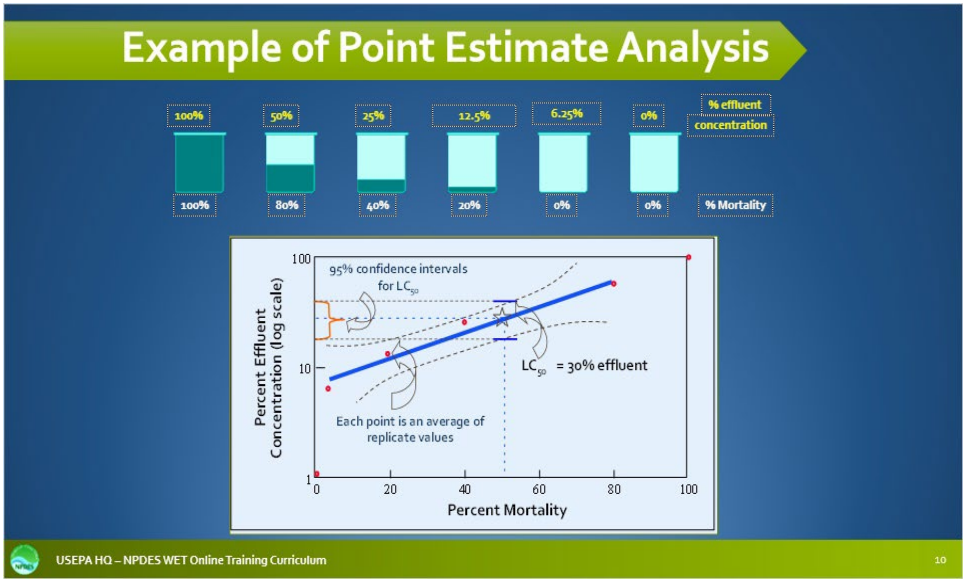
Notes:

There are different types of statistical analyses that may be used with the hypothesis approach depending on whether the data meet certain statistical assumptions. Parametric tests can be used to analyze the data if two assumptions are met: 1) the valid test data are normally distributed, and 2) the data have similar variance among the replicates. An example of a parametric hypothesis analysis would be Dunnett's multiple t-Test. When using parametric analyses, data transformation may be appropriate in some cases. If either one of the statistical assumptions above are not met, then non-parametric statistical analysis, such as Steel's Many-one Rank Test, are used to evaluate data using the hypothesis approach. Non-parametric statistical analysis approaches tend to be more conservative than parametric statistical analyses. This means that a greater difference in the test organism response between effluent test concentrations and the control treatment are needed to indicate a statistically significant difference. USEPA's WET test methods provide flow-charts that highlight the recommended decision process to use when determining which statistical analysis, parametric or non-parametric, to use. There are software packages that can be purchased for running these statistical analyses. Also, USEPA Headquarters' NPDES website provides a publicly available Excel-based statistical evaluation spreadsheet that can be downloaded for use by permitting authorities and the public. It is based on USEPA's statistical analysis decision tree, which selects the appropriate recommended statistical analysis approach to use.



Notes:

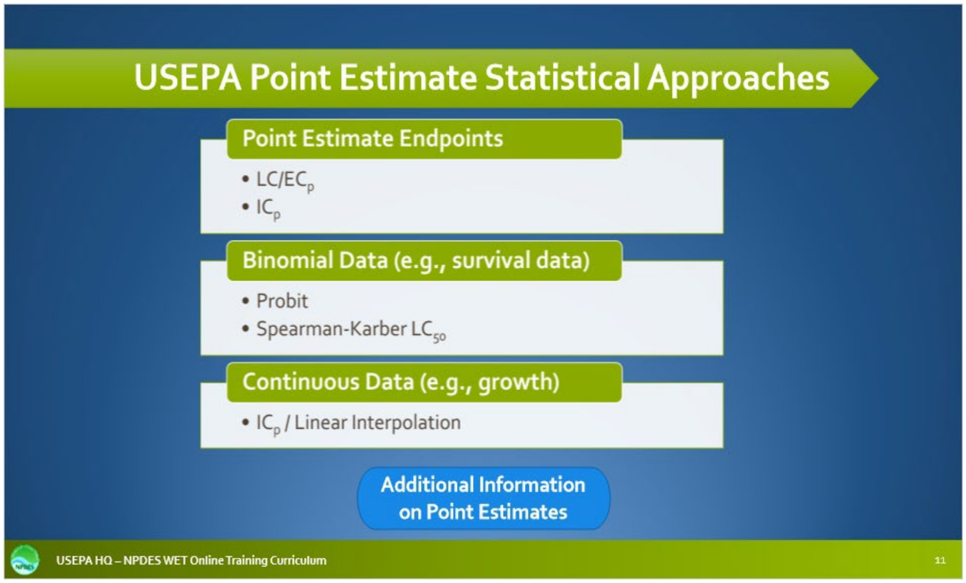
One of the statistical approaches for evaluating valid WET test data recommended in the USEPA test methods manuals is point estimation. As we indicated earlier in this presentation, the point estimate approach determines the effluent concentration at which a particular measured effect occurs. For example, if the desired endpoint is the LC_{50}, using the point estimation approach, the effluent concentration that should result in a 50% effect on organism survival is extrapolated from the observations made in all the effluent concentrations tested. The identified point estimate effluent concentration is then compared to the permittee's in-stream waste concentration, or IWC, to determine whether or not the effluent sample is toxic. Control precision is important in the point estimate analysis approach. The point estimation approach also requires that multiple effluent test concentrations as well as a control treatment be used in order to conduct the statistical analysis.



Notes:

Now let's look at an example of how the point estimation approach works. In the top part of the example, the response observed in each of the effluent test concentrations and the control treatment is illustrated. The effluent test concentrations are a control treatment, or 0% effluent, and 6.25%, 12.5%, 25%, 50%, and 100% effluent. Below the beakers is the observed percent mortality observed in each WET test concentration. On the graph, the concentrations from 0 to 100% effluent have been plotted on a log scale on the y-axis with corresponding percent mortality on the x-axis. These data are represented on a log scale so the data points can be graphed in a linear fashion. If the data were not represented on a log scale, they would appear as a curve. Point estimation of WET data, such as percent mortality, can be readily analyzed using a variety of statistical approaches if the data are presented as a straight line.

The test organism response in the control treatment, or 0% effluent, was 0% mortality, while there was 100% mortality observed in the 100% effluent test concentration. The dotted lines within the graph indicate the 50% mortality threshold, which when extrapolated from the line to the y-axis is approximately 30% effluent. USEPA recommends statistical analysis approaches that guide the user to the correct statistics for deriving an accurate point estimate, in this case the LC_{50}. Using the point estimate analysis provides 95% confidence limits around the point estimate endpoint. The 95% confidence intervals in this example are relatively small, 20% to 40%, indicating reasonable confidence in the LC_{50} estimate for this WET test. This analysis indicates that we are 95% confident that the LC_{50} for organism mortality in this test lies between 20% and 40% effluent.

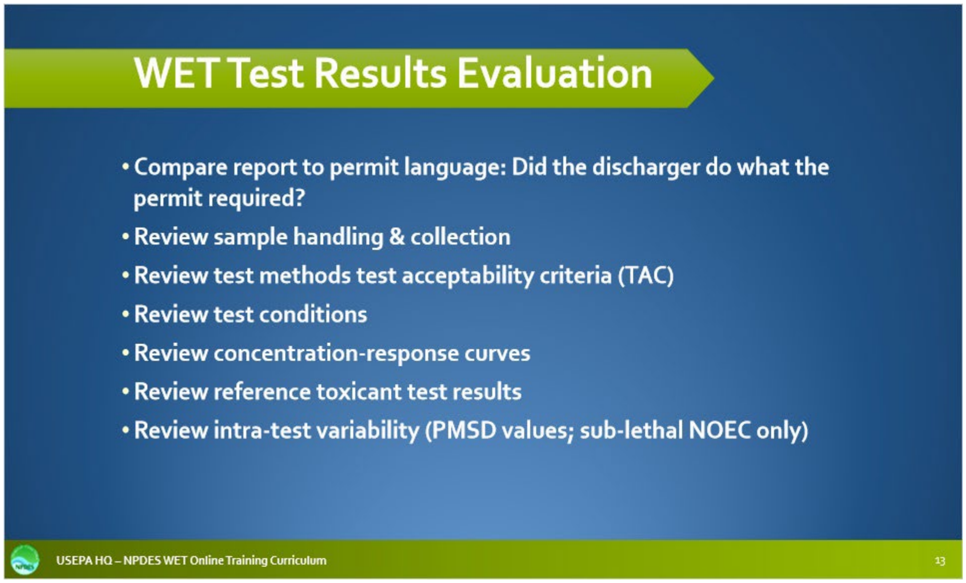


Notes:

USEPA's point-estimate statistical approach results in either an LC_p or EC_p, when interpreting survival data for acute WET testing this is typically an EC_{50} or LC_{50}. Chronic point estimate test endpoints are expressed as IC_p, with the most common being the IC_{25}, or 25% inhibition concentration. There are multiple ways that a point estimate can be calculated, which depend on the data that are being evaluated. Binomial data, which are typically applicable to percentage data, such as percent organism survival or percent normal development, may be evaluated using statistical approaches such as the Probit or Spearman-Karber analysis. These approaches are used to generate a point estimate depending on the concentration-response data. Continuous endpoints are not yes or no data, they can be any number between certain boundaries, and are evaluated using linear interpolation to generate the IC_p. Some examples are fish growth or water flea reproduction.

Notes:

So far in this module we have discussed two types of statistical analysis approaches used in evaluating WET test data: hypothesis statistics and point estimation. For the remainder of this module, we are going to examine some of the steps that should be used when reviewing WET test data.

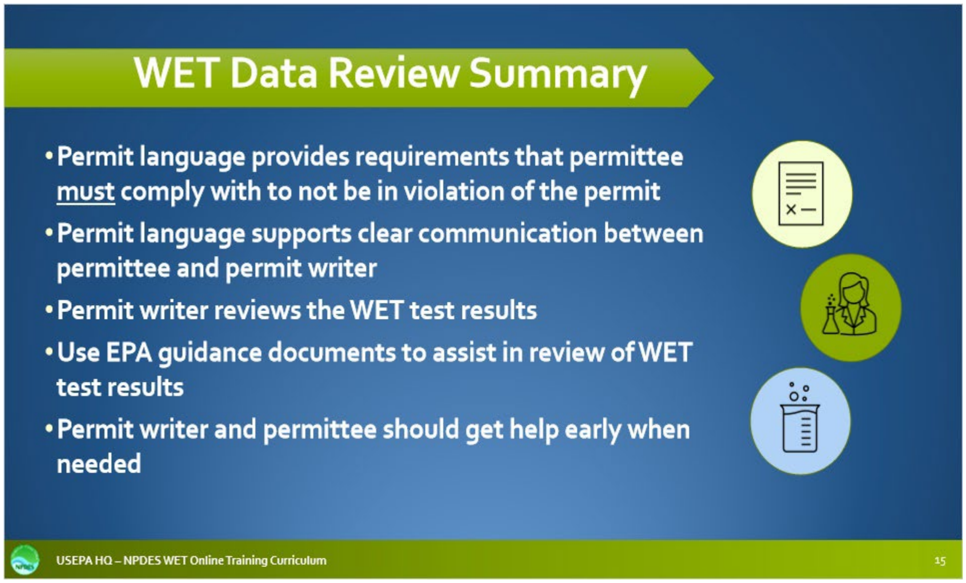


Notes:

There are many important factors that need to be considered in evaluating WET test results. The first step is verifying that the permit conditions, including monitoring triggers, WET limits, and specified test requirements in the NPDES permit are adhered to by the permittee and their laboratories. For example, did the permittee and their laboratory do what was required in the permit in terms of the specified WET test conditions, such as the required USEPA test method, test species, and the specified dilution series? After it has been established that the permittee properly adhered to the WET testing specifications required in the permit, a review of the USEPA WET test method requirements and recommendations should be checked. This would include reviewing sample handling and collection records, reference toxicant results, and verifying compliance with USEPA Test Acceptability Criteria. In addition, a review of concentration-response patterns of test results and intra-test variability assessments are also very important when evaluating WET test results and will be reviewed in more detail in another module. Adequate quality control throughout effluent sampling, the WET test procedures, and data analysis are very important to ensure that the quality of data and an accurate interpretation of results are used when implementing NPDES permit WET requirements and making NPDES permit decisions.

Notes:

After reviewing WET test results, if data have met all the required quality control requirements and are considered valid, the reported test endpoint is compared to the permit limit or trigger to decide whether follow-up permit actions are necessary. Possible follow-up actions may include: maintaining a historical record of WET test results to be used for future reasonable potential analyses, maintaining a record of test and permit requirement violations, providing direction to the permittee when a violation has been determined, evaluating whether a permittee needs to consider conducting a Toxicity Reduction Evaluation/Toxicity Identification Evaluation, and, when appropriate, administering applicable NPDES permit enforcement evaluations, next steps, or actions.



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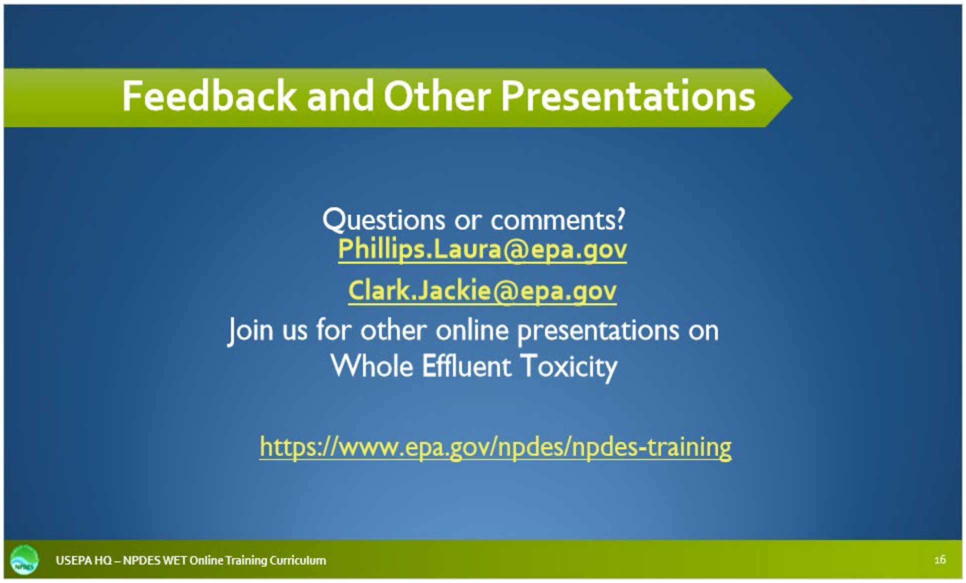
In summary, the NPDES permit language needs to comprehensively and clearly indicate the WET requirements a permittee must comply with in regard to testing and data analysis. The NPDES permit language should provide clear and enforceable written permit communication between the permit writer and the permittee. Once the permittee conducts the WET tests as required under the NPDES permit, including the statistical evaluation of the data and the calculation of the required WET test endpoints, the permit writer should review the results and determine compliance with the NPDES permit. The permit writer and the permittee should use USEPA guidance to assist in the analysis and review of the results generated under the permit. If needed, the permit writer and permittee should seek help early and often to avoid confusion surrounding the NPDES permit requirements and generated WET test results.



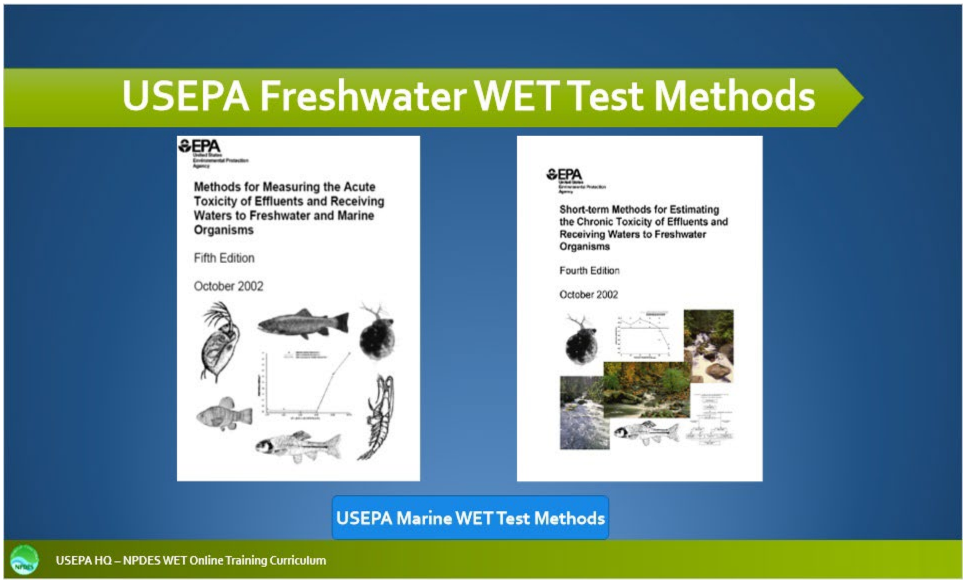
Notes:

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.

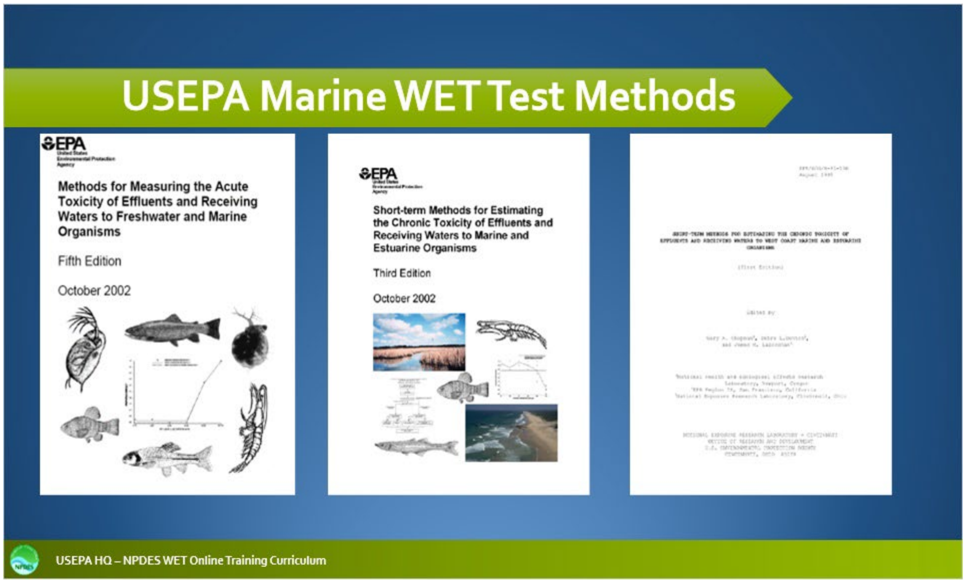
Remember, you will find all of the USEPA's NPDES WET online training presentations, under the USEPA's NPDES training section found on the Office of Wastewater Management's NPDES website.

See you next time!



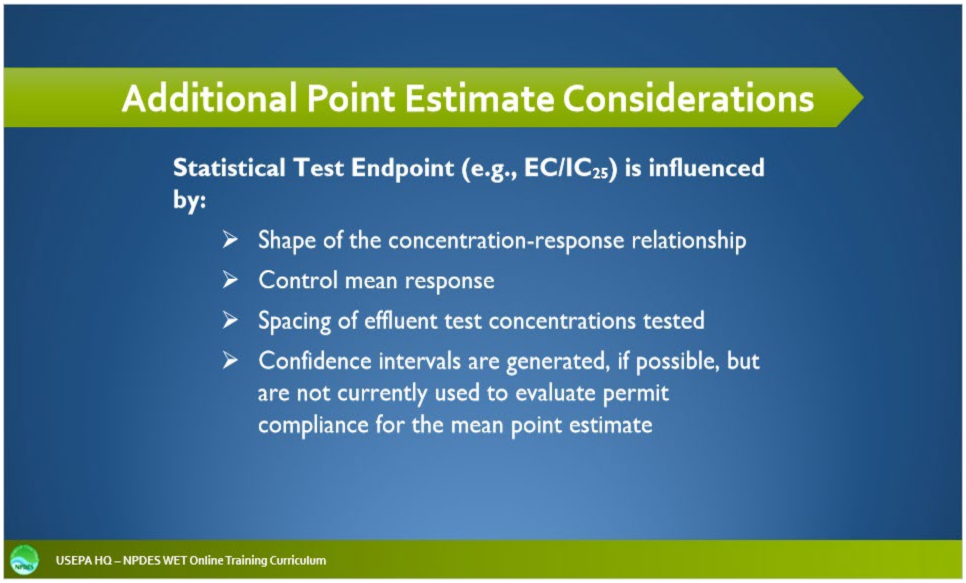
Notes:

The 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.



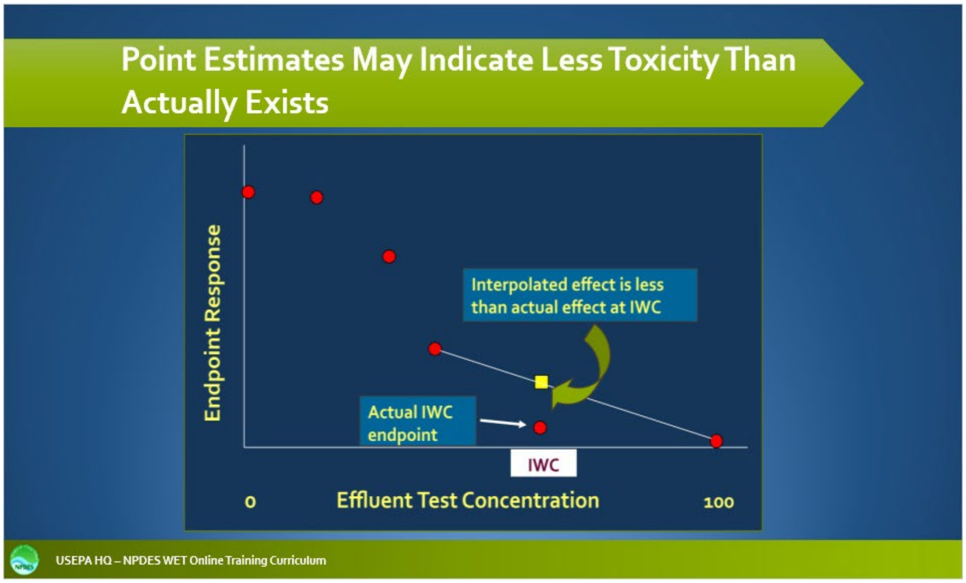
Notes:

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.



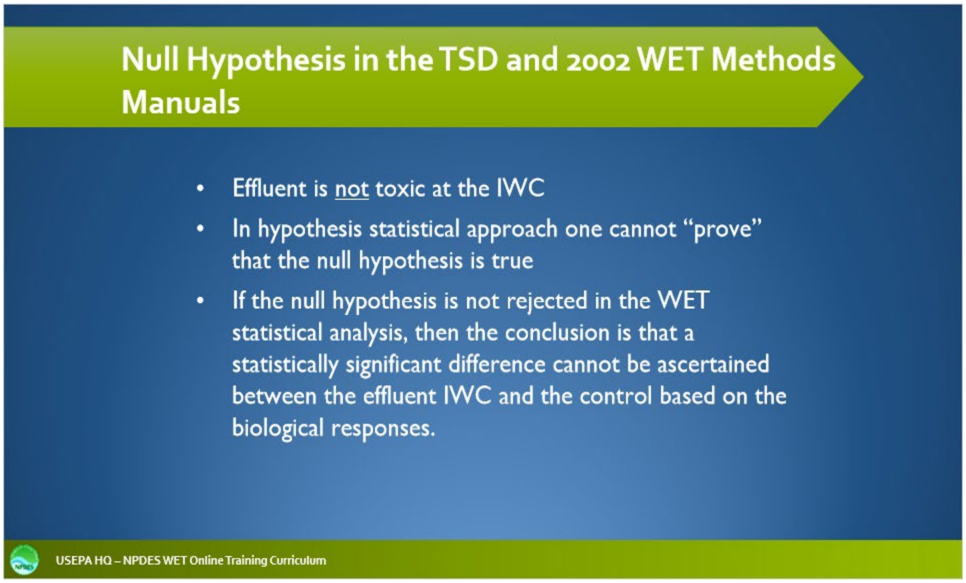
Notes:

Some of the main considerations when evaluating WET test results using the point estimate statistical approach include: the shape of the concentration-response relationship or CRR, the mean response observed in the control concentration, the spacing of effluent test concentrations, and the generated confidence intervals. The shape of the CRR relates to the reliability of the WET statistical test endpoint that is discussed in the WET Testing Data Interpretation and Evaluation module. If the mean response in the controls is well below the laboratory's normal range of control performance, then point estimate statistical test endpoints, (e.g., LC_{50} or IC_{25}), may be influenced. The test concentration series used when conducting a WET test can influence the point estimate statistical result if the test concentrations are not spaced properly. While 95% confidence intervals of the point estimate (e.g., IC_{25}) are typically generated under the point estimate analysis approach, the confidence intervals are not currently required in permit compliance but could be used by states if included as part of the permit or water quality standards.



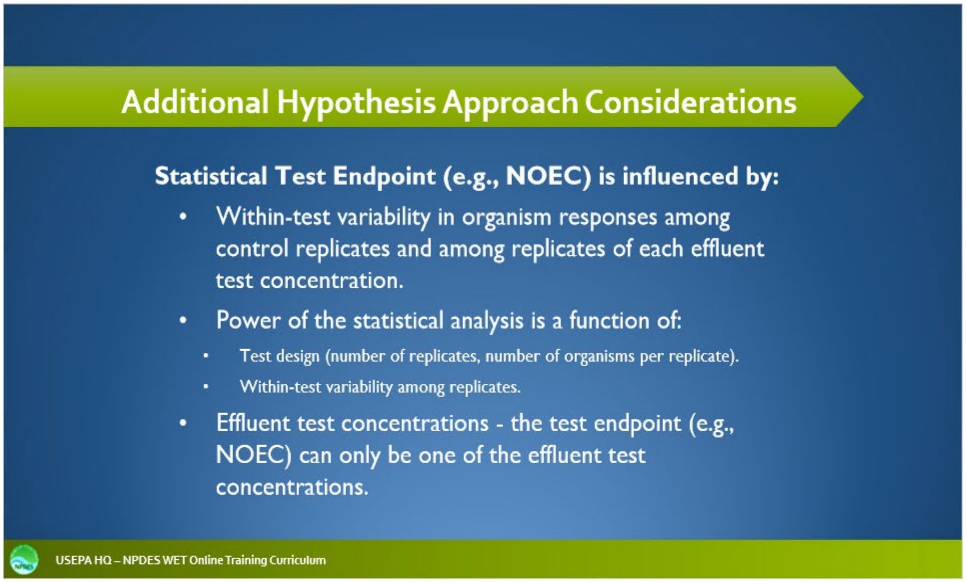
Notes:

The point estimate statistical approach in USEPA's 2002 short-term chronic WET test methods manuals, describes how to calculate an IC_{25} using linear interpolation. This statistical approach calculates the IC_{25} by drawing a line connecting the two effluent test concentrations results recorded that most closely bracket a 25% biological reduction effect (survival, growth, or reproduction) due to exposure to the effluent as compared to the controls. While this approach has the advantage of being relatively easy to calculate, it may indicate a different measured effect due to the effluent's toxic impact at the IWC than was observed in the toxicity test, depending on the actual test concentration response relationship or CRR.



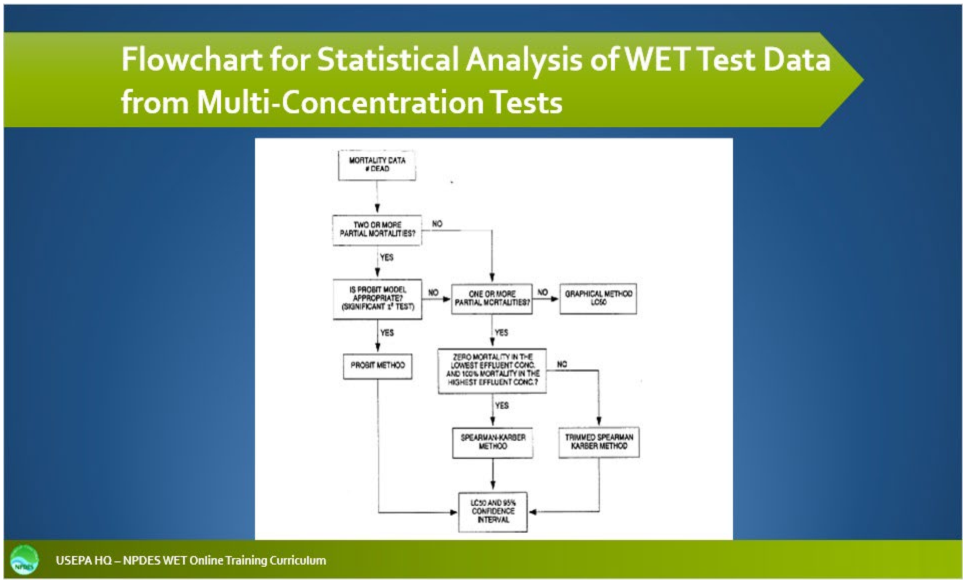
Notes:

One of the main considerations when evaluating WET test results using the hypothesis statistical approach described in USEPA's 2002 WET test methods manuals centers around the null hypothesis selected. In the hypothesis statistical approach, the analysis tests whether the null hypothesis should be rejected or not. If the statistical analysis does not reject the null hypothesis, then the correct statistical interpretation of the toxicity test results is that the null hypothesis may or may not be true. In other words, the null hypothesis cannot be proven. The null hypothesis in the WET test methods manuals is that the effluent is **not** toxic at the IWC. If this null hypothesis approach is rejected then a statistically significant difference has been ascertained between the biological responses observed in the controls and those observed in the IWC and, therefore, the effluent is declared toxic. However, if the null hypothesis is not rejected, this means that a statistically significant difference could not be ascertained between the control and the IWC and, therefore, the effluent may or may not be toxic at the IWC.



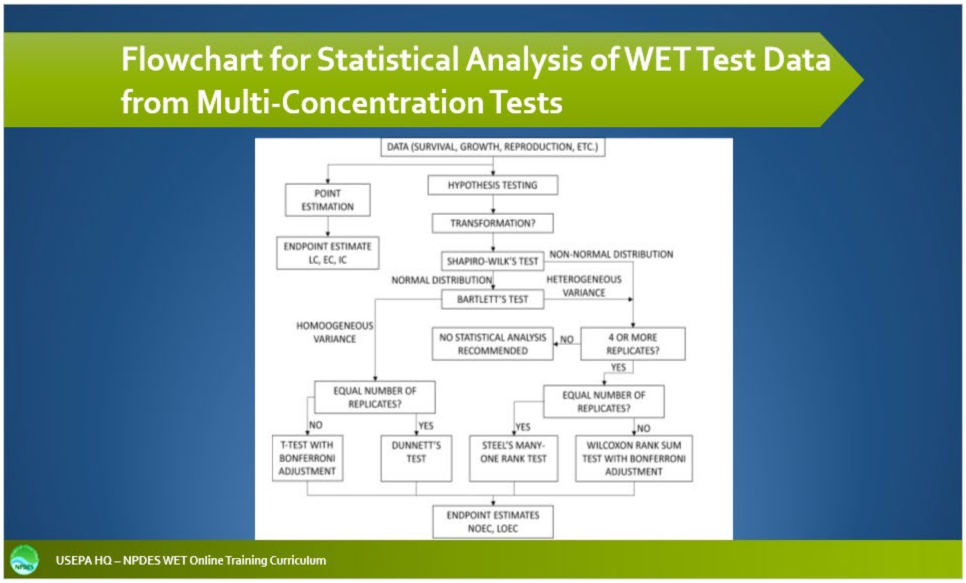
Notes:

Additional considerations with respect to the hypothesis statistical approach described in USEPA's 2002 WET test methods manuals include the influence that within-test variability, test power, and the effluent test concentration series selected have on the statistical test endpoints. The within-test variability surrounding the organism response among the control replicates and the replicates in each effluent test concentration can have a significant effect on the ability of the hypothesis test to determine a statistically significant difference. The power of the statistical analysis is a function of the test design, including the number of replicates per test concentration and the number of organisms per replicate, as well as the within-test variability among replicates of each test concentration. The test design, including the effluent test concentration series selected, can be critical because under the hypothesis statistical approach the statistical test endpoint described in USEPA's 2002 WET test methods manuals (e.g., the No Observed Effect Concentration or NOEC) can only be one of the effluent test concentrations used.



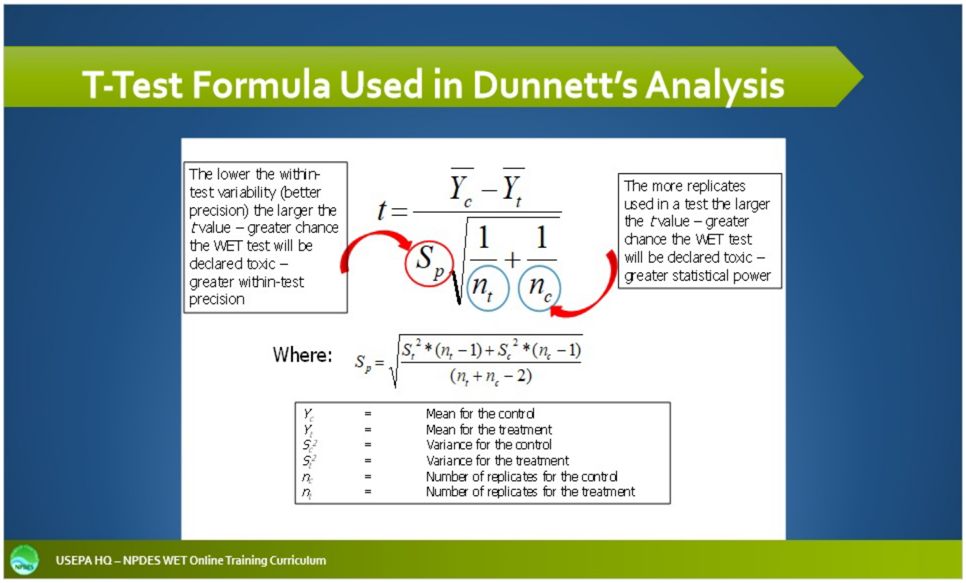
Notes:

In USEPA's 2002 WET test methods manuals, USEPA includes flowcharts for the statistical analysis of valid WET test data using either the point estimate or hypothesis statistical approach. The flowchart for the acute point estimate (e.g., LC_{50}) includes several alternative analyses depending on the test data. For IC_{25} and short-term chronic tests, the flow chart includes recommendations for data smoothing and linear interpolation.



Notes:

The flowchart for the hypothesis statistical approach outlines steps for which data analyses are appropriate to use to derive the NOEC. The recommended steps include determining whether data transformation is advisable, whether the test data are normally distributed, if the variance is homogeneous among the controls and effluent test concentrations, and whether an equal number of replicates are available in each effluent test concentration.



Notes:

In USEPA's 2002 WET test methods manuals, Dunnett's statistical analysis is described using the hypothesis statistical approach when the data are normally distributed, the variance is homogeneous, and there are an equal number of replicates among the test concentrations. Examining the t-test formula used in Dunnett's analysis shows how the within-test variability and test design, in this case the number of replicates, can influence the results of the data analysis. As noted in this slide, the lower the within-test variability the larger the t-value, which increases the chance that the sample will be significantly different from the control and, therefore, declared toxic. This type of test, a t-test, is used especially for stormwater and ambient samples, as well as for effluents. The same is true with respect to the number of test replicates in a test. For example, an increase in the number of test replicates or replication often results in a larger t-value, which can increase the chances that the biological response for the tested sample will be significantly different from the control and, therefore, declared toxic.