Statistical Analysis of Acute Toxicity Test Data for LC_{50}

1. SCOPE AND APPLICATION

1.1. This method is based on EPA, 2002. *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Edition, EPA-821-R-02-012. It is primarily used for the determination of lethal concentrations in acute toxicity bioassay tests, but can be used to determine a LC_{50} in a chronic test.

2. SUMMARY OF METHOD

2.1. Bioassay data is entered into statistical programs. Program input and output is verified, and results are entered into the Laboratory Information Management System (LIMS) for dissemination to clients.

2.1.1. The user is expected to have read the appropriate sections of the EPA Methods Manual and the "read me" files that accompany each statistical program. The *Trimmed Spearman-Karber* and the *Probit* programs are located in the X:\Labs\Lab5\Biology\Bench Common\ToxCommon\Epa Stat package folder. *ToxCalc*™ and the *Comprehensive Environmental Toxicity Information System*™ (*CETIS*™) programs are licensed for a single PC located in Room A216L. The USEPA WET (2012) Spreadsheet can also be used to calculate endpoints for acute tests. It is also located in X:\Labs\Lab5\Biology\Bench Common\ToxCommon\EPA WET Spreadsheet.

NOTE: Due to operating system updates and the age of the programs, the EPA Stat package *TSK* and *Probit* programs do not function on all PCs in the Bench Biology work group.

2.2. Definitions:

2.2.1. LC_{50}-: Calculated or observed concentration in which mortality occurs in 50% of the organisms within the concentration.

3. EQUIPMENT AND SUPPLIES

3.1. Copy of bioassay survival bench sheet

3.2. Computer with access to statistical programs

4. METHOD FOR ACUTE SCREENING TEST RESULTS:

4.1. Statistical analyses need not be performed on results from an acute screening test consisting of a control and a 100% sample concentration. Enter the results into LIMS as 100 L (LC_{50} >100% if mortality is less than 50%), 100 K (LC_{50} <100% if mortality is greater than 50%), or 100 (LC_{50} = 100% if mortality is 50%).

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5. METHOD FOR ACUTE DEFINITIVE LC_{50} CALCULATION USING TSK

5.1. Determine by visual inspection of the data whether or not the LC_{50} should fall within the range of the test concentrations (mortality in highest concentration > 50%).

5.1.1. If the mortality in the highest test concentration is >50%, continue to **Step 5.2**.

5.1.2. If there is < 50% mortality in the highest test concentration of a facility acute bioassay (presumption is < 50% mortality in all test concentrations if mortality is <50% in the highest concentration), a LC_{50} calculation should not be necessary since the compliance requirement for a facility permitted for an acute test is generally a LC_{50} > the highest permitted test concentration. By inference a LC50 would generally be greater than the concentration having < 50% mortality. If a LC_{50} calculation is desired, follow the *Probit* Method described in **Section 6**.

Reminder: The user is expected to have read the appropriate sections of the EPA Methods Manual and the "read me" files that accompany each statistical program.

5.2. Open the *Trimmed Spearman-Karber* program by double-clicking the *TSK* icon.

5.3. Enter test information/data in response to program prompts. Entry errors can be corrected prior to the [**Enter]** key being pressed; else, the program will need to be restarted.

5.4. Save the output from the TSK program:

5.4.1. Open the icon at the top left of the *TSK* screen.

5.4.2. Click *Edit*. Click *Select all*. Click *Copy*.

5.4.3. Open MS Word. Paste the file from Step 5.3. Select MS-Word *Edit-Paste* (or [Control] + [V]).

5.4.4. Save the Word file with an appropriate name such as the facility name, organism and date of tests; or SRT organism and date of test. (Ex. for an Acute *C. dubia* SRT performed on July 15, 2013, file name would be *C. dubia SRT TSK 7 15 13*.)

5.4.5. SRT files are saved in X:\Labs\Lab5\Biology\Bench Common\ToxCommon\Bioassay Charts and Tables (SRT)\TSK word files. Facility files are saved in the appropriate facility folder in X:\Labs\Lab5\\Biology\FYI Materials & Reports.

5.5. To end the *TSK* program, respond with *N* to the "*Would you like to continue*" prompt.

5.6. Enter the LC_{50} into LIMS.

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6. ACUTE DEFINITIVE TEST LC_{50} CALCULATION *PROBIT* METHOD

6.1. Examine test data to ensure that mortality in the highest test concentration is < 50% and that there is partial mortality in a minimum of two test concentrations. If both conditions are not met, then *Probit* is not the appropriate method.

6.2. Open the *Probit* program.

6.3. At "Abbreviated or full input/output," type *A* [Return].

6.4. At "Output to printer or disk file (P/D)?" type *D* [Return].

6.5. At "File name for output?" type your *filename* [Return]. The filename must follow DOS criteria: 5-8 letters or numbers; no punctuation, symbols or spaces.

6.6. At "Title?" type any descriptive wording desired. (Ex., *facility name effluent.*) The title is not subject to DOS criteria. Then press [Return].

6.7. The program will ask about the test data. Start with the lowest concentration and respond accordingly.

6.8. At "Do you wish to modify your data?" type *N* [Return] or *Y* [Return], depending on your need. When any data are modified, the program will show the corrections made and ask again for any modifications. The program will continue asking for any modifications until the final response is *N*. Only then will it move to the next step.

6.9. When the last response has been entered, press [Return] and the program will automatically execute. The message on screen will be "Output stored in FILENAME."

6.10. To view the results on the screen, open the file within the *Probit* folder using *Microsoft Notepad* or other text editing software.

6.11. Enter the LC_{50} into LIMS.

7. DATA ARCHIVAL

7.1. Use a black, waterproof pen for all bench sheets and logbooks. Enter the LC_{50} into LIMS.

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

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

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

7.2.2.1. 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.).

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8. REFERENCES

8.1. EPA-821-R-02-012 Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Ed. October 2002.

8.2. TOXCALC Toxicity Data Analysis Software User's Guide--Tidepool Scientific Software.

8.3. CETIS User's Guide (Comprehensive Environmental Toxicity Information System—Tidepool Scientific Software

8.4 USEPA WET (2012) Spreadsheet instructions.

APPENDIX OF CHANGES

12/17/2007 Added Scope and Application; Summary of Method, and Appendix of Changes.

07/31/2013 Reformatted the SOP.

02/12/2015 Formatted for 508 compliance.

08/17/2015 Updated links and added USEPA 2012 spreadsheet to calculation programs.

09/19/2017 Updated program names.

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