

Draft User Guide for
Laboratory Analysis of Toxicity -- CDF Runoff (**LAT-R**) Program

Paul R. Schroeder
Anthony C. Gibson
Environmental Laboratory
U.S. Army Engineer Waterways Experiment Station
Vicksburg, MS 39180-6199

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PURPOSE

This user guide describes how to use the LAT-R computer program. The LAT-R module is used to perform the appropriate statistical methods for analyzing data from suspended phase water column bioassay tests for acute toxicity (EPA/USACE 1994). These tests are used to evaluate the effects on water quality from discharges of water from a confined dredged material disposal facility (CDF).

BACKGROUND

Water Column Bioassay Test Setup

Samples of dredged material elutriate are collected from slurries of sediment and site water. The elutriate samples are placed in test vessels. The test vessels of dredged material elutriate are diluted, forming various concentrations or treatments. For example, a control treatment, 100, 50, 25, and 12.5 percent concentrations are typical. (The LAT-R Model can accept data for a maximum of 10 treatment concentrations). The control treatment test vessels typically contain only the dilution water or site water and are termed the dilution water treatment in the LAT-R Model. Each treatment concentration must have at least 3 replicates but not more than 10 replicates. Guidance on water column bioassay procedures is provided in Appendix D of the Draft Inland Testing Manual (EPA/USACE 1994).

Initially, a constant number of a single specie of organisms is placed in the sample test vessels at various treatment concentration levels. For example, 20 organisms of a single, unique specie are placed in each sample test vessels with the dredged material elutriate at the various treatment concentrations.

Column Test Results

The organisms are exposed to the various runoff concentrations for a number of hours or days, typically 96 hours. At the end of the test the organisms are counted to determine the number of survivors. The number of survivors is input into the LAT-R program.

Statistical Overview

The LAT-R program can be executed to perform statistical analyses on the bioassay test results as described in Appendix D of the Draft Inland Testing Manual (EPA/USACE 1994). The LAT-R program permits the user to perform statistical analyses without any knowledge of the complex statistical analyses being performed by requesting only information about the bioassay tests. Two FORTRAN compilers, LPI-Ryan McFarland Version 3.0 (1991) and Microsoft Fortran Power Station Version 1.0 (1992), were used to develop this program, and the Microsoft Integrated Mathematical and Statistical Libraries (IMSL) Version 2.0 (1994) were used to perform the statistical analyses.

There are three outcomes which the statistical analyses in the LAT-R program provide, and only one of the three outcomes will apply to organisms placed in the water column toxicity test. The three outcomes are:

- i) Water column bioassay test must be repeated.
- ii) Dredged material meets water column toxicity standards.
- iii) The dredged material exhibits a Median Lethal Concentration (LC50) which is then calculated and reported by the LAT-R program.

Figure 1 shows the water column toxicity test decision tree and Figure 2 shows the LC50 decision tree. However, since the LAT-R program make these decisions, the user need only to enter the data, save the data, and execute the calculation options.

The LAT-R Program will print "Repeat water column toxicity test" for the organism of interest when either of these conditions are true:

- i) The number of organisms that survived does not meet EPA standards.
- ii) The elutriate concentrations need to be lower.

The LAT-R Program will print "Dredged material meets guidelines of water column toxicity test" for the organism of interest when either of these conditions are true:

- i) The number of organisms that survived in the 100% elutriate concentration treatment is at least 10% less than the number of organisms that survived in the dilution water treatment.
- ii) The number of organisms that survived in the 100% elutriate concentration treatment is significantly lower than the number of organisms that survived in the dilution water treatment.

The LAT-R program will calculate an LC50 value by one of three methods. The three methods are:

- i) PROBIT Method (EMRD, 1992a)
- ii) TSK Method (EMRD, 1992b)
- iii) Linear Interpolation Method (Schroeder and Gibson, 1997)

Program developers recommend the LC50 value calculated by the PROBIT Method be used as a first alternative. However, if the PROBIT Method can not be calculated then the LC50 value from the TSK or Linear Interpolation Methods should be used.

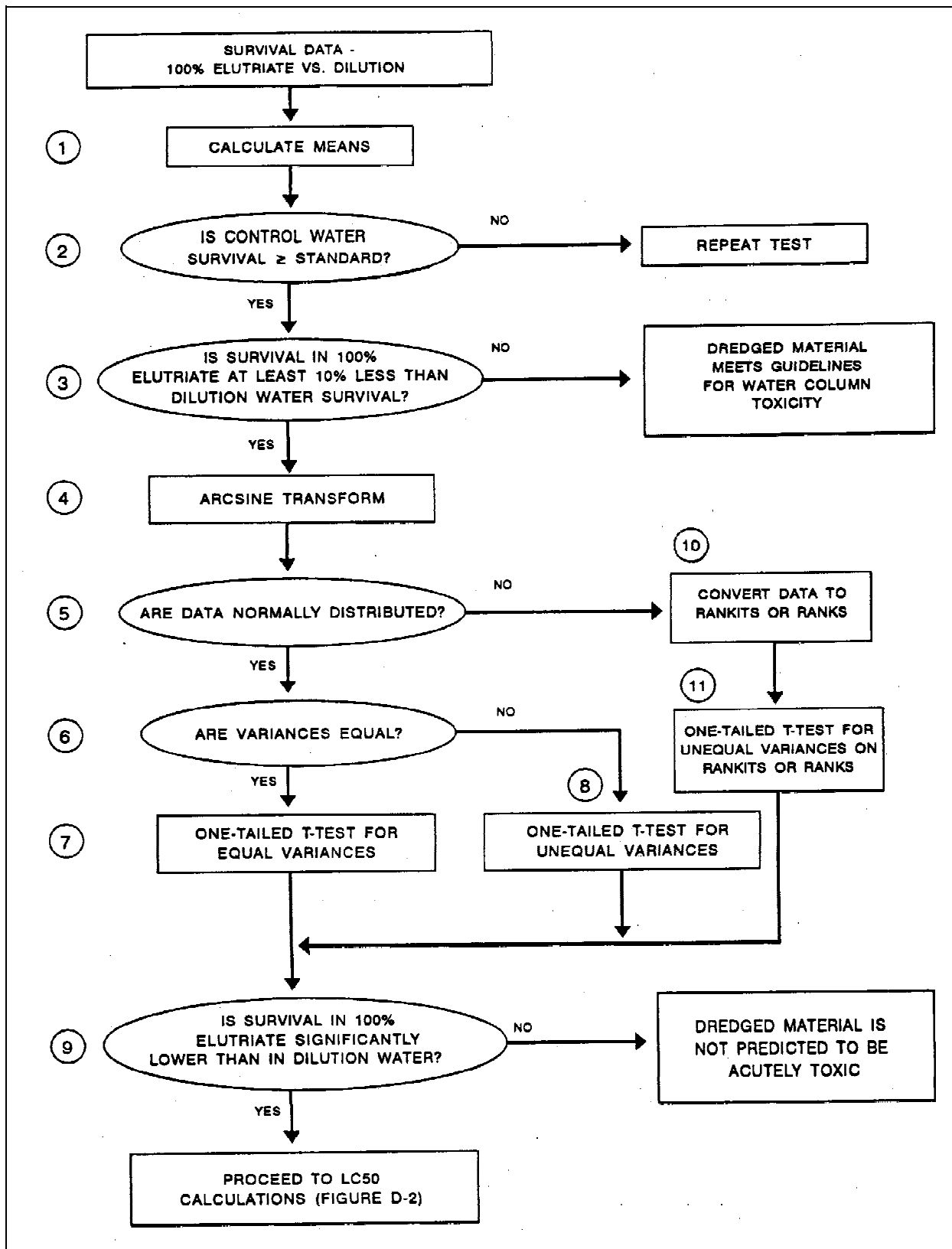


Figure 1. Water Column Toxicity Test Decision Tree.

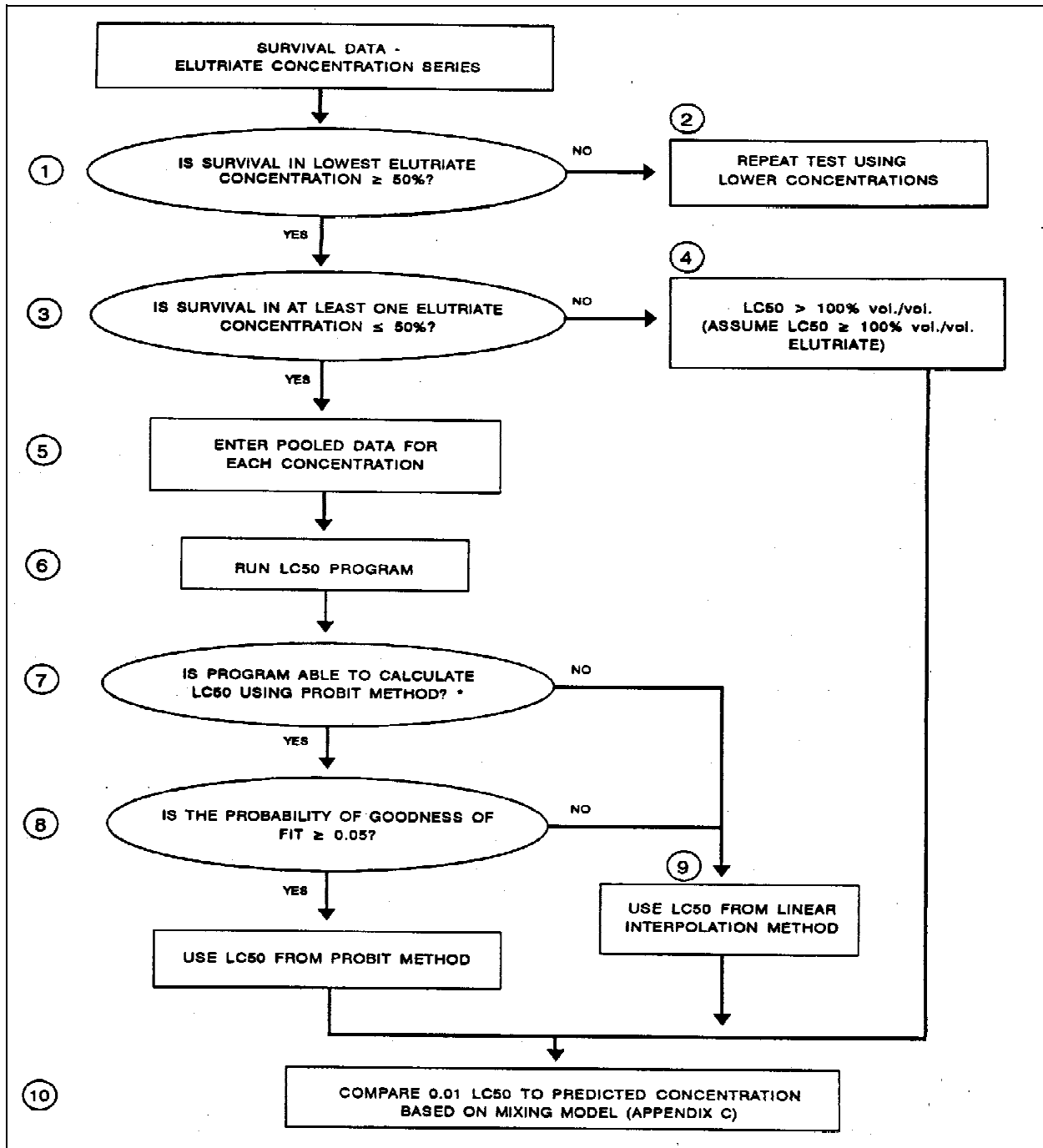


Figure 2. LC-50 Decision Tree.

ADDITIONAL INFORMATION

The user guide was written by Paul Schroeder and Anthony Gibson of the U.S. Army Engineer Waterways Experiment Station. Mr. Gibson should be contacted at (601) 634-3710 for inquiries concerning data entry and model development procedures.

GETTING STARTED

a. Hardware Requirements

This statistical model LAT-R require 2 megabytes of hard disk space, with 640 kilobytes of conventional random access memory. The LAT-R Model must be executed under the DOS (Disk Operation System).

b. Installation And Initiation of LAT-R

To execute the LAT-R Computer Program model, the user must perform these tasks:

- A. Install Model
- B. Enter/Edit Data
- C. Save Data
- D. Run Model
- E. Print Results

Task A. Install Model

Step 1 - Insert diskette 1 of 2 in drive A or B.

Step 2 - At the DOS prompt, type A:\INSTALL or B:\INSTALL.

Step 3 - Follow the instructions given by the installation program to complete the installation and start the model.

Task B. Enter/Edit Data

Step 1 - Start LAT-R.

At this time the program will give you information on technical support. Next, the main menu and its options will appear. The main menu has 6 options which are:

- Option 1 - Enter/Edit data.
- 2 - Select data file or perform file operation.
- 3 - Edit organism list and standards.
- 4 - Perform calculations & view output.
- 5 - Print output.
- 6 - Exit.

- Step 2 - Press the number 1 to enter/edit data.
- Step 3 - Type the title of study.
- Step 4 - Press Page Down key to continue to next page.
- Step 5 - Use the up and down arrow keys to select the organism of interest.
- Step 6 - Press the Enter key when the organism of interest is highlighted. (If you cannot find the organism of interest on the list, you must first edit the list of organisms. Instructions for editing the list of organisms are given at the end of this section on data entry/editing.)
- Step 7 - Enter values for these statements:
- Enter the number of dilution water replicates _____ .
 Enter the acceptable standard survival rate _____ % .
 Enter the length of exposure _____ .
- Step 8 - Press the Page Down key.
- Step 9 - Enter the number of elutriate treatments _____ .
- Step 10 - Enter the elutriate values for treatment 1 _____ .
 - Enter the elutriate values for treatment 2 _____ .
 - " " " " " " . _____ .
 - " " " " " " . _____ .
 - " " " " " " 9 _____ .
- Step 11 - Press the Page Down key to continue.
- Step 12 - Enter the number of replicates for treatment 1 _____ .
 - " " " " " " " " 2 _____ .
 - " " " " " " " " . _____ .
 - " " " " " " " " . _____ .
 - " " " " " " " " 9 _____ .
- Step 13 - Press the Page Down key to continue.
- Step 14 - Enter the number of organisms per replicate at initiation of test.
- Step 15 - Press the Page Down key to continue.
- Step 16 - Enter the number of survivors per replicate _____ .
- Step 17 - Press the Home key to return to main menu.

(Data entry is completed.)

Edit Organisms List and Standards

To edit the organisms list and standards the user should press the number 3 on the main menu options list. The list of default organisms and EPA's test standard of acceptance are shown. The user can page down to view the next page or follow the instructions found on the last line on the page to edit the list. These instructions give the user the option to:

- PgUp - PrvScr - Page up to previous screen or page.
- PgDn - NxtScr - Page down to next screen.
- Home - Menu - Return to main menu option.
- Ins - AddPrm - Add an organism to this list.
- Del - DelPrm - Delete an organism from this list.

The up and down arrow keys can be used to highlight the organism of interest. However, the user can press the Insert Key to add an organism to the list, and the model will prompt the user to type the organism name and the EPA's test standard. Test standards are survivor percentages which EPA has set for various organisms. In other words, EPA requires a significant percentage of the organisms to survive in the control for the bioassay test to ensure the test is performed correctly and to ensure that this testing method is appropriate.

Task C. Save Data

To save the data the user must select option 2 from the main menu.

Step 1 - Press the number 2.

Step 2 - Press the number 1 for select name of file to be saved.

Step 3 - Type the name of file to be saved.

Step 4 - Press the Enter key.

Step 5 - Press the number 5 to save the entered data.

Step 6 - Next, the program lists the title. If the title is correct, press the Enter key.

Step 7 - Press the Escape key.

Step 8 - The title is printed again and the user must press the Enter key to continue.

(The input data has been saved.)

Task D. Perform Calculations and View Output

To perform calculations the user should press the number 4 from the main menu options. After the calculations are completed, the output results appear on the screen. The user can press the Escape key (Esc) to exit or use the Page Up or Page Down keys to view other parts of the results.

Step 1 - Press the number 4.

Step 2 - Press Escape Key to exit view of output.

(Calculations are completed.)

E. Print Results

To print output of the calculations the user must enter the number 5 from the main menu option list.

Step 1 - Turn your printer on.

Step 2 - Press the number 5 to print output.

Step 3 - Press the number 3 and output information will be sent to your printer. However, the user has 3 other options which are:

Enter the number 1 to print default organism and EPA's standard of survivor list.

Enter the number 2 to print input data file.

Enter the number 3 to print statistical results.

Enter the number 4 to exit.

Step 4 - Press the number 5 to return to the main menu.

(Printing of output is completed.)

Step 5 - Press the number 6 to exit the LAT-R program.

Example Run of the LAT-R Model for Water Column Toxicity Test Data Analysis

The LAT-R Model is a program to compare dilution water survival vs. 100% elutriate, using an arsine-square root transformation on the data. The program performs all statistical analyses in Figure 1, The Water Column Toxicity Test Decision Tree, and Figure 2, the LC50 Decision Tree. Included in these analyses are:

mean survival for dilution water and elutriates
Shapiro-Wilk's Test for normality
t-test for equal or unequal variances
t-test for unequal variances on data converted to rankits

Refer to the decision tree in Figure 1 and 2 to determine which test results are to be used. Minimum significant difference and some other power calculations for parametric t-test are also provided.

Input Screens with Example Data

This example problem is found in Appendix D of EPA's Testing Manual entitled "Evaluation of Dredged Material Proposed for Ocean Disposal" (EPA/USACE 1994). Once the user executes the LAT-R program, the title page, Figure 3, and the main menu, Figure 4, appear.

Laboratory Analysis of Toxicity: CDF Effluent (LAT-E)

By
Dr. Charles Lee, Dr. Paul Schroeder, Dennis Brandon, and Anthony Gibson
Environmental Laboratory
USAE Waterways Experiment Station

POC: Mr. Dennis Brandon 601 634-2807
Dr. Paul Schroeder 601 634-3709

EPA/USACE. 1992. "Evaluating Environmental Effects of Dredged Material Management Alternatives -- A Technical Framework," EPA-842-B-92-008, US Environmental Protection Agency, Washington, DC.

EPA/USACE. 1994. "Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. - Testing manual (Draft)," EPA-823-B-94-002, US Environmental Protection Agency, Washington, DC.

Lee, C. R., et al. 1991. "General Decisionmaking Framework for Management of Dredged Material," Miscellaneous Paper EL-91-1, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

Press any key to continue...

Figure 3. The Title Page.

MAIN MENU OPTIONS	
1	Enter/Edit Data
2	Read/Save Data File
3	Edit Organism/Species List and Test Standards
4	Perform Calculations & View Output
5	Print Output
6	Exit
ACTIVE DATA FILE: EXAMPLE1.LEI	
TITLE: Example Run 1 (10 Dec. 1996)	
<p style="text-align: center;"> <↑↓> Select Option <CR Enter> Execute </p>	

Figure 4. The Main Menu Options.

Figure 5 shows how the user may input the title. The example input title is " Example Run 1 (10 Dec. 1996)".

WATER SAMPLE IDENTIFICATION	
Enter Site Description for the Water Samples.	
<p style="text-align: center;"> Example Run 1 (10 Dec. 1996) </p>	
<p style="text-align: center;"> <Home>Menu <↑>MoveUp <↓>MoveDown <PgUp>PrevScr <PgDn>NextScr </p>	

Figure 5. The Entry for the Title.

Figure 6 shows the default list of organisms which currently are available for analyzing the bioassay test results. Other organisms which are not on this list can be added to the default list by selecting option 3 from the main menu, "Edit organism list and standards". In Figure 6 the up and down arrow keys were used to highlight the organism " DAPHNIA PULEX ". The ENTER Key is pressed to select this organism for analysis. A maximum of 10 organisms can be selected per run from the default list.

Page 1 of 1 Pages	
ORGANISMS	
C. STIGMEUS	*MYTILUS EDULIS
CERTODAPHNIA DUBIA	NEOMYSYS AMERICANA
CITHARICHTHYS STIGMAEUS	ONCORHYNCHUS MYKISS
CRASSOSTREA SP.	PALAEEMONETES SP.
CYPRINODON VARIEGATUS	PENAEUS SP.
DAPHNIA MAGNA	PIMEPHALES PROMELAS
*DAPHNIA PULEX	STRONGYLOCENTROTUS SP.
DENDRASTER SP.	
H. SCULPTA	
HOLMESIMYSIS COSTATA	
*ICTALURUS PUNCTATUS	
LEPOMIS MACROCHIRUS	
LEURESTHES TENUIS	
LYTECHINUS PICTUS	
MENIDIA SP.	
MYSIDOPSIS SP.	

<Enter>Execute <↑>MoveUp <↓>MoveDown <PgUp> PrvScr <Home> Menu <ESC> EXIT

Figure 6. The Default List of Organisms.

Figure 7 shows input for the number of dilution water replicates, the standard survivor rate, and the time of exposure. Figure 8 shows input for the number of elutriate treatments and their values. Figure 9 shows input for the number of replicates for each treatment. Figure 10 shows the input of the initial number of organisms at the start of the test. Figure 11 shows input for the number of survivors at the end of the test.

```

┌──────────────────────────▶ELUTRIATE TOXICITY TEST◀──────────────────────────┐
│                                                                              │
│ The organism selected is:    DAPHNIA PULEX                                │
│                                                                              │
│ Enter the number of dilution water replicates.    5                    │
│                                                                              │
│ Enter the acceptable standard survival rate.        90.0 %                 │
│                                                                              │
│ What is the length of exposure?                    96.0 hrs                │
│                                                                              │
└──────────────────────────<Esc> Exit <Home> Main Menu <Pg Up> Previous Menu <Pg Dn> NEXT Menu──────────────────────────┘

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Figure 7. The Entries for Dilution Water Replicates, Survival Rate, and Exposure.

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┌──────────────────────────▶ELUTRIATE TOXICITY TEST◀──────────────────────────┐
│                                                                              │
│ Treatments are percent concentrations of elutriate dilutions:             │
│      (Examples)                                                            │
│ 100% = 1 part elutriate plus 0 part dilution water                       │
│ 50%  = 1 part elutriate plus 1 part dilution water                       │
│ 25%  = 1 part elutriate plus 3 part dilution water                       │
│                                                                              │
│ Enter the number of elutriate treatments. (Maximum of 9) 4              │
│                                                                              │
│ Enter the elutriate treatment values.                                     │
│                                                                              │
│      Treatment 1      100.0%                                             │
│      Treatment 2      50.0%                                              │
│      Treatment 3      25.0%                                              │
│      Treatment 4      12.5%                                              │
│                                                                              │
└──────────────────────────<Esc> Exit <Home> Main Menu <Pg Up> Previous Menu <Pg Dn> NEXT Menu──────────────────────────┘

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Figure 8. The Entries for Elutriate Treatments.

▶ELUTRIATE TOXICITY TEST◀

The organism selected is: DAPHNIA PULEX

(A minimum of 3 replicates is required per treatment.)
 (A maximum of 9 is allowed.)

Enter the number of replicates for treatment 100.0%

Enter the number of replicates for treatment 50.0% 5

Enter the number of replicates for treatment 25.0% 5

Enter the number of replicates for treatment 12.5% 5

<Esc> Exit <Home> Main Menu <Pg Up> Previous Menu <Pg Dn> NEXT Menu

Figure 9. The Entries for the Number of Replicates per Treatment.

▶ELUTRIATE TOXICITY TEST◀

Enter the number of organisms per replicate at test initiation.

Replicate Number	Dilution Water	Treatments (%)			
		100.0	50.0	25.0	12.5
1	<input type="text" value="20"/>	20	20	20	20
2	20	20	20	20	20
3	20	20	20	20	20
4	20	20	20	20	20
5	20	20	20	20	20

<Esc> Exit <Home> Main Menu <Pg Up> Previous Menu <Pg Dn> NEXT Menu

Figure 10. The Entries for the Initial Number of Organisms.

▶ELUTRIATE TOXICITY TEST◀

Enter the number of survivors per replicate.

Replicate Number	Dilution Water	Treatment (%)			
		100.0	50.0	25.0	12.5
1	20	6	8	12	17
2	19	7	8	18	17
3	20	9	9	15	18
4	20	5	10	14	16
5	19	8	11	13	18

<Esc> Exit <Home> Main Menu <Pg Up> Previous Menu <Pg Dn> NEXT Menu

Figure 11. The Entries for the Number of Survivors.

Output Results of Example Data

The output from the LAT-R Model is presented with results from the example data. Figure 12 consists of the output printed as a result of the example data run. The date, time, title, and the name of the organism are printed. Next, the number of organisms per replicate at initiation of test, the number of replicates, the dilution water, and the concentration treatments are printed.

The mean percent survival in the dilution water and EPA's acceptable standard survival rate are printed. Also at this time the program compares these two rates to test if percent mean survivor in the dilution meets EPA's standard.

Next, the percent treatment concentration, the number of survivors, and the number of replicates are printed, along with the mean survival and the percent mean survival for the dilution water and the treatment concentrations.

Finally, the median lethal concentrations (LC50) are printed from calculations produced by the PROBIT and the TSK Models. However, 1 percent of the LC50 value is used as the toxicity standard concentration to be entered in a mixing zone model (Chase, 1994).

12/10/96 11:26:37

Example Run 1 (10 Dec. 1996)

DAPHNIA PULEX

Test Duration: 96.0 Hours

NUMBERS OF ORGANISMS PER REPLICATE AT INITIATION OF TEST.

DILUTION					
REPLICATE	ATER	100.0	50.0	25.0	12.5
1	20	20	20	20	20
2	20	20	20	20	20
3	20	20	20	20	20
4	20	20	20	20	20
5	20	20	20	20	20

----- TREATMENTS & NUMBER OF SURVIVORS -----					
DILUTION					
REPLICATE	WATER	100.0	50.0	25.0	12.5
1	20	6	8	12	17
2	19	7	8	18	17
3	20	9	9	15	18
4	20	5	10	14	16
5	19	8	11	13	18
Mean	19.6	7.0	9.2	14.4	17.2
% SURVIVAL	98.0	35.0	46.0	72.0	86.0

Figure 12. Output Results.

THE PERCENT SURVIVORS IN THE DILUTION WATER ARE: 98.0%
THE ACCEPTABLE STANDARD SURVIVOR PERCENTAGE IS: 90.0%

PROBIT METHOD: The LC50 value is 54.50 %
TSK METHOD: The LC50 value is 49.93 %

COMPARE 0.01 LC50 TO PREDICTED CONCENTRATION BASED ON MIXING MODEL.

Figure 13. LC50 Output.

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