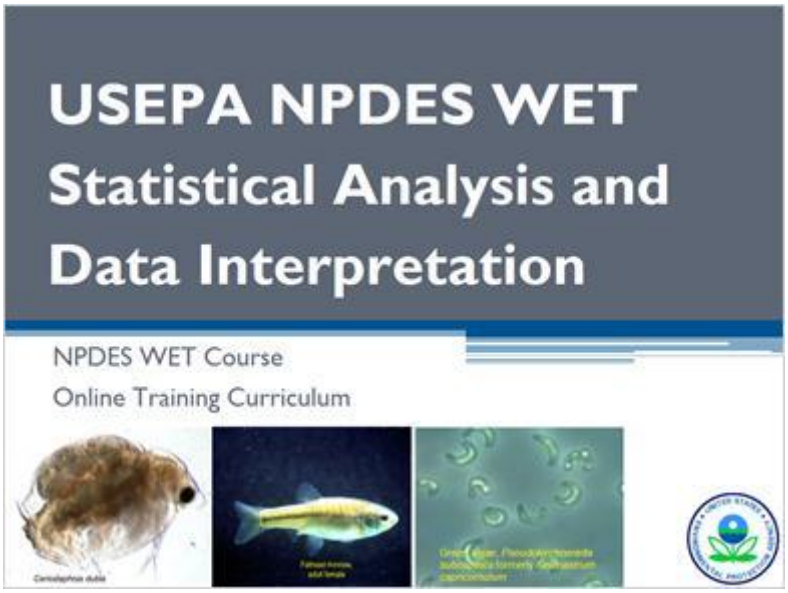
Module 4: USEPA NPDES WET Statistical Analysis & Data Interpretation



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 Statistical Analysis and Data Interpretation.

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, or WET, in the NPDES permits program.

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

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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 Jerry Diamond, USEPA HQ 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 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 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.

Alright. Let me turn this over to Jerry and we will take a look at USEPA WET statistical analysis and data interpretation.

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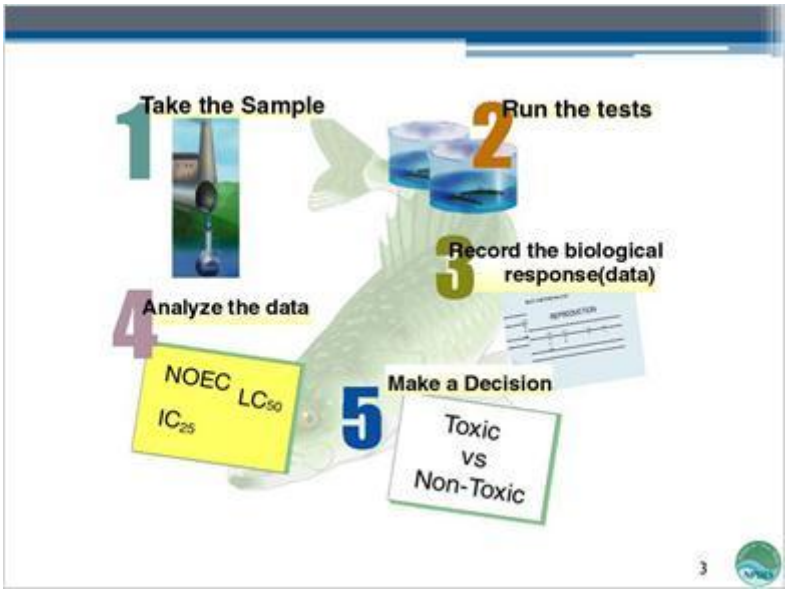

Notes:

Thanks Laura. The first step during the process of conducting Whole Effluent Toxicity 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 methods. Third, the organism responses, including mortality, and chronic sublethal endpoints according to each test method are recorded. Fourth, valid WET test data are analyzed using recommended statistical approaches that are used for the fifth or final step to determine whether the permitted effluent is in compliance with a NPDES permit's WET triggers or limits. This module will discuss the analysis of WET test data and provide a detailed explanation of the necessary steps when evaluating whether a permitted effluent is toxic or not with respect to state water quality standards. In addition, the review of WET test data for Quality Assurance and Quality Control will be covered later in this module.

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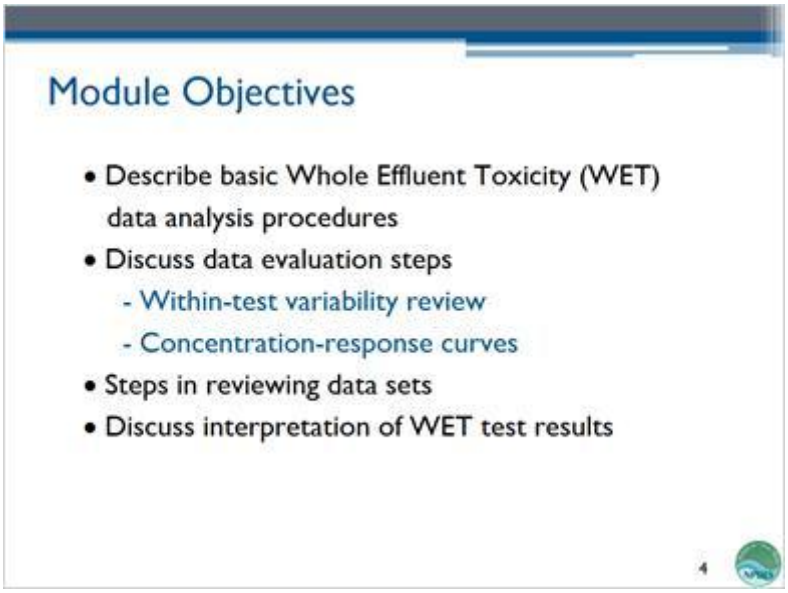

Notes:

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. Other recommended data evaluation steps, provided in the USEPA WET test methods, will be discussed in this module including: the review of within-test variability evaluated through the use of the Percent Minimum Significant Difference, or PMSD, and the evaluation of WET test concentration-response patterns.

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Notes:

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 a point estimation technique determines the effluent concentration at which a certain effect occurs, such as a 50% effect on aquatic organism survival. The statistical 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, which are typically used when evaluating chronic WET test data. In contrast, hypothesis statistical approaches evaluate whether the test organism response in a given effluent concentration is significantly different than in the control treatment. The statistical 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.

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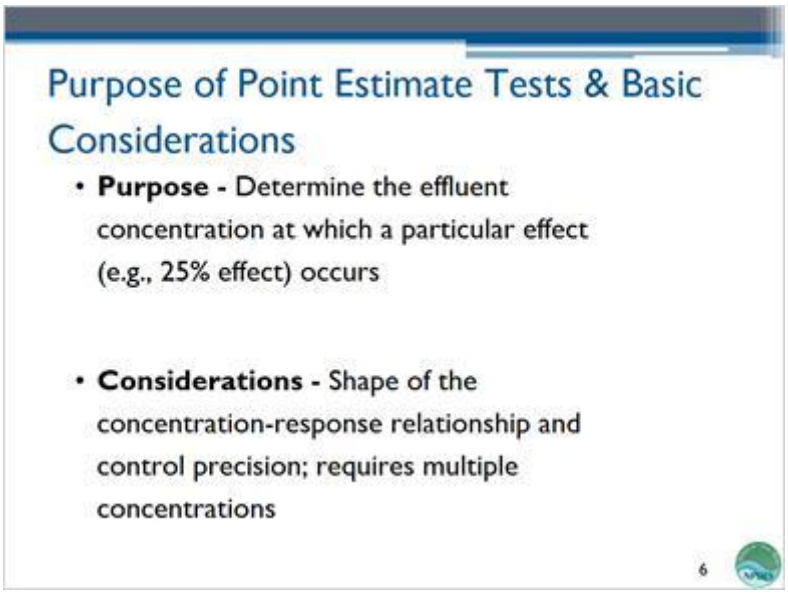

Notes:

One of the recommended statistical approaches for evaluating valid WET test data recommended in the USEPA 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 of the effluent concentrations tested. The identified point estimate effluent concentration is then compared to the permittee's IWC to determine whether or not the effluent sample is toxic. Control precision is important in the point estimate analysis approach. Also, the point estimation approach requires that multiple effluent test concentrations as well as a control treatment be used in order to conduct the statistical analysis.

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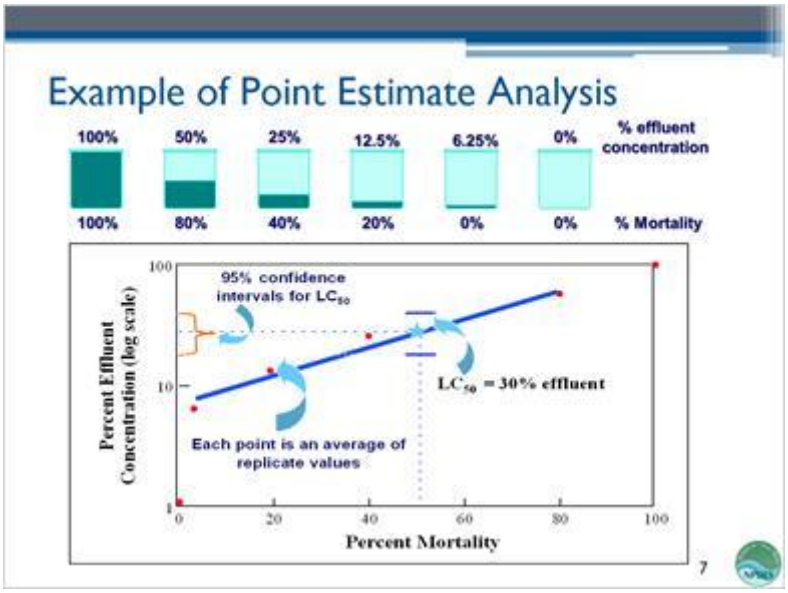

Notes:

Now let's take a 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 that the data points can be graphed in a linear fashion. If the data were not represented on a log scale, then 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 - 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.

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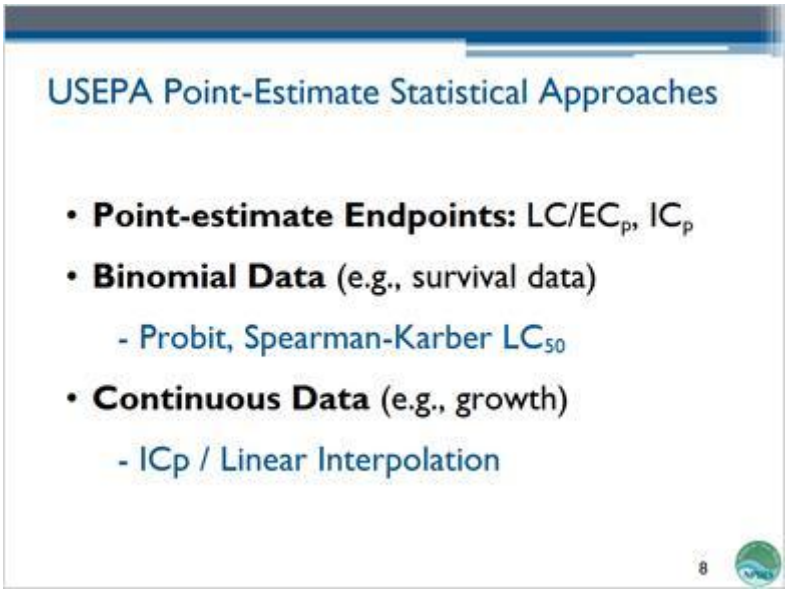

Notes:

USEPA's recommended 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}), while chronic point-estimate 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 *Ceriodaphnia* reproduction.

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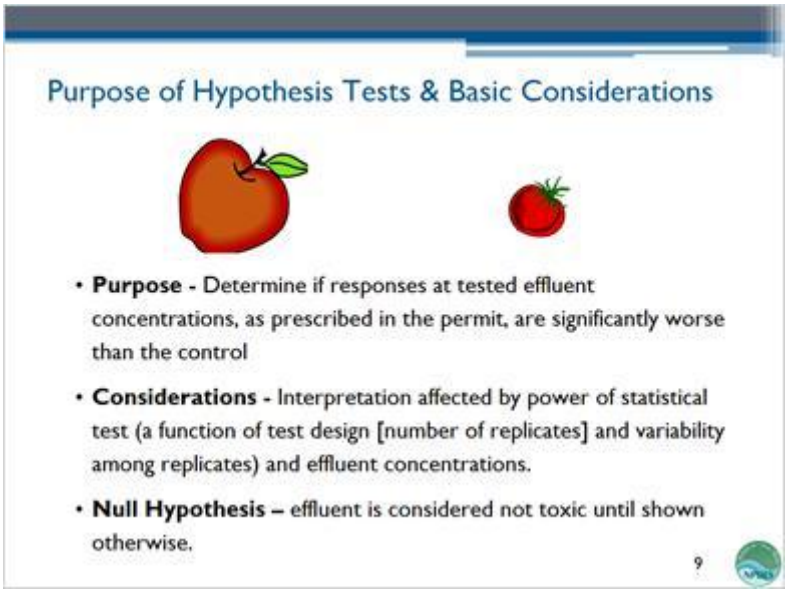

Notes:

When determining a statistically significant test organism response from WET test data using a hypothesis approach, whether it is survival, reproduction, or any other 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 concentration. The more variability that exists among replicates within a given 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 the null hypothesis is that there is no difference in organism response

between each effluent WET test concentration and the control treatment, then if the statistical analysis cannot reject this null hypothesis, 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.

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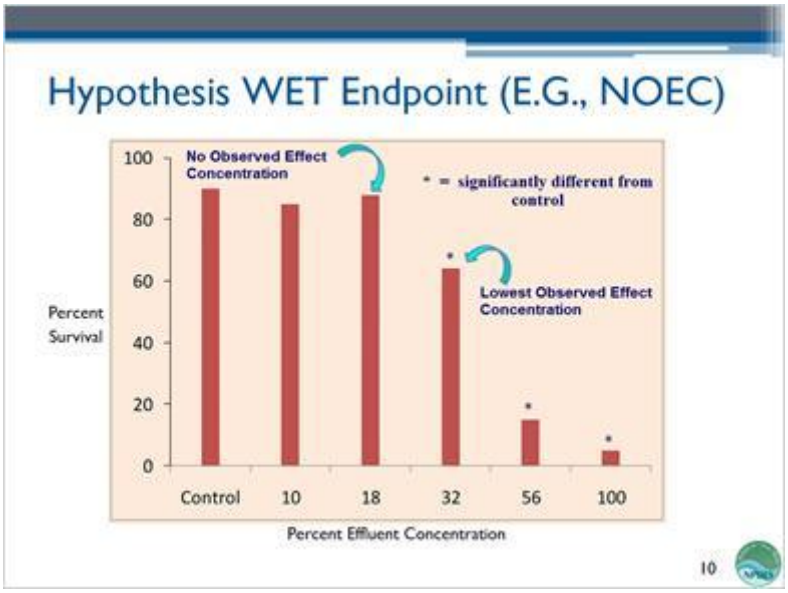

Notes:

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%. 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 concentration tested in which the organism response is not statistically different from the control treatment. Therefore, in this example, 18% effluent is identified as the NOEC concentration.

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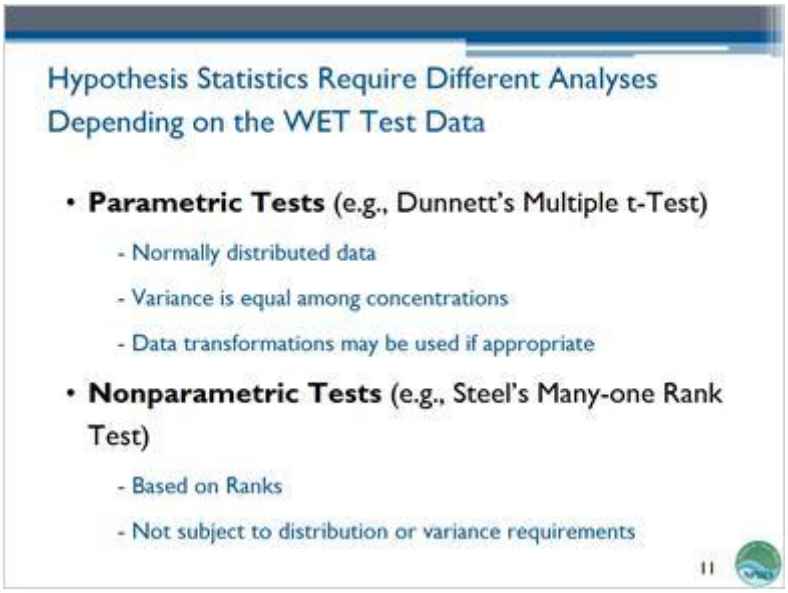

Notes:

There are different types of statistical analyses that may be used with the hypothesis approach depending on whether the data meet certain statistical assumptions. If the valid test data are normally distributed and have similar variance among the replicates, then parametric tests can be used to analyze the data. 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 publically available Excel-based statistical evaluation spreadsheet that can be downloaded for use by USEPA

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Regions, NPDES states, and the public. It is based on USEPA's statistical analysis decision tree, which selects the appropriate recommended statistical analysis approach to use.

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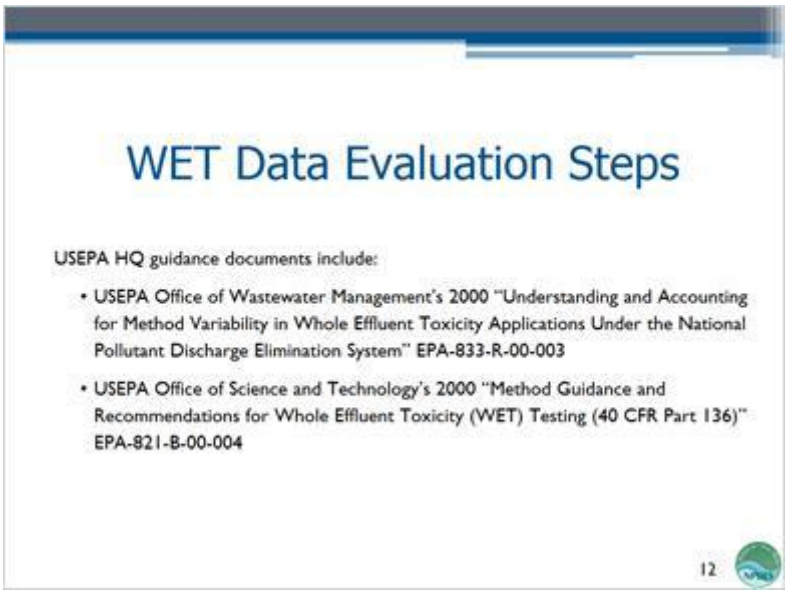

Notes:

Over the next couple of slides, we are going to turn our attention to the steps in evaluating WET test data based on USEPA guidance documents. The USEPA Headquarters guidance documents include: the Office of Wastewater Management's 2000 "Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System" this is EPA document number 833-R-00-003, and the Office of Science and Technology's 2000 "Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136)" (EPA document number 821-B-00-004). Both of these USEPA guidance documents are available in the resources tab at the top of the module and are also available on the respective USEPA Headquarters offices' websites.

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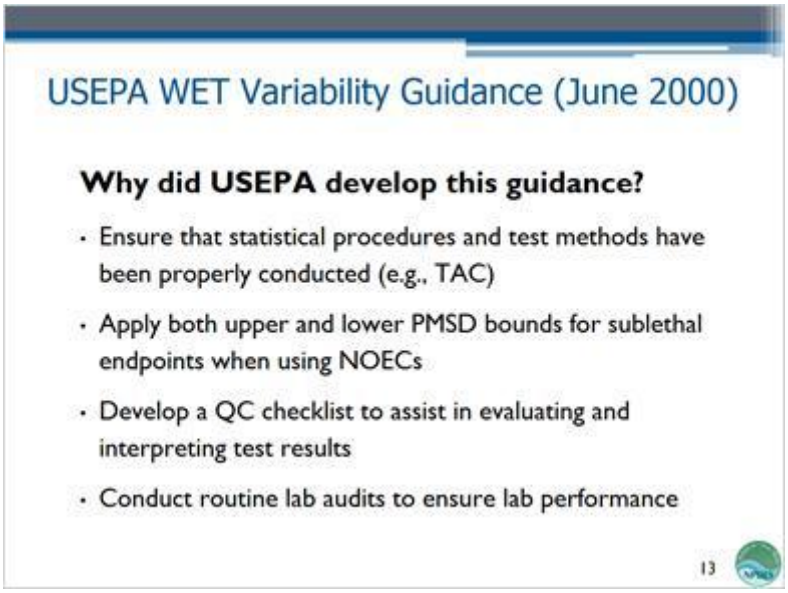

Notes:

In June of 2000, USEPA's Water Permits Division in the Office of Wastewater Management released a guidance document entitled, "Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications under the National Pollutant Discharge Elimination System," hereafter referred to as USEPA 2000 WET variability guidance. This guidance was developed after USEPA had evaluated the quality of WET test results generated throughout the U.S. to help permittees understand how to increase the quality of data they were generating and thereby WET test performance. Another important reason that USEPA released this NPDES WET guidance was to ensure that the statistical analysis approaches and USEPA methods used were properly conducted. USEPA included recommended upper and lower Percent Minimum Significant Difference, or PMSD, bounds for each USEPA chronic WET test method endpoint (including sublethal endpoints) to provide guidance on acceptable within-test precision for these methods when analyzed using the NOEC approach. This ensures that permitting decisions regarding whether the effluent is toxic or not with respect to state aquatic life protection criteria and WET water quality standards can be made with confidence. This USEPA guidance also includes a quality control checklist to assist in the evaluation and interpretation of valid

results. In addition, procedures are included on how to appropriately conduct laboratory audits to help ensure that laboratory performance meets USEPA WET test method Test Acceptability Criteria and PMSD requirements. This guidance includes a list of suggested questions that permittees should ask their laboratory to help ensure that high quality, valid data are being generated for their effluent samples submitted under NPDES permit applications and for WET permit limit compliance.

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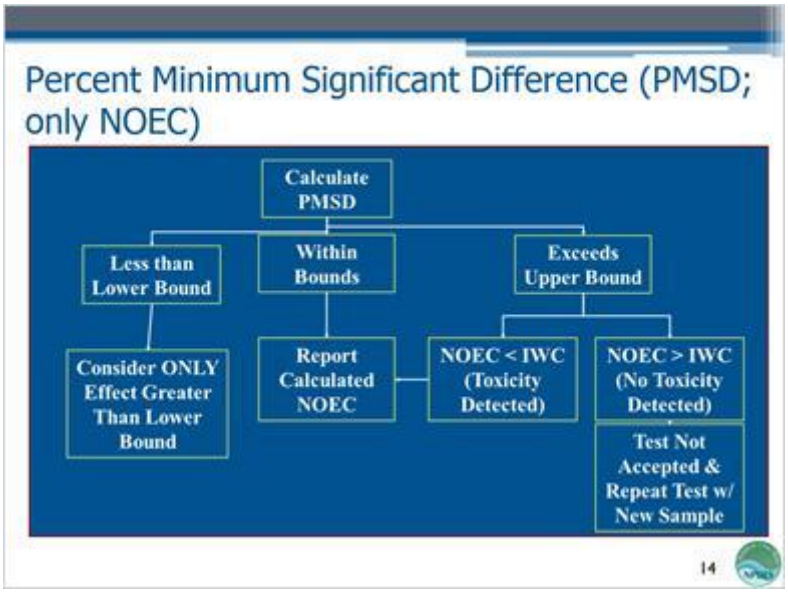

Notes:

The USEPA decision tree presented here was developed as part of USEPA 2000 WET variability guidance. It helps permittees and permit writers determine whether the reported NOEC and LOEC endpoints submitted are statistically robust so that a permitting decision can be made with confidence as to whether the effluent is declared toxic or not. The PMSD determination is only applied when using the hypothesis approach, as in the derivation of an NOEC. As shown in the decision tree, the results of the PMSD evaluation will either be less than the lower bound, within the bounds, or exceed the upper bound of acceptable difference for each respective USEPA WET test method type and endpoint.

If the calculated PMSD is less than the lower bound for a given endpoint, then the USEPA 2000 WET variability guidance indicates that only effects greater than the lower bound should be considered. In this case, the PMSD indicates that the data are unusually precise such that a very small effect can be detected using the data. When the PMSD is within the lower and upper bounds, then the data are considered statistically robust and the calculated NOEC should be reported.

When the calculated PMSD exceeds the upper bound, there are two potential

outcomes. If the calculated NOEC is less than the IWC, then toxicity has been detected despite the high within-test variability, and the NOEC should be reported with the decision that the effluent is toxic. In cases where the PMSD is greater than the upper bound and the reported NOEC is greater than the IWC, this indicates that the variability of the data is so large that it could not be determined whether the effect observed at the IWC was significantly different from the control response. This result would be considered invalid and a new WET test using a fresh effluent sample should be conducted.

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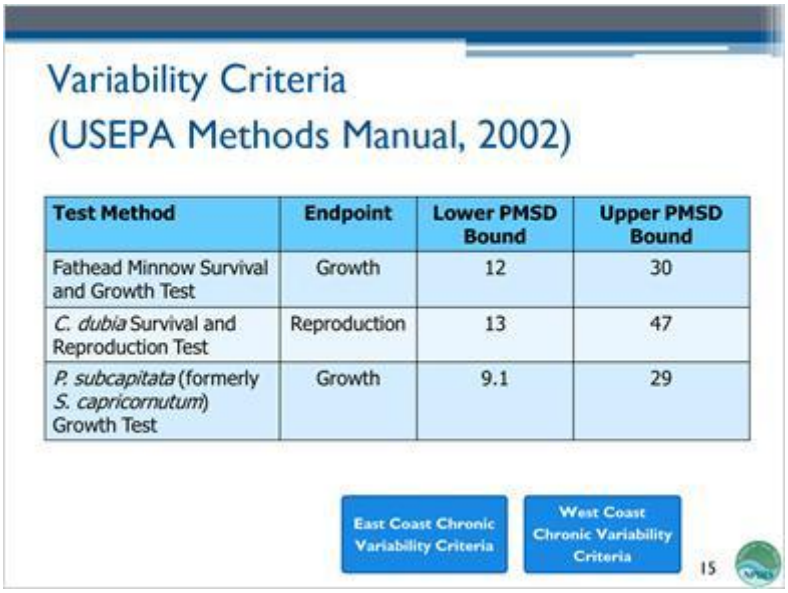

Notes:

Using previous WET test data, USEPA developed appropriate lower and upper PMSD bounds for each type of USEPA chronic test method and endpoint. The lower and upper PMSD bounds for the freshwater fathead minnow (*Pimephales promelas*) chronic sublethal endpoint of growth are 12% and 30%, respectively. The freshwater water flea (*Ceriodaphnia dubia)* chronic WET test method has lower and upper PMSD sublethal reproduction endpoint bounds of 13% and 47%, respectively. The chronic sublethal endpoint of cell density measured in the freshwater algae (*Pseudokirchneriella subcapitata)* chronic WET test has lower and upper PMSD bounds of 9.1% and 29%, respectively.

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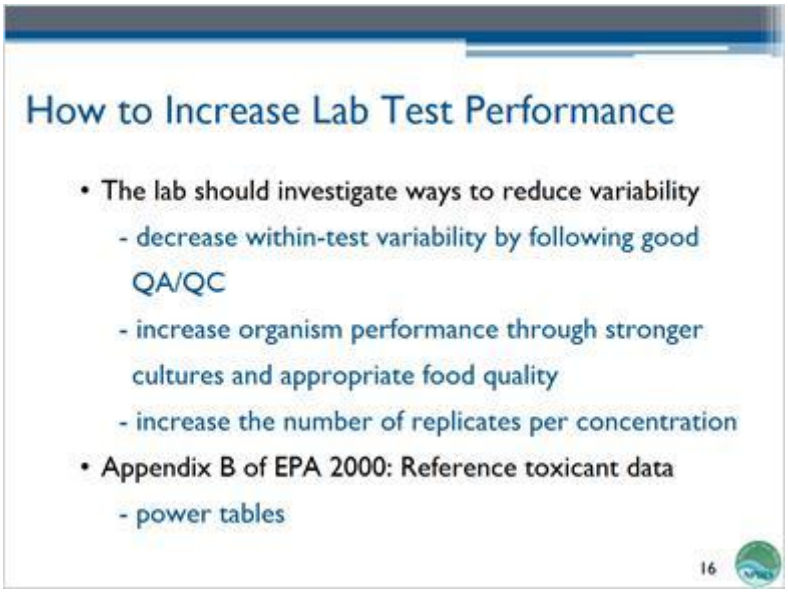

Notes:

If a laboratory is having trouble meeting the USEPA PMSD WET test method requirement, or frequently experiencing high control variability within a test, or high variability in a given endpoint between reference toxicant tests, USEPA's WET 2000 variability guidance discusses ways that laboratories can: reduce their with-in test variability due to laboratory performance, develop and implement a rigorous QA/QC program, increase test organism performance, use test organism food of the appropriate quality, and, if need be, increase the number of test replicates for each effluent concentration and control treatments within a WET test. Remember that the number of replicates per test concentration given in the USEPA WET test methods is a required minimum number. This means that a laboratory could increase the number of replicates to reduce within test variability, and thereby increase performance and resulting data quality. Other recommendations provided in USEPA's 2000 WET variability guidance include an appendix that discusses appropriate reference toxicants and reference toxicant testing procedures, as well as a system that laboratories can use to track endpoint-specific Coefficients of Variation (CV). The CV should be reported as part of the control chart developed for each species tested. This appendix also offers

guidance on the range of CVs that should be observed for each USEPA WET test species and endpoint.

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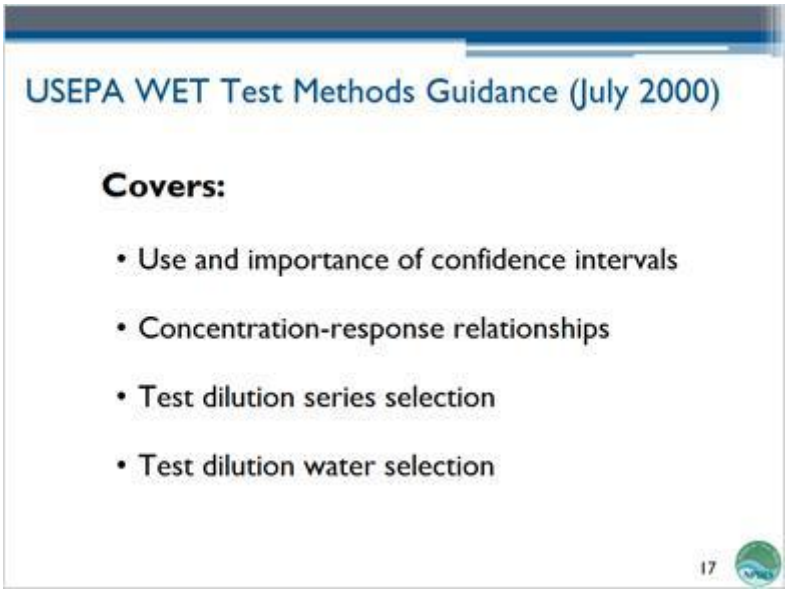

Notes:

USEPA's Office of Science and Technology's Engineering and Analysis Division's 2000 Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136), hereafter referred to as USEPA 2000 WET method guidance, is another USEPA guidance document that provides useful information for permittees and laboratories regarding WET data interpretation. This guidance discusses the importance of the confidence intervals when interpreting point estimate endpoints and how to properly apply confidence intervals in estimate analyses. Another topic of interest in this guidance includes examples of different types of concentration-response relationships and how to evaluate data from those concentration-relationships. In addition, this guidance discusses recommended effluent dilution series for different effluent scenarios and how to select the proper test dilution water for NPDES WET permit monitoring. As explained in the WET Methods Module and in the WET Permitting Module, both of these factors can have a profound effect on the endpoints reported and the confidence in those endpoints.

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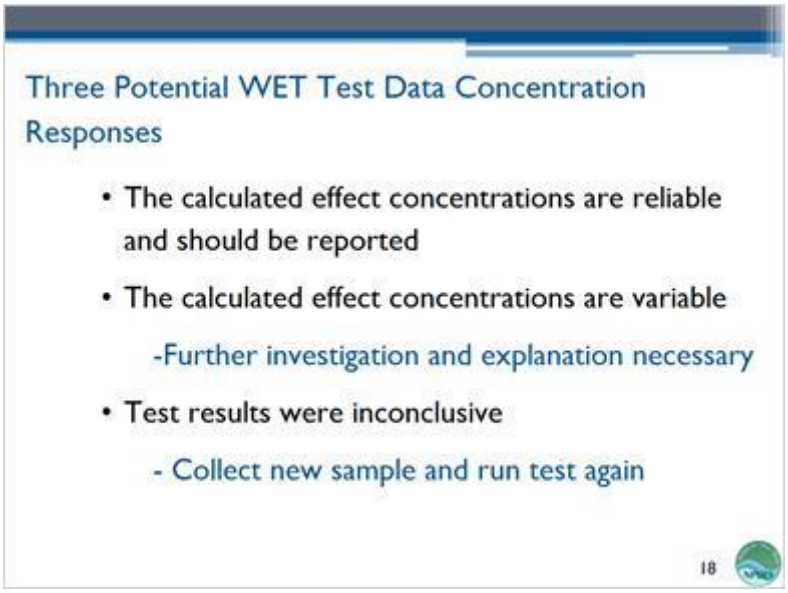

Notes:

As we noted in the previous slide, USEPA's 2000 WET method guidance describes different potential concentration-response patterns and how they should be evaluated to determine if results are reliable and should be used in NPDES permitting decisions. Three main types of concentration-response patterns are identified: (1) the calculated effect concentration is reliable and should be used, (2) the calculated effect concentration is questionable and further investigation and explanation is necessary before it should be used, and (3) the WET test results are inconclusive and a new test should be initiated using a new effluent sample. These three types of concentration-response test patterns will be examined in more detail over the next couple of slides.

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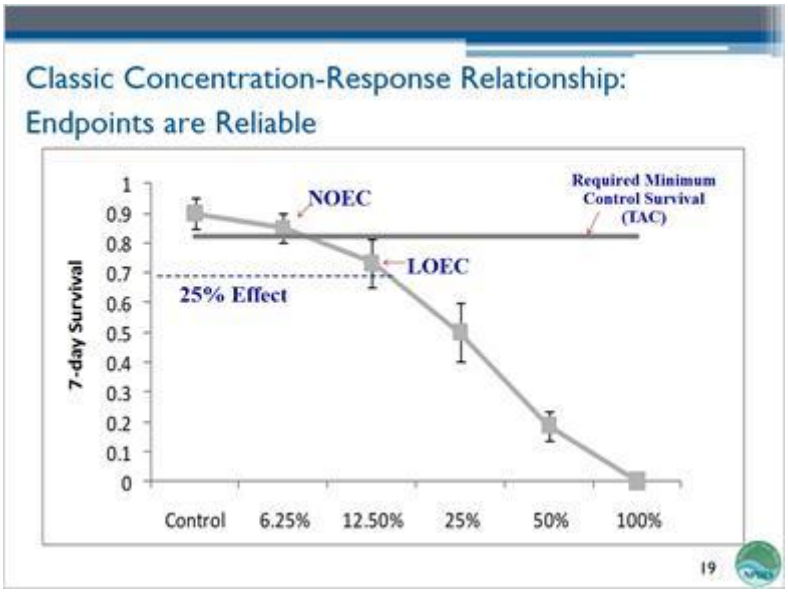

Notes:

The first concentration-response relationship, illustrated here, is a classic example of an increasing organism effect, in this case chronic survival, with increasing effluent concentration. The effluent concentration is expressed here as a percentage plotted on the x-axis, and 7-day fish survival is plotted on the y-axis. The control treatment resulted in an average of approximately 90% survival of the test organisms. Percent survival of the test organisms decreased as the effluent test concentration increased. This is referred to as a monotonic concentration-response pattern, in which each increasing effluent concentration has more effect on the test organisms as compared to the lower test concentrations. The results in this example indicate that the IC_{25} is similar to the effluent concentration that has been identified as the LOEC, and both of these endpoints are at higher effluent test concentration than the NOEC. The bars surrounding the average effect in each test concentration demonstrate low variability within the replicates of each test concentration. Therefore, given the monotonic concentration-response pattern and the fairly high within-test precision observed in this example, the results for any of the USEPA recommended endpoints should be considered reliable and should be reported as calculated.

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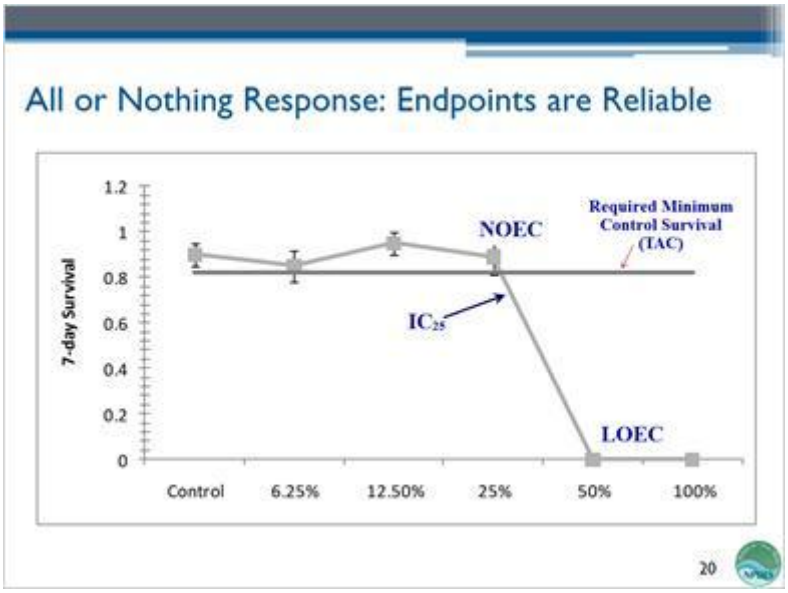

Notes:

The concentration-response relationship illustrated here is an example of what is commonly referred to as an "all or nothing" response. The control treatment resulted in an average of approximately 90% survival of the test organisms. As the effluent test concentration increased, the percent survival of the test organisms is relatively constant at around 90% until an apparent threshold is reached between the 25% and 50% effluent test concentrations. The results indicate that the IC_{25} is between the 25 and 50% effluent concentrations and that the NOEC is 25% effluent. The bars surrounding the average effect in each effluent test concentration indicate low variability (high precision) among replicates within each effluent test concentration. Since the IC_{25} or NOEC can be calculated with statistical confidence given the concentration-response pattern and the within-test precision is satisfactory, either the IC_{25} or the NOEC in this WET test should be considered reliable and should be reported as calculated.

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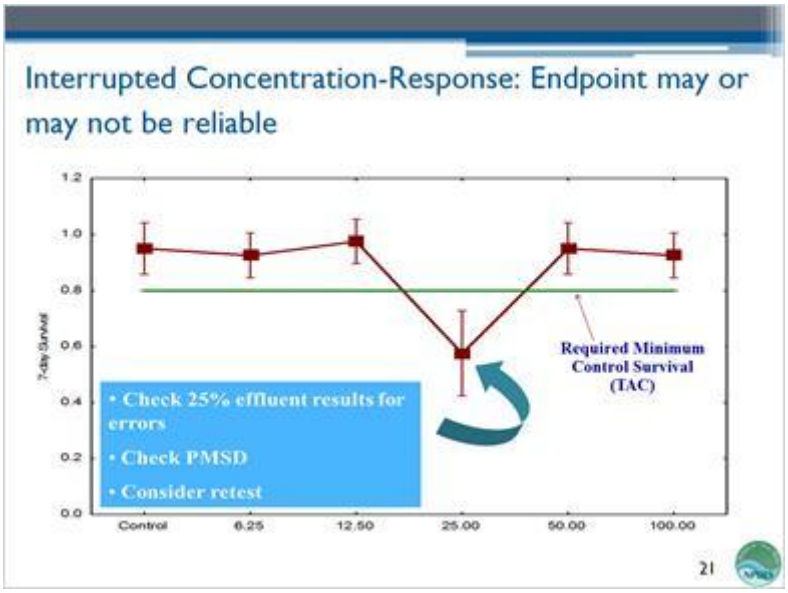


Notes:

This last concentration-response pattern is an example of an interrupted dose response. Once again, the control treatment resulted in an average of approximately 90% survival of the test organisms and as the effluent concentration increased, the percent survival of test organisms in all effluent test concentrations, with the exception of the 25% effluent concentration, is relatively constant around 90%. The observed response in the 25% effluent concentration is significantly different from the controls according to an NOEC analysis and in fact represents approximately a 35% effect as compared to the controls. Note that for this example, the NOEC would be either 12.5% or 100% effluent, depending on how the permitting authority interprets interrupted WET concentration-response data.

When effects occur at an intermediate effluent test concentration but not at effluent test concentrations closer to or at 0 (the control treatment) or 100% effluent, this type of concentration-response can be very difficult to interpret. USEPA's 2000 WET method guidance suggests that the results of the 25% effluent concentration should first be evaluated for possible laboratory errors, such as data transcription errors, incorrect make-up of the 25% effluent concentration in the laboratory, or other method performance errors that could affect the data. Also, if the NOEC statistical approach is

used to interpret these data, the Percent Minimum Significant Difference (PMSD) is evaluated to determine whether it is below the lower bound and therefore, the test is capable of identifying a very small difference as being significantly different from the control. USEPA's 2000 WET method guidance notes that with this type of concentration-response pattern, the permit writer should consider requiring the permittee to conduct a new test using a new effluent sample to determine if the effluent continues to demonstrate an interrupted concentration-response pattern and a toxic effect that is an excursion of a state's WET water quality standards.

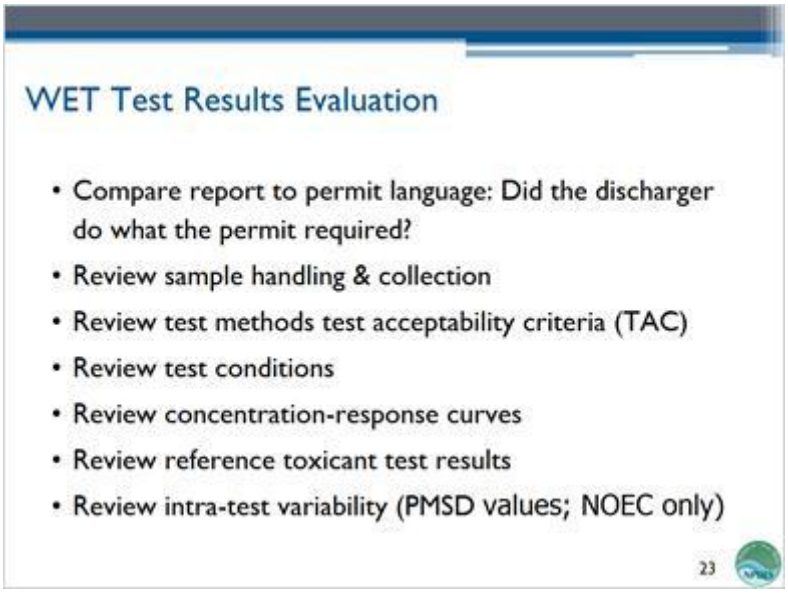
Note that, using USEPA's recommended linear interpolation approach, the IC_{25} point estimate would include the effect observed in the 25% effluent concentration and the IC_{25} would be around 40% effluent in this example.

Module 4: USEPA NPDES WET Statistical Analysis & Data Interpretation

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. We have reviewed some steps for evaluating data, including the percent minimum significant difference; and we've looked at the different types of concentration-response patterns. For the remainder of this module we are going to examine some of the steps that should be used when reviewing WET test data.

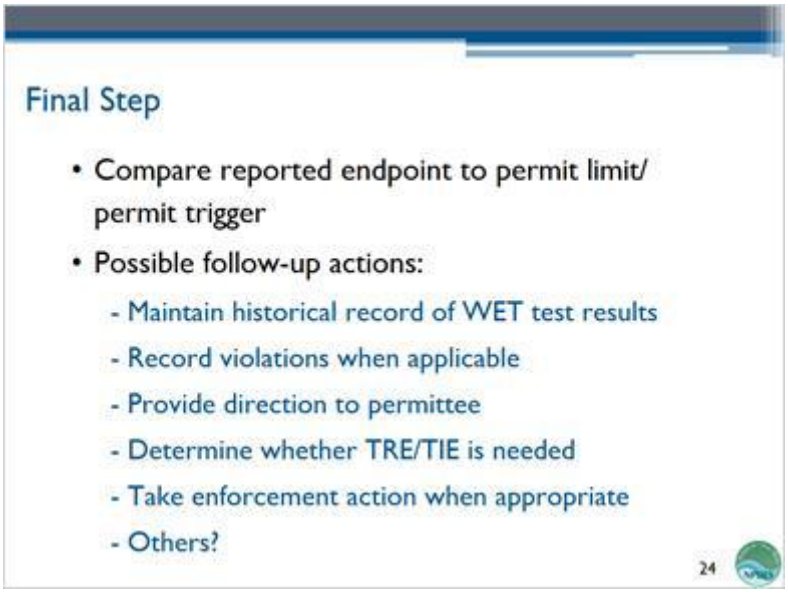
Module 4: USEPA NPDES WET Statistical Analysis & Data Interpretation



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 required 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, and verifying compliance with USEPA Test Acceptability Criteria. In addition, a review of concentration-response patterns of test results, reference toxicant results, and intra-test variability assessments are also very important when evaluating WET test results. 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.

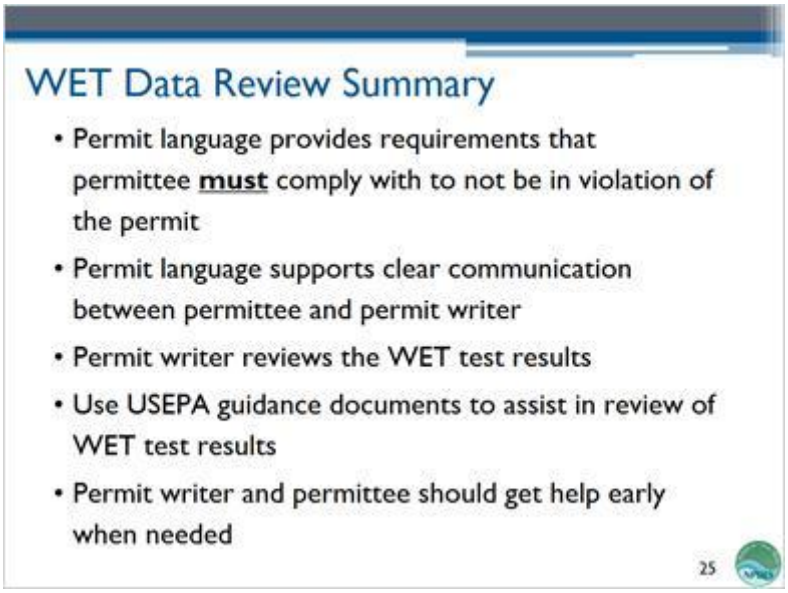
Module 4: USEPA NPDES WET Statistical Analysis & Data Interpretation



Notes:

After reviewing WET test results, if data have met all the required quality control requirements and are considered valid, the reported endpoint is compared to the permit limit or trigger to decide whether or not 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.

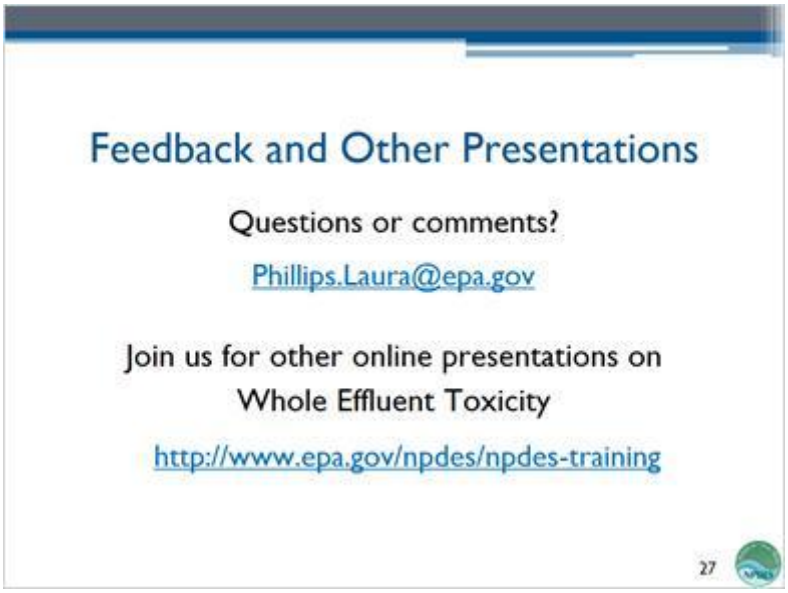
Module 4: USEPA NPDES WET Statistical Analysis & Data Interpretation



Notes:

In summary, the NPDES permit language needs to comprehensively and clearly indicate the WET requirements with which a permittee must comply in regards to testing and data analysis and interpretation. The NPDES permit language should provide clear and enforceable written permit communication between the permit writer and the permittee. Once the permittee conducts the Whole Effluent Toxicity tests as required under the NPDES permit, including the statistical evaluation of the data and the calculation of the required 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.

Module 4: USEPA NPDES WET Statistical Analysis & Data Interpretation



Notes:

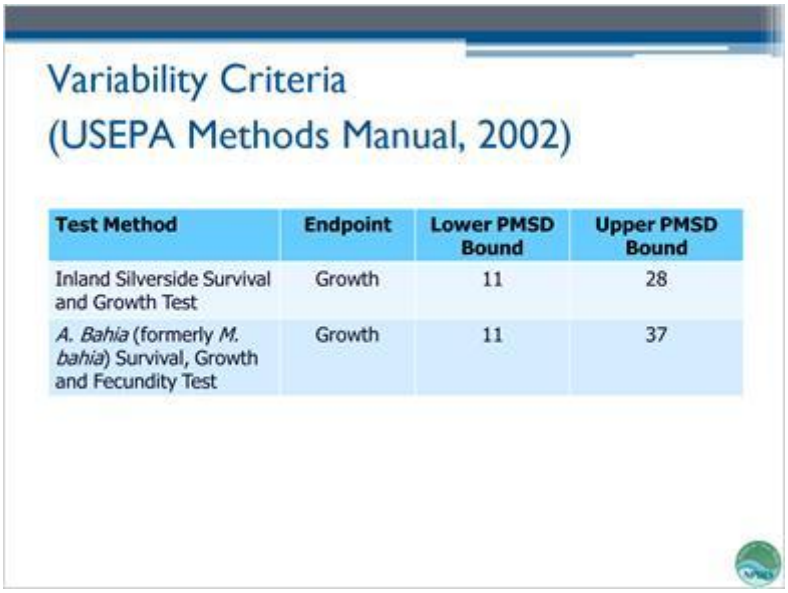
Thank you for joining us for this USEPA's NPDES Whole Effluent Toxicity training presentation. We hope that you have enjoyed it!

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!

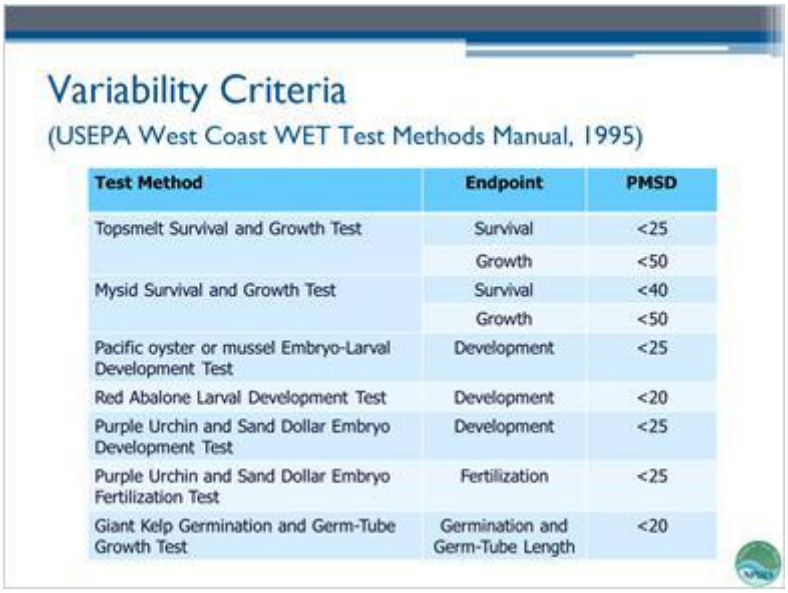
Module 4: USEPA NPDES WET Statistical Analysis & Data Interpretation



Notes:

The PMSD bounds for the USEPA East Coast marine WET test methods have only been calculated for the inland silverside fish and the mysid shrimp WET tests. The lower and upper PMSD bounds for inland silverside (*Menidia beryllina*) fish growth chronic sublethal endpoint are 11% and 28%, respectively. The chronic sublethal endpoint for the mysid shrimp (*Americamysis bahia*) WET test method has lower and upper PMSD growth bounds of 11% and 37%, respectively.

Module 4: USEPA NPDES WET Statistical Analysis & Data Interpretation



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 PMSD bounds for 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 the specific PMSD species 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 chronic sublethal endpoints are all 25%. The red abalone larval development and the giant kelp germination and germ-tube length PMSD chronic sublethal endpoints are all 20%.