# 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 v1.6.1.

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). This 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 (any previous entries are deleted). A "Generated Report" is created and saved in the appropriate folder. All statistics (LC50, NOEC, LOEC, PMSD, IC25, and Control %CV) are recorded in the appropriate space in the Bioassay Bench Sheet logbooks. A second analyst redoes the statistics to verify that the data was entered 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 Toxcommon folder and Excel

3.1.1. The EPA WET Analysis Spreadsheets are in the "EPA WET Spreadsheet" folder within Toxcommon. Located here; X:\Labs\Lab5\Biology\Bench Common\ToxCommon\EPA WET Spreadsheet

3.4. Bioassay Bench logbooks and SRT logbooks.

## 4. METHOD

4.1. Chronic Growth Statistics (P. promelas, M. beryllina, A. bahia)

4.1.1. In the "EPA WET Spreadsheet folder, go to the "Organism Final Weights" folder. Open a blank template (fish species or A. bahia). Transcribe pan



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weight and pan plus organism weight from the *Chronic Weights* logbook to the excel template. The organism weights should autofill the rest of the template. Transcribe organism weight, mean weight (mg), and mean weight for concentration in the bioassay bench sheets.

4.1.2. Go to the "EPA Stats SRT and Facility" folder and open a previously saved Chronic Growth EPA WET spreadsheet. Click "File" and Save As with the name of the test facility or SRT and date. Click on the tab labeled "Start" and fill in the boxes of test information; Facility (for SRT put 'Lab SRT'), analyst, Test ID (facility name, Chronic), date, species, test type (growth), and IWC (should be set to 100). Hit the "Enter Data" button.

4.1.3. Under the "Input1" tab fill in the concentrations in the blue boxes at the top of the data table. Use "0" for control. Enter the data endpoints (the organism weights in grams) for each concentration's replicates. Press the "Calculate the result" button and the "Output1" tab should appear.

4.1.4. Transcribe the NOEC, LOEC, IC25, PMSD, 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. Press the "Save to Statistics" button and the "Statistics" tab should have the current data in it. Delete any previous saved statistics on the "Statistics" tab. Record relevant statistics into the appropriate logbook.

4.1.5. Go back to the "Output" tab and click the "Generate Report" button. A new tab named "OutputReport1" will open, copy and paste the information and graph to a Word file. Save the Word file under the "Generated Reports" folder within the "EPA WET Spreadsheet" folder.

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

4.2.1. Go to the "EPA Stats SRT and Facility" folder and open a previously saved Chronic Survival EPA WET spreadsheet. Click "File" and Save As with the name of the test facility or SRT and date. Click on the tab labeled "Start" and fill in the boxes of test information; Facility (for SRT put 'Lab SRT'), analyst, Test ID (facility name, Chronic), date, species, test type (Chronic Survival), and IWC (should be set to 100). Hit the "Enter Data" button.

4.2.2. Under the "Input2" tab fill in the concentrations in the blue boxes at the top of each data table. Use "0" for control. Enter the number of organisms exposed or counted per replicate into the first data table. Enter the data endpoints (number of organisms surviving or responding per replicate) into the second data table. Press the "Calculate the result" button and the "Output" tab should appear.

4.2.3. Transcribe the NOEC and LOEC into the bioassay or SRT bench sheets. Those are the only two statistics needed for the survival portion of the test. Press the "Save to Statistics" button and the "Statistics" tab should have



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the current data in it. Delete any previous saved statistics on the "Statistics" tab. Record relevant statistics into the appropriate logbook.

4.2.4. Go back to the "Output" tab and click the "Generate Report" button. A new tab named "OutputReport1" will open; copy and paste the information and graph to a Word file. Save the Word file under the "Generated Reports" folder within the "EPA WET Spreadsheet" folder.

4.3. Chronic Reproduction Statistics (C. dubia)

4.3.1. 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.

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

4.3.2.1. 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.

4.3.3. 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.

4.3.4. Go to the "EPA Stats SRT and Facility" folder and open a previously saved C. dubia Chronic Reproduction EPA WET spreadsheet. Click "File" and Save As with the name of the test facility or SRT and date. Click on the tab labeled "Start" and fill in the boxes of test information; Facility (for SRT put 'Lab SRT'), analyst, Test ID (facility name, Chronic), date, species, test type (reproduction), and IWC (should be set to 100). Hit the "Enter Data" button.

4.3.5. Under the "Input1" tab fill in the concentrations in the blue boxes at the top of the data table. Use "0" for control. Enter the data endpoints (number of neonates) for each concentration's 4 replicates. Press the "Calculate the result" button and the "Output1" tab should appear.

4.3.6. Transcribe the NOEC, LOEC, IC25, PMSD, 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. Press the "Save to Statistics" button and the "Statistics" tab should have the current data in it.



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Delete any previous saved statistics on the "Statistics" tab. Record relevant statistics into the appropriate logbook.

4.3.7. Go back to the "Output" tab and click the "Generate Report" button. A new tab named "OutputReport1" will open; copy and paste the information and graph to a Word file. Save the Word file under the "Generated Reports" folder within the "EPA WET Spreadsheet" folder.

4.4. Acute Statistics (P. promelas, M. beryllina, C. leedsi, C. dubia, A. bahia)

4.4.1. Survival is the only endpoint for acute tests. Go to the "EPA WET Spreadsheet folder" and open a previous acute test spreadsheet for the organism being used. Click on the tab labeled "Start" and fill in the boxes of test information; Facility (for SRT put 'Lab SRT'), analyst, Test ID (facility name, Acute), date, species, test type (Acute Survival), and IWC (should be set to 100). Hit the "Enter Data" button.

Note: For C. leedsi test: select "Cyprinodon variegatus (sheepshead minnow)" as the species. The EPA spreadsheet does not include C. leedsi.

4.4.2. Under the "Input2" tab fill in the concentrations in the blue boxes at the top of each data table. Use "0" for control. 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 the result" button and the "Output" tab should appear.

4.4.3. Transcribe the NOEC, LOEC and LC50 into the bioassay or SRT bench sheets. Those are the only statistics needed for the survival portion of the test. Press the "Save to Statistics" button and the "Statistics" tab should have the current data in it. Delete any previous saved statistics on the "Statistics" tab.

4.4.4. Go back to the "Output" tab and click the "Generate Report" button. A new tab named "OutputReport1" will open; copy and paste the information and graph to a Word file. Save the Word file under the "Generated Reports" folder within the "EPA WET Spreadsheet" folder.

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.



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5.4. To correct raw data entries, place a single line through the incorrect entry, write the corrected entry near the error and initial and date the correction. Do not overwrite entries.

5.4.1. The corrected entry is written as near its appropriate space as possible or else in the comment box on the appropriate bench sheet.

5.4.2. If the correction requires an explanation, write a comment and reference the error. Initial and date the explanation.

5.4.3. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

## 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 transcribed to the excel spreadsheets from the logbooks correctly.

## 7. SAFETY/HAZARDOUS WASTE MANAGEMENT

7.1. Proper laboratory protection, including lab hood or ventilation system, lab coat, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

7.2. Refer to the Laboratory Quality Manual for sample disposal

8.3 Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management

## 8. REFERENCES

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

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

8.3. Laboratory Quality Manual, https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

8.4. Laboratory Health and Safety Manual and Hazardous Waste Management, Health and Safety Manual

**APPENDIX OF CHANGES**

08/29/2019 Updated links

03/26/2020 Added note for C. leedsi



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