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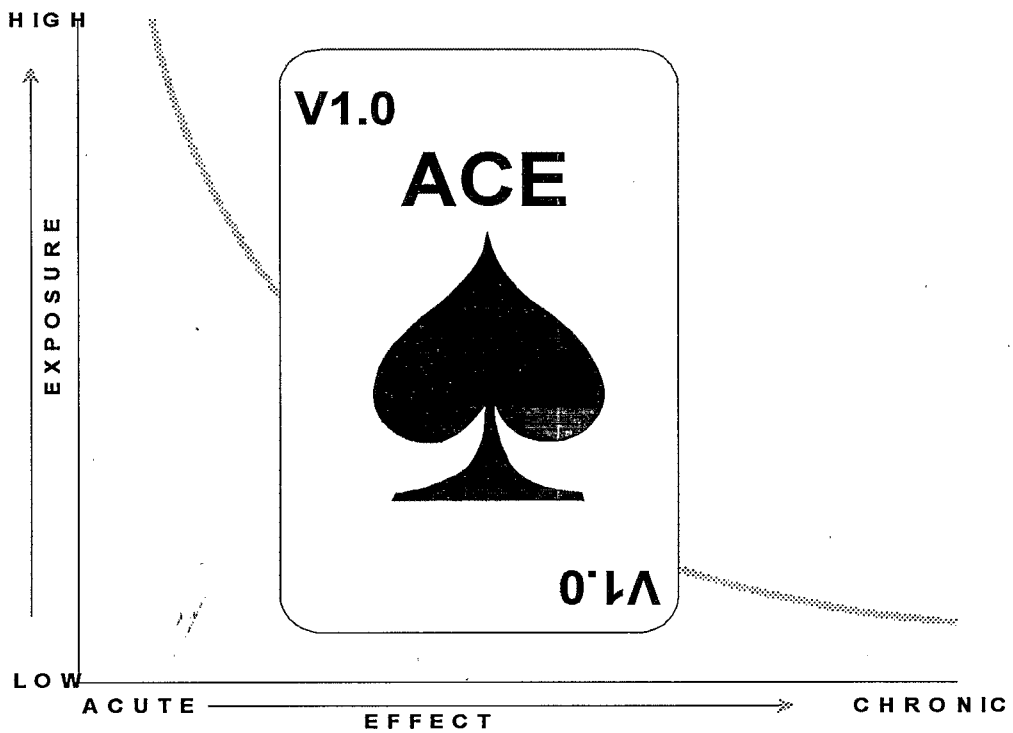
# User Guide
# Acute to Chronic Estimation



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# User Guide
# Acute to Chronic Estimation

Foster L. Mayer, Jr.
U.S. Environmental Protection Agency
Gulf Ecology Division, NHEERL, ORD
1 Sabine Island Drive
Gulf Breeze, FL 32561

Kai Sun, Gunhee Lee, Mark R. Ellersieck, and Gary F. Krause
University of Missouri-Columbia
Agricultural Experiment Station
Columbia, MO 65211

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# 1. INTRODUCTION

Acute and chronic toxicity testing play a major role in ecological risk assessment requirements involved in several environmental laws. Chronic toxicity tests commonly include measurement of long-term effects of a contaminant on survival, growth, and reproduction of test organisms. Such studies generally are expensive, high-risk investigations, sometimes requiring months to a year to conduct. Consequently, development of alternative estimation methods that provide similar information on chronic toxicity with less effort and expense is highly desirable. The Acute to Chronic Estimation (ACE) software application involves a major advancement in the area of ecological risk assessment and provides a reliable tool and technical basis to improve chronic prediction assessment for hazard.

Environmental toxicologists are often interested in determining no-observed-effect concentrations of a chemical or effluent to an organism exposed for extended (chronic) periods of time. In the past, various acute-chronic ratio and correlation analyses of acute (EC and LC50s) and chronic data (maximum acceptable toxicant concentrations, MATC) were used and refined to estimate chronic toxicity from acute data. Using acute lethality data to estimate chronic toxicity involved deriving an application factor, AF (Mount and Stephan 1967), or an acute-chronic ratio, ACR (Kenaga 1982); both require acute and chronic testing. The AF is derived by dividing the MATC for a compound, as determined in a chronic test with a given species, by the LC50 value determined with the same compound and species in an acute flow-through toxicity test. The MATC is used as a range [no-observed-effect concentration, (NOEC) to lowest-observed effect concentration, (LOEC)] or the geometric mean of the NOEC and LOEC. The ACR is essentially the inverse of the AF. The AF or ACR is then used to estimate the MATCs for other species for which only acute toxicity data exist. Both the AF and ACR approaches have worked reasonably well, but both have limitations and a degree of uncertainty in estimating chronic toxicity.

One limitation is that biological endpoints and degrees of response may not be comparable between acute and chronic toxicity data. When the AF or ACR method is used, the acute median lethal concentration (LC50) is compared with the MATC, which may be an endpoint other than lethality (e.g., growth or reproduction). Secondly, although different degrees of response (acute 50% versus chronic 0%) may be used when the response slopes are similar, the slopes can be different. Additionally, the AF or ACR method does not take into consideration the progression of lethality through time that occurs in acute toxicity tests. The acute toxicity value represents only one point in time (e.g., 96-h LC50), and duration of exposure is essential when one predicts chronic toxicity from acute toxicity data with any degree of certainty.

New alternatives and more comprehensive statistical approaches have been devised recently (Mayer et al. 1994, Sun et al. 1995). The two new methods give consideration simultaneously to concentration of toxicant, degree of response, and time course of effect. These new methods use all acute data -- not just one point in time. The result is a function which can predict a toxicant concentration at a specified percent survival and the exposure time required to observe that response. Thus, a toxicant concentration can be calculated that will kill only a small percent of a population

(e.g., 0.01%) at chronic exposure times. These calculations are based solely on acute toxicity test data, and do not require conducting a chronic toxicity test.

The ACE software package contains two statistical methods for predicting chronic lethality of chemicals to aquatic organisms from acute toxicity test data. The package was cooperatively developed by the U.S. Environmental Protection Agency (Gulf Ecology Division, NHEERL, ORD) and the University of Missouri-Columbia (Agricultural Experiment Station). Two articles describing the scientific basis and explaining the two methods were published in the *Journal of Environmental Toxicology and Chemistry*.

The first method in ACE is a two-step Linear Regression Analysis (LRA). This method estimates LC values at each time period of observation and regresses the LC values as the dependent variable versus the reciprocal of time as the independent variable (Mayer et al. 1994). The point of interest is the Y intercept which is interpreted as the LC value at time infinite or chronic time.

The second method is a survival analysis approach based on Accelerated Life Testing (ALT) theory (Sun et al. 1995). This method originally was used for mechanical devices which were placed under sort-term or "acute" stress (e.g., a generator runs constantly at full power in high heat) to predict long-term or "chronic" time to failure. In this software, the method is applied to biological organisms which are placed under acute stress (i.e., toxicant), and the variable measured is time to failure or death.

The computer program of the LRA method was written by Gunhee Lee (Lee et al. 1992), and the computer program of the ALT method was written by Kai Sun (Sun et al. 1994). Documentation for both programs are also included in the respective references. The projects were funded by the U.S. Environmental Protection Agency and are combined here into one software package (Acute to Chronic Estimation or ACE).

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FOOTNOTE: A third method (not included in ACE) is called Multifactor Probit Analysis or MPA (Lee et al. 1995), and is a multiple regression model. It uses several linear models that simultaneously evaluate the relationship among chemical concentration, time, and probit mortality to predict chronic response. Most toxicologists are familiar with probit analysis in which LC values are computed at a specific time. The MPA method uses all times and concentration data simultaneously. If data are taken at different times (e.g. 24, 48, 72, 96 h), the MPA forecasts LC values to chronic times at a low LC percent (e.g., 0.01% mortality). This method can also be used as an alternative procedure of estimating acute toxicity values.

The MPA model is most appropriate when different experimental units are present for concentration-time combinations (i.e., where one complete replicate is removed at one or more time intervals to conduct a measurement different than survival; only the remaining replicates are used for the remainder of the toxicity test). Also, the MPA requires three partial kills. The MPA program can be obtained from the NTIS (Lee et al. 1992).

## 2. DISCUSSION OF METHODS

The two chronic lethality prediction methods in the ACE package were tested using a real data base of a variety of chemicals and fish species (Mayer et al. 1992). The data from the acute tests were analyzed to predict chronic no-effect values for lethality, and actual chronic test data (lethality) for the same chemical-species combinations (28) were used to check and validate the predicted results. The NOECs predicted by the methods were well matched, in most cases, with actual NOECs from chronic toxicity experiments. NOEC values that did not match well were mainly due to a lack of partial kills, depending on the model used. Although the acute-to-chronic models in ACE generally perform well in predicting NOECs, a number of questions remain. For example, model diagnosis, plot of model adequacy, and criteria for selecting each model need further investigation.

Some brief guidelines for using ACE and selecting the appropriate models are described as follows.

1. Historically, three testing techniques have been used to determine acute toxicity: flow-through, static renewal, and static. Acute toxicity test data used in ACE should be based on flow-through or static renewal techniques. Analyses based on the static technique may give erroneous results except for chemicals that are highly water soluble (see Fluridone, Mayer et al. 1994).

2. With experimental designs most commonly used in acute toxicity testing, the ALT is the method of choice followed by the LRA.

3. When using method 1 (LRA), there are six combinations of models and transformations to choose from. Associated with each case and its LC percentage, there is a R-SQUARE. The larger the R-SQUARE value, the better the model fits the data. The largest R-SQUARE among the six cases using the same data and the same LC percentage is used as the criterion to choose the final analysis in LRA.

4. The dependability of the chronic lethality value predicted is enhanced with increasing numbers of partial kills. However, the models will function with the following numbers of partial kills: LRA=0 and ALT=1. It is not uncommon to conduct high quality tests where no partial kills occur, only 0 and 100%, and it is usually not justifiable in terms of the time or effort to rerun the test with more finely graded exposure concentrations. Under these conditions, the LRA is the method of choice.

5. We recommend the following percent values for predicting chronic toxicity: LRA = 0.01% and ALT = 1.0%. The value of 0.01% represents a very close approximation to zero on the probit scale (Mayer et al. 1994). Use of 0.01% for the LRA model also corresponds well to statistically-based no-effect concentrations in chronic toxicity tests using hypothesis testing techniques (analysis of variance). ALT differs in that 1.0% is presently considered as the smallest detectable difference using this technique, due to small numbers of organisms usually exposed in each concentration. However, use of the 1.0% value does approximate chronic no-effect-values derived from hypothesis

testing. The authors believe that selection of 0.01% when using the ALT may result in the "absolute" no-effect concentration if a very large number of organisms were tested acutely. However, this question will be addressed in future research and will require validation by testing large numbers of organisms under chronic exposures.

# 3. GETTING STARTED

## Computer Requirements

1. IBM-PC or compatible computer with math-processor.
2. 500K of free RAM memory.
3. PC-DOS or MS-DOS version 3.1 or later.

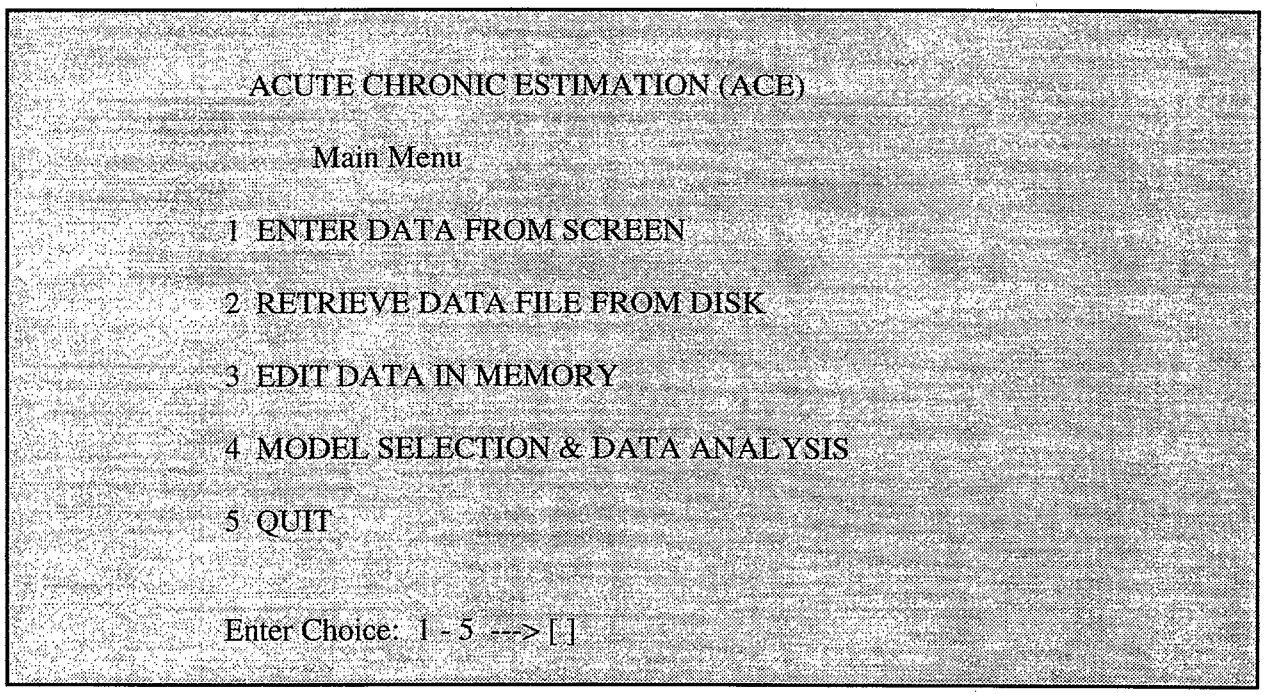
## Installation

1. Insert the program disk into your floppy disk drive A.
2. At the C:> prompt, type COPY A:*.* and press {ENTER}.
3. Type ACE and press {ENTER} to start the program.

Note: If you experience "out of memory" while running the program, reboot the computer with the program disk in drive A.

## Data Entry

Once the program started, a Logo appears. Press the <enter> key, and then the Main Menu appears:




This software has its own data entry system. The format of data is the same for all procedures. If the data set is entered for one procedure, it can be used for the other procedures. Data

can be entered from other software such as Lotus 1-2-3, Excel, Wordperfect, dBase, etc. However, the data file must be converted to a text or ASCII file in order to be read by the ACE. The following example data (Mayer et al. 1975) will be used for all methods in the ACE.

| | TIME OF OBSERVATION (h) | | | |
|---|---|---|---|---|
| CONCENTRATION | 24 | 48 | 72 | 96 |
| 0 | 0 | 0 | 0 | 0 |
| 2.0 | 0 | 0 | 0 | 0 |
| 2.9 | 0 | 0 | 0 | 4 |
| 4.2 | 0 | 0 | 9 | 22 |
| 6.2 | 0 | 10 | 26 | 26 |
| 8.2 | 0 | 23 | 26 | 26 |
| 11 | 2 | 26 | 26 | 26 |
| 16 | 25 | 26 | 26 | 26 |

To enter the data, one must use the following format:

| Concentration | Time (h) | Number of organisms | Number dead |
|---|---|---|---|
| 0 | 24 | 26 | 0 |
| 2 | 24 | 26 | 0 |
| 2.9 | 24 | 26 | 0 |
| 4.2 | 24 | 26 | 0 |
| 6.2 | 24 | 26 | 0 |
| 8.2 | 24 | 26 | 0 |
| 11 | 24 | 26 | 2 |
| 16 | 24 | 26 | 25 |
| 0 | 48 | 26 | 0 |
| 2 | 48 | 26 | 0 |
| 2.9 | 48 | 26 | 0 |
| 4.2 | 48 | 26 | 0 |
| 6.2 | 48 | 26 | 10 |
| 8.2 | 48 | 26 | 23 |
| 11 | 48 | 26 | 26 |
| 16 | 48 | 26 | 26 |
| 0 | 72 | 26 | 0 |
| 2 | 72 | 26 | 0 |
| 2.9 | 72 | 26 | 0 |
| 4.2 | 72 | 26 | 9 |
| 6.2 | 72 | 26 | 26 |
| 8.2 | 72 | 26 | 26 |
| 11 | 72 | 26 | 26 |
| 16 | 72 | 26 | 26 |
| 0 | 96 | 26 | 0 |
| 2 | 96 | 26 | 0 |

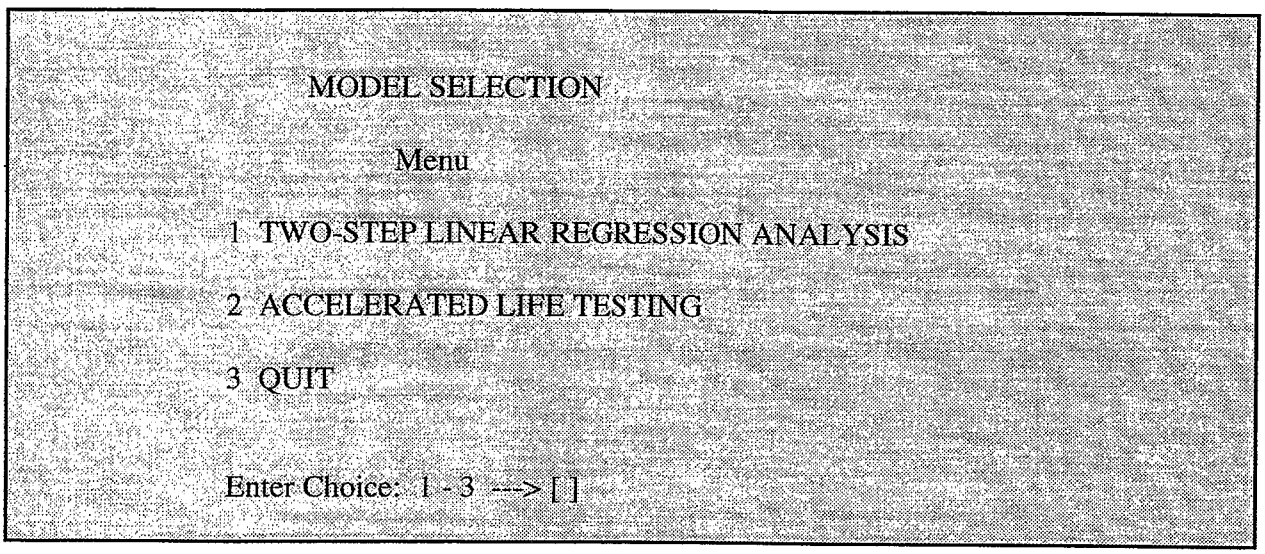
Table continued:

| Concentration | Time (h) | Number of organisms | Number dead |
|---|---|---|---|
| 2.9 | 96 | 26 | 4 |
| 4.2 | 96 | 26 | 22 |
| 6.2 | 96 | 26 | 26 |
| 8.2 | 96 | 26 | 26 |
| 11 | 96 | 26 | 26 |
| 16 | 96 | 26 | 26 |

**Requirements of data quality**

1. Number of partial kills: method 1 works with data having only 0 and 100% responses; method 2 works with data having at least one partial kill.
2. Use number dead, do not use % mortality.
3. For acute test data, time must be in hours.
4. The numbers of total test organisms need to be the same across doses.
5. Control group (i.e., zero concentration group) is needed.

After entering data, press Esc and go back to the main menu. Press 4 and then go to the MODEL SELECTION menu.



## 4. TWO-STEP LINEAR REGRESSION ANALYSIS (METHOD 1)

If number 1 is entered from the MODEL SELECTION menu, the following menu appears.

```
PREDICTING CHRONIC LETHALITY USING LINEAR REGRESSION
          MAIN PROGRAM MENU


1  DEFINE A TITLE
2  STATISTICAL ANALYSIS
3  QUIT

CHOOSE 1-3 (enter a single number, you do not need to press <enter>)
_____________________________________________________

          CURRENT PROGRAM STATUS

LAST DISK FILE READ .....
LAST DISK FILE WRITTEN ON ..
TITLE .....
```

This method performs a probit analysis and a simple linear regression of concentration versus probit percent responding at each time. One of the requirements is that some partial responses occur. If at a specific time, the probit analysis fails and/or the regression analysis fails, the following prompt appears.

```
ESTIMATION OF LEAST SQUARE REGRESSION HAD LESS THAN 3 OBS. AT __
HOURS.

DO YOU WISH TO INCLUDE MAXIMUM CONCENTRATION WITH NO MORTALITY
FOR FURTHER REGRESSION ANALYSIS ? (Y/N)

NOTE: MAXIMUM CONCENTRATION WITH NO MORTALITY IS ___
```

If the data at a time period is not all 0% responding or 100% responding, it is suggested to enter Y for YES. This approach allows for use of data having only 0 and 100% responses, with no partial responses required.

In some cases, the individual response at dose level 0 control is not 0%, and then the following menu appears.

```
NON-ZERO RESPONSE IS PRESENT AT DOSE LEVEL 0.

1  STOP PROCESSING.
2  IGNORE RESPONSE AT DOSE LEVEL 0.
3  ADJUST RESPONSE USING ABBOTT'S FORMULA.

CHOOSE 1-3 (enter a single number, you do not need to press <enter>)
```

Finney (1971) suggests that the data be adjusted using Abbott's formula. At this point you may choose that option. However, based on the authors' knowledge, if the control mortality is not greater than 10%, do not adjust data and enter number 2 (IGNORE RESPONSE AT CONCENTRATION LEVEL 0). This is an accepted practice in toxicity testing. If the percent responding at concentration level 0 is greater than 10%, the entire experiment should be rerun. If all control values at all times have a percent mortality between 0 and 10%, Abbott's formula is suggested. Once the statistical analysis is completed by entering number 6 from the MAIN PROGRAM MENU, the following output menu appears.

```
OUTPUT MENU

1  ON THE SCREEN
2  ON A PRINTER
3  ON A DISK
4  QUIT

CHOOSE 1-4 (enter a single number, you do not need to press <enter>)
```

This method produces 6 pages of output. The basic equation of CONCENTRATION = INTERCEPT + SLOPE/TIME is the same except that the value of dose is either based on probit or least square analysis from the first analysis and log10 transformation may or may not be used for DOSE and TIME.

**Example Output**

A probit analysis and a least square regression analysis are performed separately for each time. After LC percent value of 0.01, 0.1, 1, 5, 10, 20 and 50 percent have been generated by the first analysis (either least square or probit), a second regression equation (CONCENTRATION = INTERCEPT + SLOPE/TIME) is calculated along with confidence intervals on the slope and intercept (Mayer et al. 1994). The output includes:

A. The description of the model and data transformation. The first step is a least square or a probit analysis. The second one describes the transformation used on the second step regression equation CONCENTRATION = INTERCEPT + SLOPE/TIME.

Since the equation is based on 1/TIME as the X axis; as time approaches infinity, the axis approaches 0. Thus, Y intercept is interpreted as LC value at time infinity which is reflective of chronic exposure.

B. The LC percentage or mortality.

C. The predicted concentration (Y intercept or the LC value at X% mortality and time infinity).

D. 95% confidence interval represented by ± 2 standard errors (SE). The ± 2SE are based on the last analysis of this procedure and none of the variances from the first analysis is included. Thus, SE may be smaller than actual.

E. $R^2$ describes how well the model fits the data. The analysis selected should be the one having the highest $R^2$ at the % mortality of interest.

EXAMPLE

For 0.01% mortality in the following example (data on disk as fish.dat), the first model should be selected since it has the highest $r^2$ value. For this particular data set, however, results for the second method (Accelerated Life Testing or ALT) should be used since it is the model of choice when adequate data exists.

## LINEAR REGRESSION ANALYSIS MODEL 1

REGRESSION ANALYSIS OF CONCENTRATION VERSUS TIME
MODEL: CONCENTRATION = INTERCEPT + SLOPE/TIME
(LEAST SQUARE REGRESSION AT EACH TIME)

| % Mortality | Predicted Concentration (Infinite Hours) | 95% Confidence Intervals[a] | | $R^2$ |
|---|---|---|---|---|
| 0.01% | .041196 | -.569211 | .651603 | .998326 |
| 0.1% | .062094 | -.410405 | .534593 | .999136 |
| 1% | .093015 | -.198878 | .384907 | .999725 |
| 5% | .126563 | -.085054 | .338179 | .999877 |
| 10% | .147069 | -.126421 | .420559 | .999811 |
| 20% | .174722 | -.249060 | .598504 | .999591 |
| 50% | .236612 | -.584811 | 1.058035 | .998741 |

[a] ± 2 Standard Errors

## LINEAR REGRESSION ANALYSIS MODEL 2

REGRESSION ANALYSIS OF LOG10 (CONCENTRATION) VERSUS TIME
MODEL: LOG10 (CONCENTRATION) = INTERCEPT + SLOPE/TIME
(LEAST SQUARE REGRESSION AT EACH TIME)

| % Mortality | Predicted Concentration (Infinite Hours) | 95% Confidence Intervals[a] | | $R^2$ |
|---|---|---|---|---|
| 0.01% | 1.498081 | .720607 | 3.114382 | .950105 |
| 0.1% | 1.631971 | .820941 | 3.244241 | .955264 |
| 1% | 1.810830 | .955353 | 3.432349 | .960481 |
| 5% | 1.986849 | 1.085468 | 3.636742 | .964090 |
| 10% | 2.087588 | 1.158007 | 3.763380 | .965589 |
| 20% | 2.216940 | 1.248328 | 3.937126 | .966994 |
| 50% | 2.485526 | 1.423314 | 4.340463 | .968354 |

[a] ± 2 Standard Errors

LINEAR REGRESSION ANALYSIS MODEL 3

REGRESSION ANALYSIS OF LOG10 (CONCENTRATION) VERSUS LOG10 (TIME)
MODEL: LOG10 (CONCENTRATION) = INTERCEPT + SLOPE/LOG10 (TIME)
(LEAST SQUARE REGRESSION AT EACH TIME)

| % Mortality | Predicted Concentration (Infinite Hours) | 95% Confidence Intervals[a] | | $R^2$ |
|---|---|---|---|---|
| 0.01% | .096474 | .017815 | .522456 | .977930 |
| 0.1% | .107020 | .023624 | .484809 | .982044 |
| 1% | .121395 | .032292 | .456359 | .985930 |
| 5% | .135838 | .041000 | .450053 | .988294 |
| 10% | .144229 | .045638 | .455811 | .989108 |
| 20% | .155129 | .050841 | .473339 | .989662 |
| 50% | .178189 | .057986 | .547565 | .989346 |

[a] ± 2 Standard Errors

LINEAR REGRESSION ANALYSIS MODEL 4

REGRESSION ANALYSIS OF CONCENTRATION VERSUS TIME
MODEL: CONCENTRATION = INTERCEPT + SLOPE/TIME
(PROBIT ANALYSIS AT EACH TIME)

| % Mortality | Predicted Concentration (Infinite Hours) | 95% Confidence Intervals[a] | | $R^2$ |
|---|---|---|---|---|
| 0.01% | -.532398 | -4.571520 | 3.506725 | .924033 |
| 0.1% | -.979421 | -10.948431 | 8.989588 | .983776 |
| 1% | -.666491 | -8.475493 | 7.142510 | .991689 |
| 5% | -.387357 | -6.269628 | 5.494914 | .995941 |
| 10% | -.238531 | -5.093533 | 4.616470 | .997438 |
| 20% | -.057631 | -3.663962 | 3.548701 | .998707 |
| 50% | .286474 | -.944669 | 1.517616 | .999872 |

[a] ± 2 Standard Errors

LINEAR REGRESSION ANALYSIS MODEL 5

REGRESSION ANALYSIS OF LOG10 (CONCENTRATION) VERSUS TIME
MODEL: LOG10 (CONCENTRATION) = INTERCEPT + SLOPE/TIME
(PROBIT ANALYSIS AT EACH TIME)

| % Mortality | Predicted Concentration (Infinite Hours) | 95% Confidence Intervals[a] | | $R^2$ |
|---|---|---|---|---|
| 0.01% | 1.321393 | .643829 | 2.712024 | .957402 |
| 0.1% | 1.315983 | .193912 | 8.930916 | .986684 |
| 1% | 1.539801 | .198665 | 11.934594 | .983754 |
| 5% | 1.771380 | .203003 | 15.456851 | .980709 |
| 10% | 1.908770 | .205355 | 17.742010 | .978898 |
| 20% | 2.090198 | .208250 | 20.979281 | .976503 |
| 50% | 2.484262 | .213870 | 28.856629 | .971292 |

[a] ± 2 Standard Errors

LINEAR REGRESSION ANALYSIS MODEL 6

REGRESSION ANALYSIS OF LOG10 (CONCENTRATION) VERSUS LOG10 (TIME)
MODEL: LOG10 (CONCENTRATION) = INTERCEPT + SLOPE/LOG10 (TIME)
(PROBIT ANALYSIS AT EACH TIME)

| % Mortality | Predicted Concentration (Infinite Hours) | 95% Confidence Intervals[a] | | $R^2$ |
|---|---|---|---|---|
| 0.01% | .072678 | .009565 | .552238 | .971866 |
| 0.1% | .065626 | .014367 | .299760 | .999186 |
| 1% | .084482 | .010380 | .687607 | .998345 |
| 5% | .105831 | .007767 | 1.442007 | .997275 |
| 10% | .119338 | .006654 | 2.140169 | .996562 |
| 20% | .138099 | .005514 | 3.458495 | .995547 |
| 50% | .182310 | .003857 | 8.617365 | .993104 |

[a] ± 2 Standard Errors

## 5. ACCELERATED LIFE TESTING (METHOD 2)

If one chooses number 2 from the MODEL SELECTION menu, the following submenu appears.

```
ACCELERATED LIFE TESTING

     Menu

1  ENTER EXPOSURE TIME

2  STATISTICAL ANALYSIS

3  QUIT

Enter Choice:  1 - 3  --->[ ]
```

**Steps to Run the Program**

1. Enter the days of long-term exposure of interest. (Menu #1).
2. Run the program (Menu #2).
3. Print, view, and save results. An output menu presents after running the statistical analysis.

```
OUTPUT MENU

1  ON THE SCREEN

2  ON A PRINTER

3  GRAPH

4  QUIT

Enter Choice:  1 - 4  --->[ ]
```

A results report or graph may be viewed on the screen. The results report is

automatically saved as an output file (e.g., if a data file is fish.dat, an output file fish.out is created), and the file can be printed on a printer.

## Example Output

This procedure uses a Qasi-Newton method to find the maximum likelihood estimates of the parameters. Confidence intervals for parameters are based on normal approximations to distributions of the maximum likelihood estimates. The data set described previously (fish.dat) is used to illustrate the procedure. The default times of long-term exposure are 30, 60 and 90 days. Following data entry, the software program will carry out all of the calculations. The output includes:

A. Iteration generated as a result of solving the non-linear equations.

B. Estimate of model parameters.

C. Listing of variance covariance matrix.

D. The Predicted Concentration or No-Observed-Effect-Concentration (NOEC), including 95% confidence limits, can be the concentration causing mortalities of 0.01%, 0.05%, 0.1%, 0.5%, 1%, or 5%. The acceptable percentage is determined by the user. However, the authors recommend 1% at this time (see DISCUSSION OF METHODS).

---

ACCELERATED LIFE TESTING OUTPUT

---

**A.** FISH-OUT

| Iteration | Intercept | Shape (Concentration) | Shape (Time) |
|---|---|---|---|
| 0 | 3.59543954 | 6.55224212 | 6.42689656 |
| 1 | 3.53930751 | 6.59341717 | 6.39132355 |
| 2 | 3.63040812 | 6.72936222 | 6.31424798 |
| 3 | 3.62451964 | 7.56820538 | 7.16033003 |
| 4 | 3.62241908 | 7.39753660 | 6.98759628 |
| 5 | 3.62304127 | 7.37705121 | 6.96534248 |
| 6 | 3.62306413 | 7.37709530 | 6.96536033 |
| 7 | 3.62306381 | 7.37708749 | 6.96535205 |

---

| B. Parameter | Estimate | 95% Lower Limit | 95% Upper Limit |
|---|---|---|---|
| AA | 3.62306381 | 3.38666616 | 3.85946147 |
| B | 7.37708749 | 6.81879123 | 7.93538375 |
| C | 6.96535183 | 6.96208338 | 6.96862028 |
| A | .00007510 | .00000000 | .00016151 |
| C/B | .94418723 | .87273288 | 1.01564159 |

INTERPRETATION: A--measure of initial toxic strength; B--measure of mode of concentration-response; C--measure of mode of time-response; A=(1/AA)**b; C/B--measure of domination between concentration and time.

C. COVARIANCE MATRIX

| | AA | B | C |
|---|---|---|---|
| AA | .01454702 | .0285735 | .00000019 |
| B | .02857385 | .08113669 | .00000309 |
| C | .00000019 | .00000309 | .00000278 |

D. MAXIMUM LIKELIHOOD ESTIMATES FOR "NO-EFFECT" CONCENTRATIONS

30-day

| % Mortality | Predicted Concentration | 95% Confidence Limits | |
|---|---|---|---|
| 0.01% | .1161208 | .114125 | .208291 |
| 0.05% | .200512 | .145233 | .255791 |
| 0.1% | .220271 | .161098 | .279444 |
| 0.5% | .274047 | .204914 | .343180 |
| 1% | .301148 | .227304 | .374991 |
| 5% | .375609 | .289714 | .461504 |

60-day

| % Mortality | Predicted Concentration | 95% Confidence Limits | |
|---|---|---|---|
| 0.01% | .083783 | .055191 | .112376 |
| 0.05% | .104211 | .070358 | .138063 |
| 0.1% | .114480 | .078100 | .150860 |
| 0.5% | .142428 | .099505 | .185351 |
| 1% | .156513 | .110454 | .202572 |
| 5% | .195212 | .141003 | .249422 |

90-day

| % Mortality | Predicted Concentration | 95% Confidence Limits | |
|---|---|---|---|
| 0.01% | .057134 | .035990 | .078279 |
| 0.05% | .071064 | .045932 | .096196 |
| 0.1% | .078067 | .051010 | .105123 |
| 0.5% | .097126 | .065060 | .129191 |
| 1% | .106730 | .072251 | .141210 |
| 5% | .133120 | .092326 | .173914 |

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