# 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 v2.0.

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



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

4.1.2.1. Click on "Download file" at the top of the page.

4.1.2.2. After the download is complete, choose "Open file".

4.1.2.3. 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.

4.1.2.4. 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.

NOTE: 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.

4.1.3. 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.

NOTE: For chronic tests, survival data must be analyzed first. If there is a survival effect for a given concentration, then those data are not entered for the sublethal endpoint. EXAMPLE: Test concentration series 0, 6.25, 12.5, 25, 50, and 100. For survival the output indicates that 50 and 100 are significantly different than the control (highlighted yellow with a "N" in the statistical result column). When analyzing the sublethal endpoint (growth) from this test, **do not** enter data for the 50 and 100 concentrations – these concentrations already exhibited an effect on survival.

4.1.4. 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"



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tab will become available. In the "Statistics" tab, record the PMSD in the bioassay or SRT bench sheet.

4.1.5. 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.

NOTE: For reporting the SRT IC25 and PMSD to control charts, after the appropriate results are recorded in the bench sheets, record the IC25 and PMSD to four significant figures. To do this, the test concentration series must be entered as a percentage of 100%. For example, enter test concentrations as 12.5, 25, 50, 75, and 100 instead of 0.125, 0.25, 0.5, 0.75, and 1.0. The calculated IC25 and PMSD will now be given in a "##.##" format. Move the decimal point two to the left to report to the ten thousandths place.

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

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

4.2.1.1. Click on "Download file" at the top of the page.

4.2.1.2. After the download is complete, choose "Open file".

4.2.1.3. 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.

4.2.1.4. 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.

NOTE: 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.

4.2.2. 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 or responding per replicate) into the second data table. Press the "Calculate Results" 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.



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Press the "Save to Statistics" button and the "Statistics" tab should have the current data in it. 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 "OutputReport" will open. Save the "OutputReport" tab as a .pdf in 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 for 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 https://www.epa.gov/npdes/whole-effluent-toxicity-wet-npdes-spreadsheet and open "Whole Effluent Toxicity (WET) Analysis Spreadsheet (Excel)(xlsm)".

4.3.4.1. Click on "Download file" at the top of the page.

4.3.4.2. After the download is complete, choose "Open file".

4.3.4.3. 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.

4.3.4.4. 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.



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NOTE: 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.

4.3.5. 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.

NOTE: For chronic tests, survival data must be analyzed first. If there is a survival effect for a given concentration, then those data are not entered for the sublethal endpoint. EXAMPLE: Test concentration series 0, 6.25, 12.5, 25, 50, and 100. For survival the output indicates that 50 and 100 are significantly different than the control (highlighted yellow with a "N" in the statistical result column). When analyzing the sublethal endpoint (growth) from this test, do not enter data for the 50 and 100 concentrations – these concentrations already exhibited an effect on survival.

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 (i.e. if the number is 0.026, record the value as 2.6%). Press the "Save to Statistics" button and the "Statistics" tab should have the current data in it. 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 "OutputReport" will open. Save the "OutputReport" tab as a .pdf in 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 https://www.epa.gov/npdes/whole-effluent-toxicity-wet-npdes-spreadsheet and open "Whole Effluent Toxicity (WET) Analysis Spreadsheet (Excel)(xlsm)".

4.4.1.1. Click on "Download file" at the top of the page.

4.4.1.2. After the download is complete, choose "Open file".

4.4.1.3. 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.

4.4.1.4. 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



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starting with the Control. The control value needs to be a number ("0"). Enter "100" in the IWC field.

NOTE: 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.

NOTE: When using C. leedsi as the test species, select "Cyprinodon variegatus (sheepshead minnow)". The EPA spreadsheet does not include C. leedsi.

4.4.2. 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.

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.

4.4.4. 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.

## 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.

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.



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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.

7.3. 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. Whole Effluent Toxicity (WET) NPDES Spreadsheet

8.4. FDEP Laboratory Quality Manual

8.5. Laboratory Health and Safety Plan

## APPENDIX OF CHANGES

| | |
|---|---|
| 08/29/2019 | Updated links |
| 03/26/2020 | Added note for C. leedsi |
| 02/26/2021 | Updated folder same information for currently used and updated links. |
| 02/24/2022 | Changed method of accessing the EPA WET Analysis Spreadsheet. Updated to reflect changes to the EPA WET Analysis Spreadsheet. Updated links. |
| 03/22/2023 | Made minor grammatical updates. |
| 03/13/2024 | Updated links and added missing references; edited for grammar, clarity, and word choice. |



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