Using EPA Whole Effluent Toxicity (WET) Statistics for Acute and Chronic Bioassay Tests

1. SCOPE AND APPLICATION

1.1. This method is based on the EPA's WET Analysis Spreadsheet v2.3.

1.2. The spreadsheet is used to analyze valid WET test data to obtain acute and chronic test endpoints (e.g. survival, reproduction and biomass) identified in EPA's WET test methods under the NPDES program. EPA's WET Analysis Spreadsheet calculates WET test endpoints recommended in EPA's Technical Support Document (TSD) (e.g. LC50, NOEC, LOEC, IC25; see Chapter 1, Section 1.3 of the TSD) as well as EPA's Test of Significant Toxicity (TST; EPA 833-R-10-003, June 2010). The spreadsheet can also be used for reasonable potential analysis, permit compliance determination, and permit limit development.

2. SUMMARY OF METHOD

2.1. Information about the test is entered on the "Start" tab in the spreadsheet. On the "Input" tab, data endpoints are entered (growth, reproduction, or survival). Results are calculated by the program and put in the "Output" tab. Statistics are saved to the "Statistics" tab. A "Generated Report" is created and saved. All statistics (LC50, NOEC, LOEC, PMSD, IC25, and Control % CV) are recorded in a Bioassay Bench Sheet logbook. A second analyst checks that all the endpoint counts were entered correctly as well as performs the calculations again to verify the final stats were generated and recorded correctly.

2.2. Interferences:

2.2.1. Non-valid test data entered, or data entered incorrectly.

2.2.2. Excel files not saved properly.

3. EQUIPMENT AND SUPPLIES

3.1. Computer with access to the internet, Toxcommon folder, and Excel

3.1.1. The EPA WET Analysis Spreadsheet is located at https://www.epa.gov/npdes/whole-effluent-toxicity-wet-npdes-spreadsheet. A new spreadsheet should be obtained from EPA's website for each new test.

3.4. Bioassay Bench logbooks and SRT logbooks.

4. METHOD

4.1. All Test Statistics (*P. promelas*, *M. beryllina*, *A. bahia* and *C. dubia*)

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4.1.1. Go to https://www.epa.gov/npdes/whole-effluent-toxicity-wet-npdes-spreadsheet and open "Whole Effluent Toxicity (WET) Analysis Spreadsheet (xlsm)".

4.1.1.1. Click "Enable Editing" and save excel file in the appropriate location [year, type of test (facility, SRT, or PT)] within the "EPA WET Spreadsheet">"!EPA Stats SRT and Facility" folder.

4.1.1.2. In the "Start" tab, fill out the following fields: Facility, Analyst, Test ID, Date, Lab ID (DEP), Species, Test Type, and Dilutions. When entering concentrations, they must be entered from low to high starting with the Control. The control value needs to be a number ("0"). Enter "100" in the IWC field.

4.1.1.3. If the "Enter Data" button on the "Start" tab is not working, then you need to enable macros. To do this, select File>Options>Trust Center>Trust Center Settings>Macro Settings. Make sure the "Enable VBA macros" is selected. Select "OK", and "OK" again to clear all windows. Exit the program and re-open to use.

4.1.2. Under the "Input1" or "Input2" tab enter the data endpoints for each concentration replicates. Press the "Calculate Results" button and the "Output" tab should appear.

4.1.3. Transcribe the NOEC, LOEC, IC25, and Control % CV in the bioassay or SRT bench sheets. The % CV is the number at concentration 0 under "CV" in the green box and turned into a percentage (i.e. if the number is 0.026, record the value as 2.6%). Press the "Save to Statistics" button and the "Statistics" tab will become available. In the "Statistics" tab, record the PMSD in the bioassay or SRT bench sheet.

4.1.4. Go back to the "Output" tab and click the "Generate Report" button. A new tab named "OutputReport" will open. Save the "OutputReport" tab as a .pdf in the "Generated Reports" folder within the "EPA WET Spreadsheet" folder.

4.1.5. For reporting the SRT IC25 and PMSD in control charts, after the appropriate results are recorded in the bench sheets, record the IC25 and PMSD to four significant figures. For example, enter test concentrations as 12.5, 25, 50, 75, and 100. The calculated IC25 and PMSD will now be given in a "##.##" format. Divide by 100 and report to the ten thousandths place.

4.2. Chronic Survival Statistics (*P. promelas*, *M. beryllina*, *A. bahia*, *C. dubia*)

4.2.1. For chronic tests, survival data must be analyzed first. If there is a survival effect for a given concentration (highlighted yellow with a "N" in the statistical result column), then those data are not entered for the sublethal endpoint.

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4.2.2. Under the “Input1” tab enter the number of organisms exposed or counted per replicate into the first data table. Enter the data endpoints (number of organisms surviving per replicate) into the second data table. Press the “Calculate Results” button and the “Output” tab should appear.

4.3. Chronic Growth Statistics (*P. promelas*, *M. beryllina*, and *A. bahia*)

4.3.1. In the “EPA WET Spreadsheet” folder, go to the “Organism Final Weights” folder. Open a blank template (fish species or *A. bahia*). Enter pan weight and pan plus organism weight from the *Chronic Weights* logbook to the excel template. Transcribe organism weight, mean weight (mg), and mean weight for concentration in the bioassay bench sheets.

4.3.2. Under the “Input1” tab enter the data endpoints (the organism weights in grams) for each concentration’s replicates. Press the “Calculate Results” button and the “Output” tab should appear.

4.4.

4.5. Chronic Reproduction Statistics (*C. dubia*)

4.5.1. Open the “Chronic C. dubia Counts” folder within the “EPA WET Spreadsheet”. Open the “C. dubia Calculations Sheet” excel template. Click “File” and Save As with the name of the test facility or SRT and date. Save the file to the current year folder in the “Chronic C. dubia Counts” folder.

4.5.2. Fill in Analyst initials, Test ID, and Date at the top. Fill in the number of neonates for each replicate per day on the data table. Enter the amount of surviving original organisms for each day in the column titled “Surviving Original Organisms” for each concentration.

4.5.2.1. When there is a split brood, type in “s” and then the two-digit number (Ex. S04, S10) and that number will be added to the previous days total (line above it). When there is a fourth brood, type in “f” and the two-digit number and the number will not be added to the total. Reproduction statistics only take the first three broods for calculations.

4.5.3. When all the numbers have been filled in, have another analyst verify that all the numbers have been transcribed correctly from the bioassay bench sheets into the excel sheet. Have the analyst who verifies add their initial at the top of the spreadsheet.

4.5.4. Open “Whole Effluent Toxicity (WET) Analysis Spreadsheet (xlsm)”.

4.5.5. Under the “Input1” tab enter the data endpoints (number of total neonates in first three broods) for the replicates of each concentration. Press the “Calculate Results” button and the “Output” tab should appear.

4.6. Acute Statistics (*P. promelas*, *M. beryllina*, *C. leedsi*, *C. dubia* and *A. bahia*)

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4.6.1. Survival is the only endpoint for acute tests.

4.6.1.1. When using C. leedsi as the test species, select “Cyprinodon variegatus (sheepshead minnow)”. The EPA spreadsheet does not include C. leedsi.

4.6.2. Under the “Input2” tab enter the number of organisms counted per replicate into the first data table. Enter the data endpoints (number of organisms surviving per replicate on termination day) into the second data table. Press the “Calculate Results” button and the “Output” tab should appear.

5. DATA ARCHIVAL

5.1. Use a black, waterproof pen for all bench sheets and logbooks.

5.2. Record statistics in appropriate spaces in bench sheets and SRT logbooks.

5.3. Save EPA WET Statistics Spreadsheets in the appropriate folders under the “EPA WET Spreadsheet” folder in Toxcommon.

6. QUALITY CONTROL

6.1. Refer to QA manual for corrective action and handling out-of-control data.

6.2. Have another analyst verify that the statistical analysis was run correctly, and that the data was entered into the excel spreadsheets from the logbooks correctly.

7. REFERENCES

7.1. Chapter 1, Section 1.3 of the EPA’s Technical Support Document (TSD).

7.2. EPA’s Test of Significant Toxicity (TST; EPA 833-R-10-003, June 2010).

7.3. Whole Effluent Toxicity (WET) NPDES Spreadsheet

7.4. FDEP Laboratory Quality Manual

7.5. Laboratory Health and Safety Plan

APPENDIX OF CHANGES

02/24/2022 Changed method of accessing the EPA WET Analysis Spreadsheet. Updated to reflect changes to the EPA WET Analysis Spreadsheet.

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