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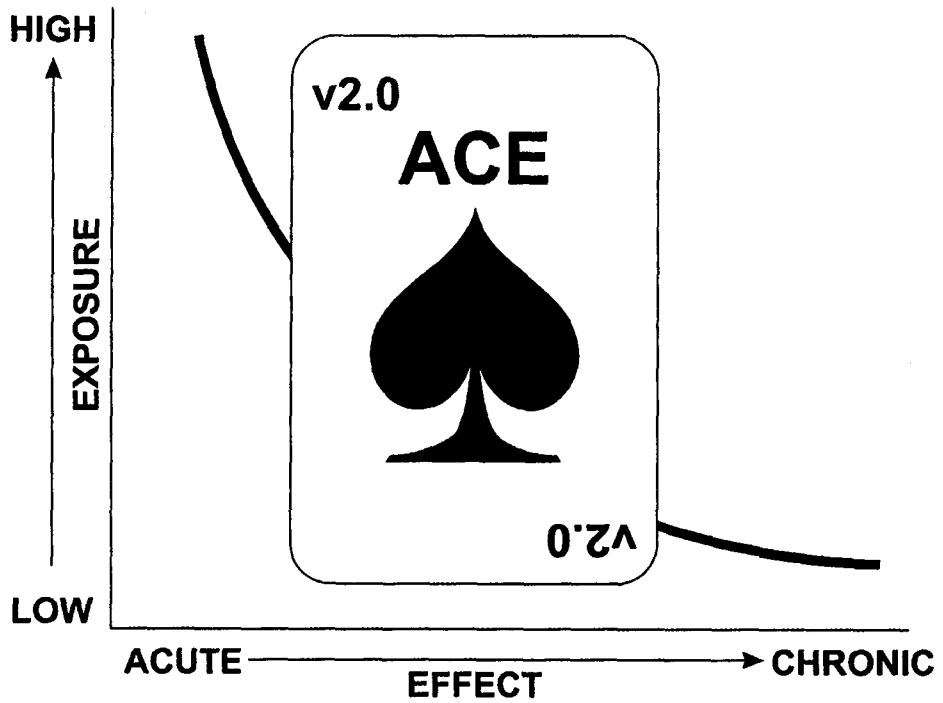
Acute-to-Chronic Estimation (ACE v 2.0) with Time-Concentration-Effect Models

User Manual and Software



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Acute-to-Chronic Estimation (ACE v 2.0) with Time-Concentration-Effect Models

User Manual and Software

By

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Notice

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Abstract

Predictive toxicological models, including estimates of uncertainty, are necessary to address probability-based ecological risk assessments. Methods and software (ACE) were developed for estimating chronic toxicity from raw acute toxicity data (all response observations at all times and exposures). Three methods were developed - - Accelerated Life Testing (ALT), Multifactor Probit Analysis (MPA), and two-stage Linear Regression Analysis (LRA). Of the three, the method of choice is ALT, in that time to failure (death) of each experimental unit is independent. It requires three partial responses over the time period of acute testing, but will function with one. The MPA is a two dimensional probit analysis using both time and concentration to produce a multiple regression equation, however, each experimental unit is not independent. Also, the MPA requires more partial responses than the ALT. The LRA calculates LC values for each time period and then regresses the LC values as the Y axis and the reciprocal of time as the X axis. The Y intercept is the chronic no-effect concentration. The LRA will function when ALT and MPA fail; no partial responses are required. All methods provide confidence limits for the point estimates. The methods have previously been shown to estimate chronic no-effect concentrations very well when validated against actual paired acute and chronic test results with fishes.

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Introduction

Both understanding and evaluating chronic toxicity of chemicals are essential to assessing their ecological hazards and making environmentally sound management decisions. Because of the large number and variety of industrial, agricultural and home-use chemicals released in the U.S. annually and the high cost and effort required for chronic tests, resources are often insufficient to obtain experimental information about long-term environmental impacts for all potentially hazardous chemicals. In comparison, acute tests are less costly and time consuming and, for these reasons, an abundance of acute toxicity data exists for numerous chemicals and organisms. Also, procedures have been developed for extrapolating effects data within classes of chemicals sharing similar chemical structures (Lipnick 1995). Thus, there is a strong rationale to relate acute and chronic toxicities of chemicals and to develop statistical and mathematical techniques to predict chronic toxicity based on data from acute experiments.

Use of short-term tests as a basis for linkage of exposure and time to response with chronic effects for ecological risk assessments is significant. The ability to accurately and precisely associate chronic effects from acute time-concentration-effect data is a powerful approach that integrates various aspects of toxicokinetics and directly addresses a variety of uncertainties in terms of chronicity. Three models were developed (Lee et al. 1995; Mayer et al. 1994, 2002; Sun et al. 1995b), tying together classical methods (e.g., probit regression) (Finney 1978) and time to event methods (Newman 1994) to provide models that predict chronic toxicity from acute toxicity data.

- Accelerated Life Testing (ALT) – A survival analysis and population-based approach (Weibull distribution) using accelerated life testing theory (Mayer et al. 2002, Sun et al. 1995b). The method was originally used for mechanical and electrical devices placed under short-term or "acute" stress (e.g., generator running constantly at full power and high heat) to predict long-term or "chronic" time to failure. In the ACE software, the model is applied to organisms placed under acute stress (i.e., toxicant), and the variable measured is time to failure or death. The model assumes that both exposure concentrations and duration affect survival probability, and hence, has the ability to summarize the entire concentration-time-response data of a toxicity test. Actual proportion responses are used; probit transformations are not applied. ALT also takes into account the spontaneous survival probability and is suitable to describe both acute and chronic lethality data. The survival function includes competing risks, with contaminant exposure being one.

- Multifactor Probit Analysis (MPA) – Multiple regression models that simultaneously evaluate the relationship among exposure concentration, time, and probit % mortality to predict chronic response (Mayer et al. 2002, Lee et al. 1995). This model is appropriate when different experimental units are present for concentration-time combinations (i.e., one complete replicate is removed at one or more time intervals for a measurement different than survival; only the remaining replicates are used for the remainder of the toxicity test). ALT and LRA models are more appropriate for predicting chronicity from standard acute toxicity data; however, multiple regression models, such as MPA, are necessary when estimating chronicity under changing conditions (e.g., varying exposure scenarios in effluents).

- Linear Regression Analysis (LRA) – A two-step linear regression analysis (Mayer et al. 1994, Mayer et al. 2002). This model combines two linear regressions: 1) estimates low lethal concentrations at each observation time period and 2) regresses those concentrations (dependent variable) against the reciprocal of time (independent variable), with the intercept being the chronic no-effect concentration. Probit transformations of percent response are used.

The software program, Acute-to-Chronic Estimation (ACE), described herein, allows the user to estimate chronic toxicity for a species from raw acute toxicity data with accuracy and precision. ACE will, therefore, greatly enhance the use of probability-based risk assessments for chemicals having minimal data sets. However, if a chronic test is to be conducted, ACE can be used to more accurately identify the range of exposure concentrations required. ACE is based on the Windows platform and is specifically designed

for estimating chronic toxicity and providing graphical and tabular presentation of results. ACE v 2.0 is an upgrade of the former DOS version (Mayer et al. 1999).

Background

Using acute mortality data to estimate chronic toxicity (survival, growth, reproduction) to aquatic organisms customarily involves deriving an application factor (Mount and Stephan 1967) or an acute-to-chronic ratio (Kenaga 1982), both of which require acute and chronic toxicity testing. Kenaga (1979) reviewed the principal measurements of the acute LC50, the maximum acceptable toxicant concentration (MATC), and the application factor (AF) used in determining chronic NOECs (highest concentration causing 0% or no statistically significant effect) for many chemicals. The AF is derived by dividing the MATC for a compound, as determined in a chronic toxicity test with a given species, by the acute LC50 for the same compound tested with the same species. The acute-to-chronic ratio (ACR) is the inverse of the AF. The AF or ACR is then used to estimate chronic NOECs for other species for which only acute toxicity data (EC or LC50s) exist (Buikema et al. 1982). These approaches have limitations.

One limitation is that the biological endpoints and degrees of responses are often not comparable between acute and chronic toxicity data. When either the AF or ACR is used, the acute median lethal concentration (EC or LC50) is compared with the MATC, often derived from an endpoint other than mortality. Although different degrees of response (acute 50% vs. chronic no-effect) could be used when response slopes are similar, the slopes may be different. Additionally, use of the AF or ACR method does not take into consideration the progression of mortality through time that is derived in acute toxicity tests. The concentration-time-response interaction has been addressed by Shirazi and Lowrie (1988), but they directed their efforts toward better defining the LC50. The acute toxicity value represents only one point in time (e.g., 96-h LC50), and the relationship of degree of response with duration of exposure should be essential when chronic toxicity is predicted from acute toxicity data.

Lethality and other toxic effects are dependent on both concentration of a chemical to which an organism is exposed and length of exposure time. It is a common practice to investigate the toxicity of new and existing chemicals and effluents using acute toxicity tests. This is done by observing mortality resulting from exposure to a series of chemical concentrations, usually at 24, 48, 72, and 96 h. Time course distinguishes acute from chronic toxicity and also relates them as an integrated and progressive process. A time to response approach gives a better understanding of the progression of toxic effects over time, and survival time modeling has shown great applicability in toxicological studies (Crane et al. 2002, Dixon and Newman 1991, Newman and Aplin 1992).

The models included here are more comprehensive approaches to predicting chronicity, both toxicologically and statistically. Simultaneous consideration is given to exposure concentration, degree of response, and time course of effect, all of which are usually included in describing the results of an acute toxicity test, but are seldom used in hazard assessment. A consistent endpoint (mortality) and degree of response (~0%) are used to predict long-term (chronic) lethality from acute toxicity test data. These calculations are based solely on raw acute toxicity test data and do not require conducting a chronic toxicity test. Estimated long-term (chronic) lethality values have previously been validated for accuracy with actual chronic no-effect values derived for 28 chemical-fish species combinations (Mayer et al. 2002).

Software Language

The ACE software is based on a Windows® platform and written in Visual Basic (Microsoft® Visual Basic 6.0 1987-2000). Subroutines (Fortran programs) in Visual Basic and Visual Fortran are required to call Fortran IMSL Routines necessary in certain calculations (Compaq Fortran 1999, Visual Numeric 1999).

Installing ACE

System Requirements

- Operates on Microsoft Windows 95, 98, 2000, NT and XP (Windows® 98 or later is suggested).
- Minimum 16 MB RAM (64 MB or greater is suggested).
- CPU speed of over 200 MHz is suggested; ACE will work with less, but is very slow.
- 6MB hard disk space.
- Mouse or pointing device.
- Printer (optional).

Remove any existing versions of ACE before installing the new one or malfunctions may occur.

To remove old ACE software:

1. Double click **My Computer**.
2. Double click **Control Panel**.
3. Double click **Add/Remove Programs**.
4. Click **ACE**.
5. Click **Delete** or **Change/Remove**.

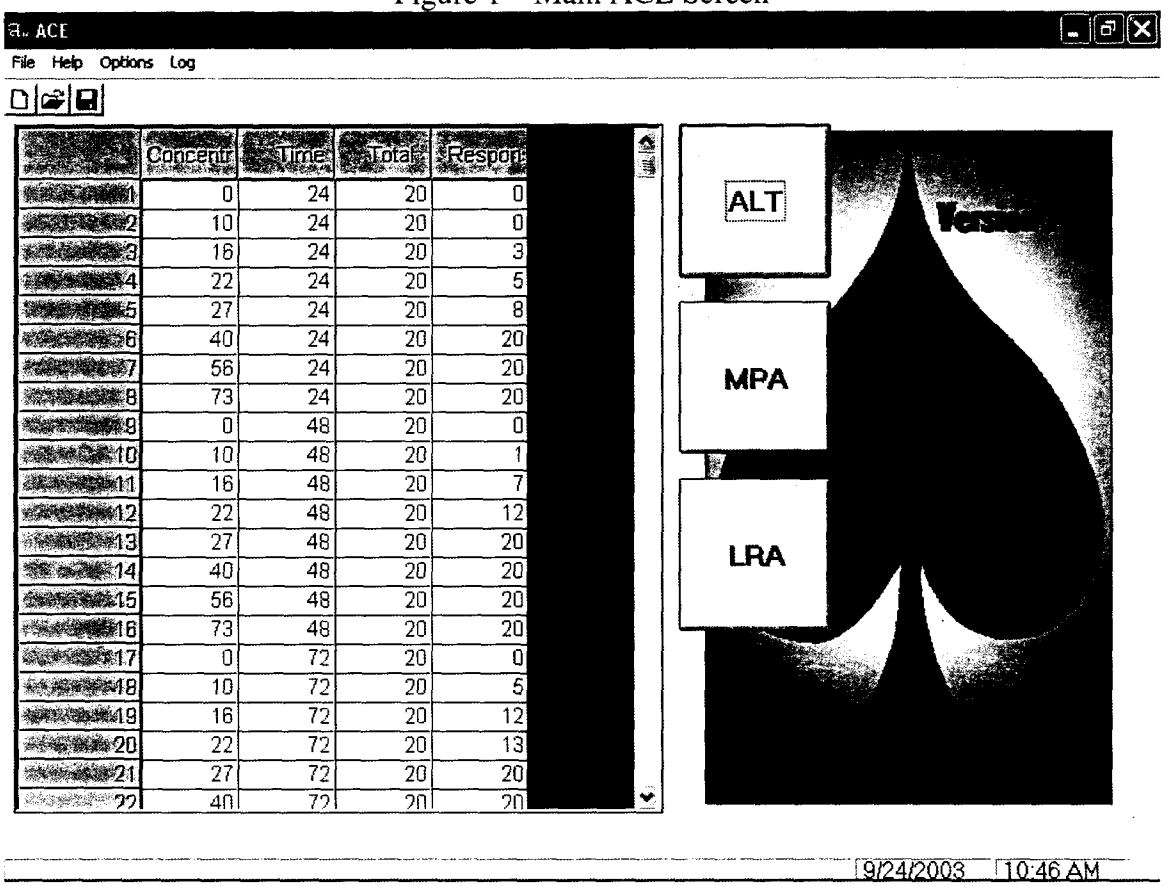
To install new ACE software:

1. Place the ACE CD in the CD ROM drive.
2. Click **Start** button.
3. Select **Run** from the menu.
4. Select **Browse** from the **Run** window.
5. Select drive letter associated with the CD drive from **Browse** window (or ACE 2003 [D:]).
6. Double-click **Setup** file or **D:\SETUP.EXE** file.
7. Click **OK**.
8. Windows now walks you through the installation process. If a "Yes" or "No" question is encountered, choose "Yes".
9. Following installation, the ACE program can be accessed by clicking **Start**, **Programs**, and then **ACE**. You can create an icon on the Desktop screen by placing the mouse pointer on the ACE icon, holding down on the control button, and dragging the icon to desired location on the screen.

Using ACE in Windows

Double click on the ACE icon in the Desktop screen and the main ACE screen will appear (Fig. 1). There are three main sections to the screen. The first section (left) is for data entry or for including data from other sources (e.g., Excel, Lotus 123, etc.). The second section (right center) represents the models available in ACE (ALT, accelerated life testing; MPA, multifactor probit analysis; LRA, linear regression analysis). The third section is the ACE logo, appearing in the background at right. Following data entry and conversion to ASCII files (see below), click on the box for the model of choice (ALT, MPA, LRA), and the analysis results and graphics will automatically be generated.

Figure 1 – Main ACE Screen



Menu Bar - Main Screen

File – Clicking on **File** provides the following drop down menu:

- **New** – Clears spreadsheet so new data can be entered.
- **Open** – Obtains a saved data set from an outside source (see **Obtaining Data from an Outside Source**).
- **Save** – Saves any changes back to the same file name.
- **Save As** – Saves a data set for the first time or saves an existing data set to a new file name.
- **Exit** – Clicking on **Exit** will end the ACE program; clicking on **X** in the upper right-hand corner of the main ACE window will perform the same function as **Exit**.
- Help – User manual.

Options – Option screen will appear; see **OPTIONS** for explanation.
Log – If the ACE program does not run, then an error list will appear; the screen will be empty if no problems occur.
Sheet icon – This is the same as **New** under the **File** drop down menu.
File icon – This is the same as **Open** under the **File** drop down menu.
Floppy disk icon – This is the same as **Save** under the **File** drop down menu.

Menu Bar - ALT, MPA, LRA

- **Print** – Allows printing of selected output (statistical output, graph, or log).
- **Save_on_file** – Saves the statistical output to a file; this is the same as **Save as** described previously.

- **Log** – Provides additional statistical output information.

Data Entry

Format

The following acute toxicity data set for Kepone (Buckler et al. 1981) is used to demonstrate data formatting. The data must be entered in column format as follows, except that columns may be in any order; each column is identified by column headers in the first window (Fig. 1). Data must be entered in the following format for rows:

Concentration	Time (h)	Total (# of Organisms Tested)	Response (# Dead)
0	24	20	0
10	24	20	0
16	24	20	3
22	24	20	5
27	24	20	8
40	24	20	20
56	24	20	20
73	24	20	20
0	48	20	0
10	48	20	1
16	48	20	7
22	48	20	12
27	48	20	20
40	48	20	20
56	48	20	20
73	48	20	20
0	72	20	0
10	72	20	5
16	72	20	12
22	72	20	13
27	72	20	20
40	72	20	20
56	72	20	20
73	72	20	20
0	96	20	0
10	96	20	5
16	96	20	12
22	96	20	13
27	96	20	20
40	96	20	20
56	96	20	20
73	96	20	20

Entering Data Directly

Acute toxicity data can be entered directly to ACE using the spreadsheet (Fig. 1) and keypad functions. The following keypad functions are operational in the spreadsheet: arrow keys, Delete key, Enter key

(functions the same as the down arrow key), and number keys. Each column has to be identified for the ACE program to function properly. Click on each of the column headers, click on arrow, and select appropriate descriptor for that column.

- ID – This is not necessary if a single data set is entered. If more than one data set is to be entered, see **Entering Data from Outside Source** below.
- Concentration – Exposure concentration or % effluent (for extremely large numbers, convert to next higher unit [e.g., µg to mg]).
- Time – Observation time in hours, usually 24, 48, 72, and 96 hours (maximum times are 12).
- Total – Number of organisms exposed per concentration.
- Response – Number of organisms dead or affected.

The ACE default order of column designation is the same as above.

Next, enter the data, click on **File** and then **Save as** and enter a data set name in the file name box. The data set will be saved as a tab delimited file unless an extension name of CSV is typed. An extension name of CSV will results in a comma delimited file. The Tab or Comma delimited file types are preferred. The data are brought back into ACE by clicking on the icon file, data set to be analyzed, and **Open**. Then click on the model of preference (ALT, MPA, LRA), and the analysis is automatically conducted. If data are not analyzed, recheck the column headers to make sure they are correct.

Entering Data from Outside Source

The software is not meant to be a sophisticated spreadsheet, and the best way to enter multiple data sets is from an outside source using softwares capable of producing ASCII text files (e.g., Excel, Word, etc.). If data sets are stacked, a fifth column (ID) must be added in order to identify the different acute data sets.

Once data have been entered, save them as an ASCII file. This is done by clicking on **File** in the upper left corner and then clicking on **Save as**. The **Save as** screen will appear with two boxes at the bottom; **File name** and **Save as type:**. Type in a name for the data set in the File name box. Click **Save as type:**, a list of file types will appear. The following file types are appropriate for the ACE software: **Space delimited, Tab delimited** and **Comma delimited (CSV).** The Tab delimited or CSV file types are preferred.

Obtaining Data from Outside Source

To obtain a data set from an outside source while in the ACE program, click on the File icon in the upper left-hand corner and the following drop down menu will appear:

New Ctrl N
Open
Save
Save As
Exit

Click **Open**; if the data set is not listed in the **Open** screen, click **Files of type:**. Click arrow and then **All(*.*)**. If the file is still not present, click on **Look in:**. This will list all of the disk drives in your computer. Once the data set has been found, double click on the data set and the data will be entered into the ACE program. Again, data sets must be converted to Tab, Comma or Space delimited file types, with Tab and CSV being preferred.

Once the data set is imported into the ACE program in the correct format, title or other descriptive lines must be removed. Click on the line number in the spreadsheet for the line that is to be deleted (left side of main ACE window) and press the Delete key on keyboard.

Each column needs to be identified by the ACE program. Check the column headers on the Main ACE Screen. If they are correct, the program is ready to run. If not, click on each of the column headers and correct (see **Entering Data Directly**).

Data Correction

If data need to be corrected, it can be done within ACE. Just click on the cell, delete the incorrect number with the Delete key, and then correct the entry. If columns are too narrow to fully observe identifiers or numbers, widen the columns by placing the cursor on the right border of the column header and, while holding down the left mouse button, drag to the right until the desired width is achieved. Reverse this process to narrow the columns. Changes are saved by clicking on **File**, selecting either **Save** or **Save as**, entering a name for the data set in **File name:**, and clicking on **Save**.

Model Selection

Brief guidelines for using ACE and selecting the appropriate models are:

1. Exposure Type - Historically, three test exposure techniques have been used to determine acute toxicity for aquatic organisms (static, static renewal, and flow-through). Acute toxicity data used in ACE should be based on static renewal or flow-through techniques, since static exposure may give erroneous results, except for chemicals that are water soluble (see fluridone, Mayer et al. 1994). Further research is needed to determine at what octanol/water or solubility values static test data begin resulting in erroneous chronic predictions.

2. Model Preference – ALT is the method of choice, followed by LRA and MPA, based on experimental designs commonly used in acute toxicity testing. MPA is a special case application and is seldom used.

3. Partial Responses – Dependability of chronicity estimates is generally enhanced with increasing numbers of partial responses (% mortality >0<100%). Recommended partial responses are: ALT $\geq$ 3, MPA $\geq$ 5, and LRA $\geq$ 1. However, ALT will generally function with one partial response; LRA will function with no partial responses as long as there is an exposure-response in time. It is not uncommon to conduct acceptable acute toxicity tests where no partial responses occur, only 0 and 100%; under these conditions, the LRA is the model of choice.

4. Percent Effect for Chronicity – Recommended percent values to be selected for estimated chronic toxicity are: ALT = 1.0%, MPA = 0.01%, and LRA = 0.01%. Use of 0.01% for the MPA and LRA represents a very close approximation to zero on the probit scale (Mayer et al. 1994, Mayer et al. 2002). ALT differs in that 1.0% is presently considered the smallest detectable difference due to the model being population-based (small numbers of organisms usually exposed in each concentration). These percentages correspond well to statistically-based chronic no-effect concentrations for mortality using hypothesis testing (i.e., analysis of variance; Mayer et al. 2002).

ACE Application Windows

Data Analysis

Download a data set to the main ACE screen and click on a model (ALT, MPA, or LRA); the data will automatically be analyzed. Click on the **X** in the upper right hand corner to return to the main screen; a different model can then be selected. When you click on a model on the main screen, a split screen will appear; statistical output on the left and graphics on the right. Double click on either to fill screen; double click again to return to split screen. Click on the **X** in the upper right-hand corner of the main screen to exit ACE.

Printing Output

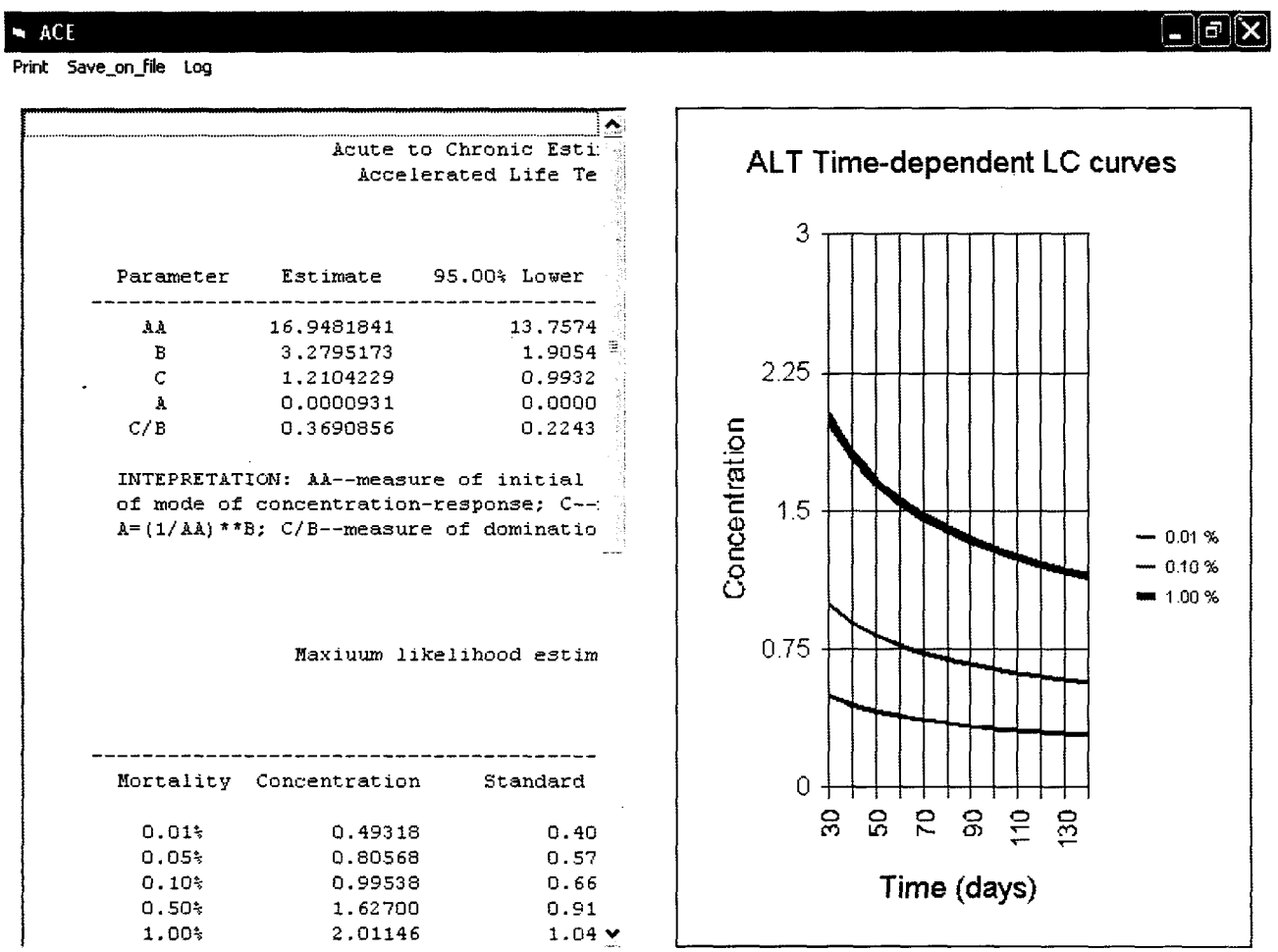
Printing of the statistical or graphics output is achieved by clicking **Print**, or the outputs can be saved by clicking **Save_on_file** (upper left-hand corner of screen). Additional statistical output can be obtained by clicking on **Log**. The output for **Log** includes the statistical output plus the additional information below and can also be printed or saved.

- ALT – Data input, iterations required to solve function estimates, variance-covariance matrix for function estimates to estimate confidence intervals, and data used in the analysis (the highest concentration having 0% response and the lowest concentration having 100% response are used for each observation time).
- MPA – Data used in the analysis as described in ALT.
- LRA – Statistical analyses for all six models including slope, estimated no-effect chronic concentration, confidence intervals, r^2, and data used in the analysis as described in ALT.

ALT- Accelerated Life Testing Model

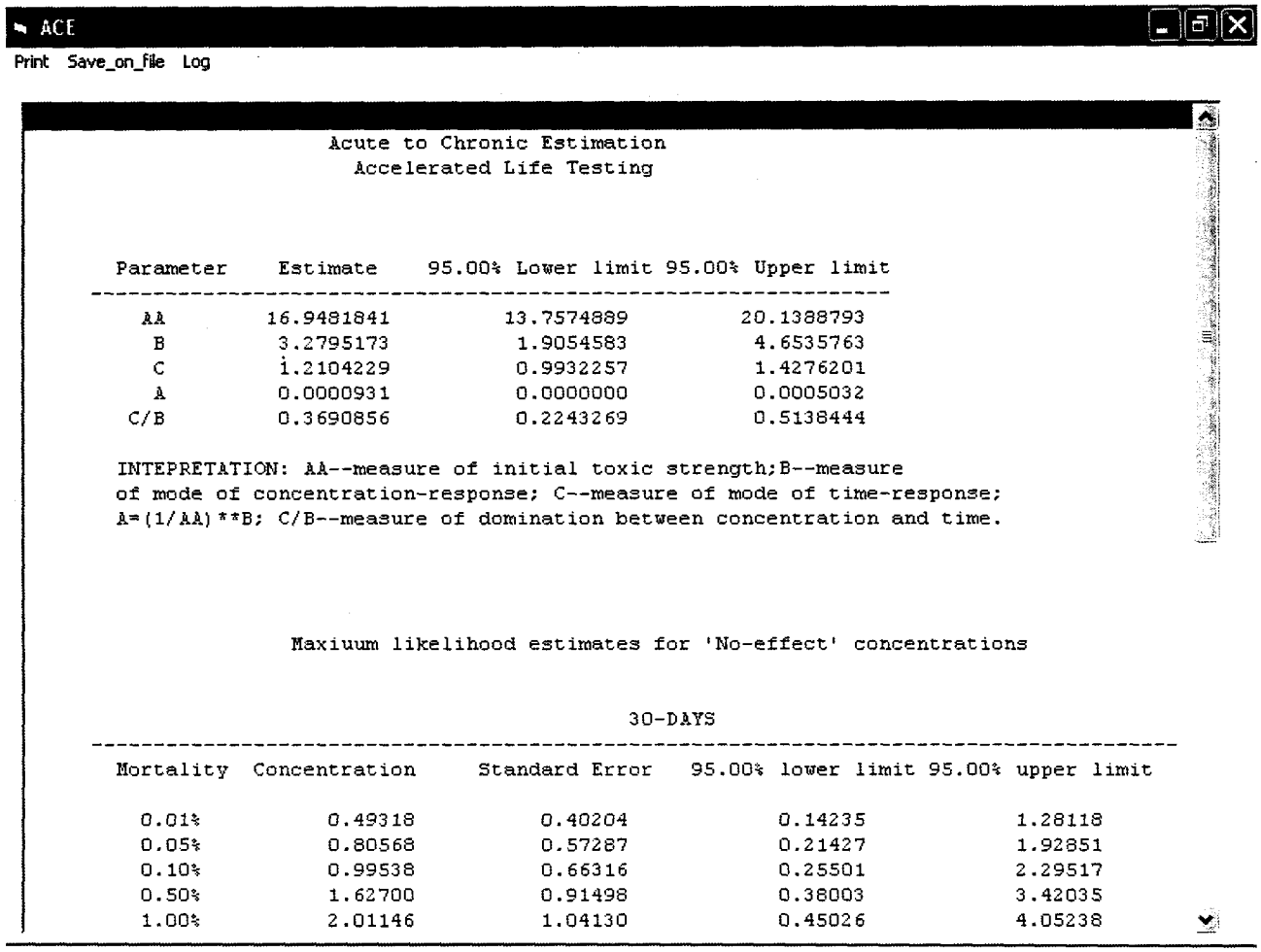
Click on the box **ALT** (Accelerated Life Testing) in the main ACE screen, and analysis of the downloaded acute toxicity data is performed (Fig. 2).

Figure 2 –Accelerated Life Testing (ALT) Screen



Double click on the statistical output screen (left side) in order to obtain the full screen (Fig. 3).

Figure 3 – ALT Full Screen



There are two main parts to the statistical output. The first part contains statistical parameter estimates, along with confidence limits. Interpretation of these parameters follows the estimates. C/B provides an indication of the importance of exposure time (C) versus exposure concentration (B); if equal to one, both are equally important.

The second part of the statistical output is the maximum likelihood estimates of chronic no-effect concentrations. By default, analyses are performed for three different chronic times (30, 60 and 90 days). Within each time period are percent level of chronic mortality (0.01 – 10.0%; 1.0% is recommended for chronic survival with ALT), predicted toxicant concentration associated with each percentage, standard error of the predicted toxicant concentration, and confidence limits (default is 95% confidence limits).

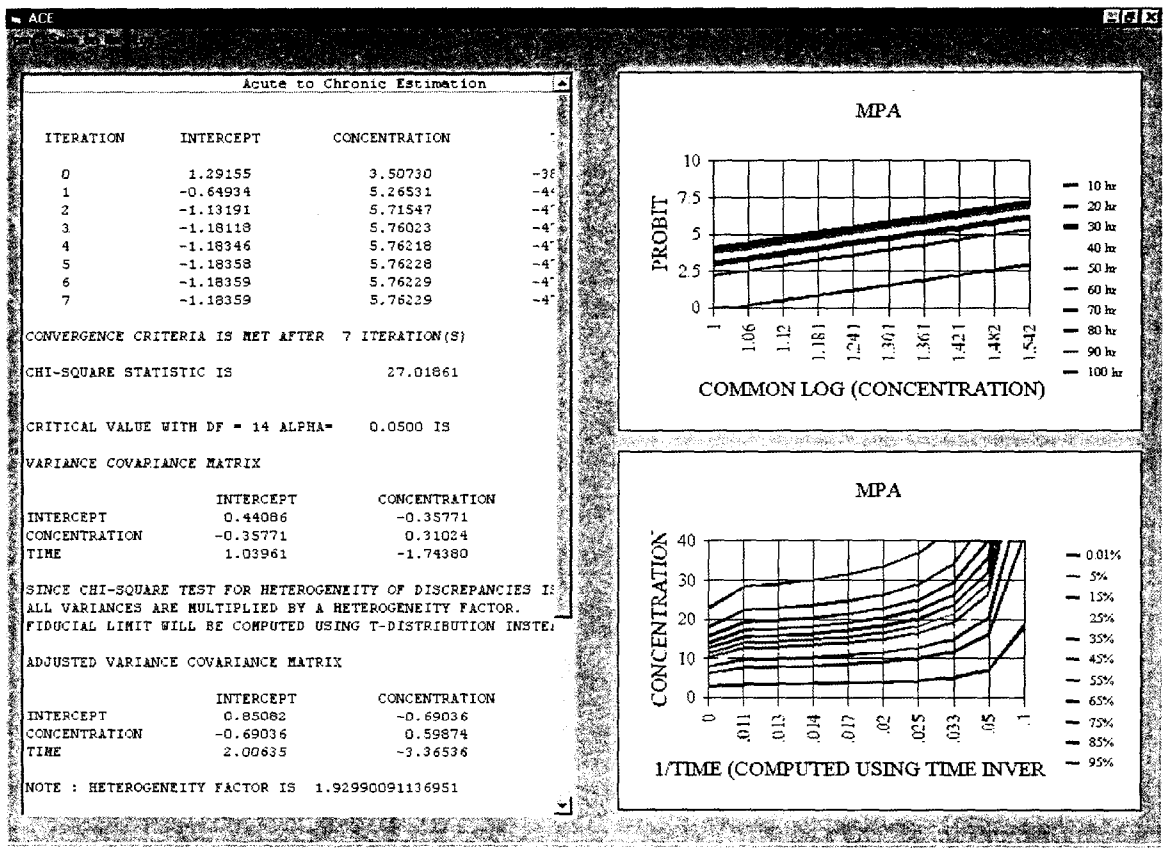
The ALT procedure will function even with a small number of partial responses in the raw acute toxicity data. However, the confidence limits may be large; an error message will appear and the ALT will fail if no partial responses are present in the data.

Additional chronic exposure times and the *alpha* level for confidence limits can be specified (see **Options**).

MPA – Multifactor Probit Analysis Model

Click on the box **MPA** (Multifactor Probit Analysis) in the main ACE screen, and analysis of the downloaded acute toxicity data is performed (Fig. 4).

Figure 4 – Multifactor Probit Analysis (MPA) Screen



Double click on the statistical output screen (left side) in order to obtain the full screen (Fig. 5).

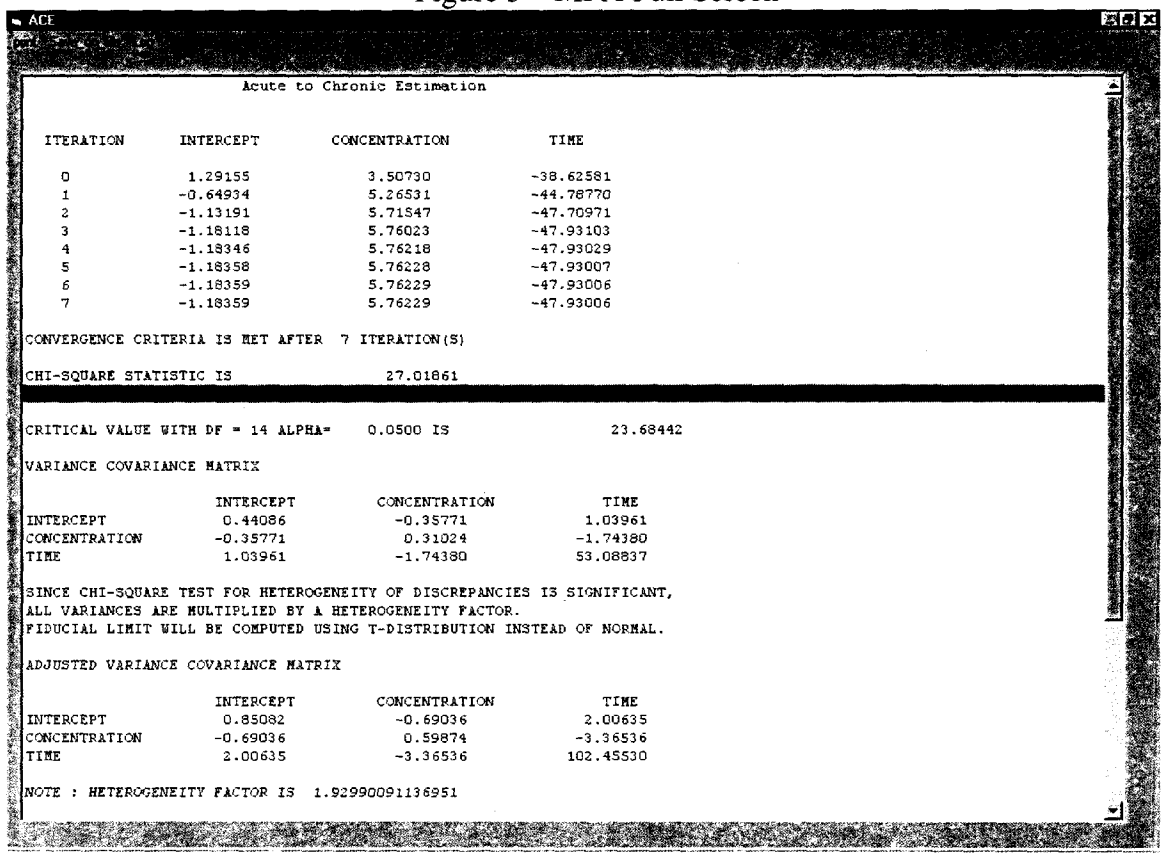
The output provides the number of iterations required to calculate factors for the MPA model, test statistics, variance-covariance matrix, and the predicted chronic no-effect concentrations along with 95% confidence limits.

The MPA includes four different models to choose from that may give different estimates of the MPA functions (see **Options**). The default model is Model 3 in **Options**:

$$Probit_p = \alpha + \beta(Concentration) + \Upsilon/Time$$

Chronic exposure time is specified and the assumption is that slopes change with a constant rate as observation times increase.

Figure 5 – MPA Full Screen



By default, there is one chronic time period (infinity). Within each time period are percent level of mortality (0.01 – 50%; 0.01% is recommended for MPA), predicted toxicant concentration associated with each percentage, and confidence limits (default = 95%). The data fit the model if the chi-square statistic is ≤ the critical chi-square value.

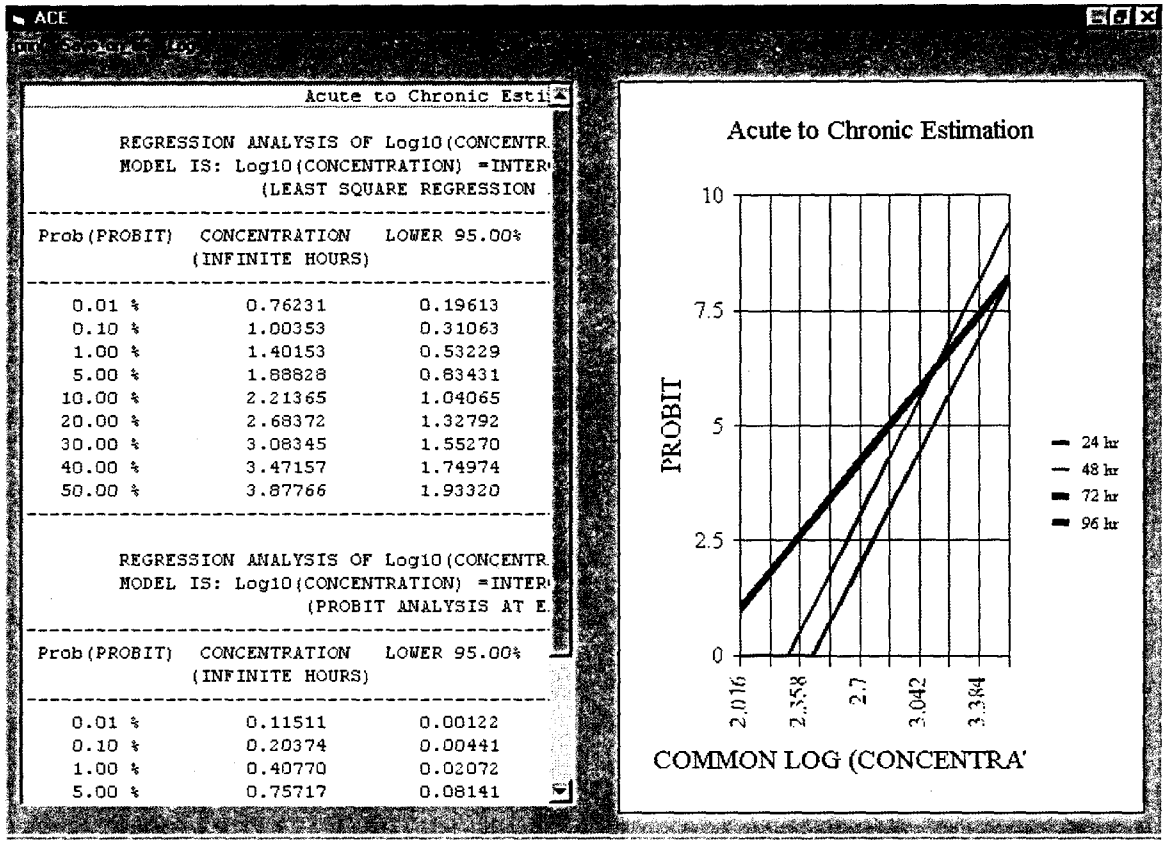
The MPA is the most sensitive to lack of partial mortalities (responses); at least five partial responses between 10 and 90% among all exposure concentrations and times are preferred. An error message will appear and MPA will fail if inadequate partial responses or an insufficient range of partial responses exist.

Additional chronic exposure times and the *alpha* level for confidence limits can be specified (see **Options**).

LRA - Linear Regression Analysis Model

Click on the box **LRA** (Linear Regression Analysis) in the main ACE screen, and analysis of the downloaded acute toxicity data is performed (Fig. 6).

Figure 6 – Linear Regression Analysis (LRA) Screen



Double click on the statistical output screen (left side) in order to obtain the full screen (Fig. 7).

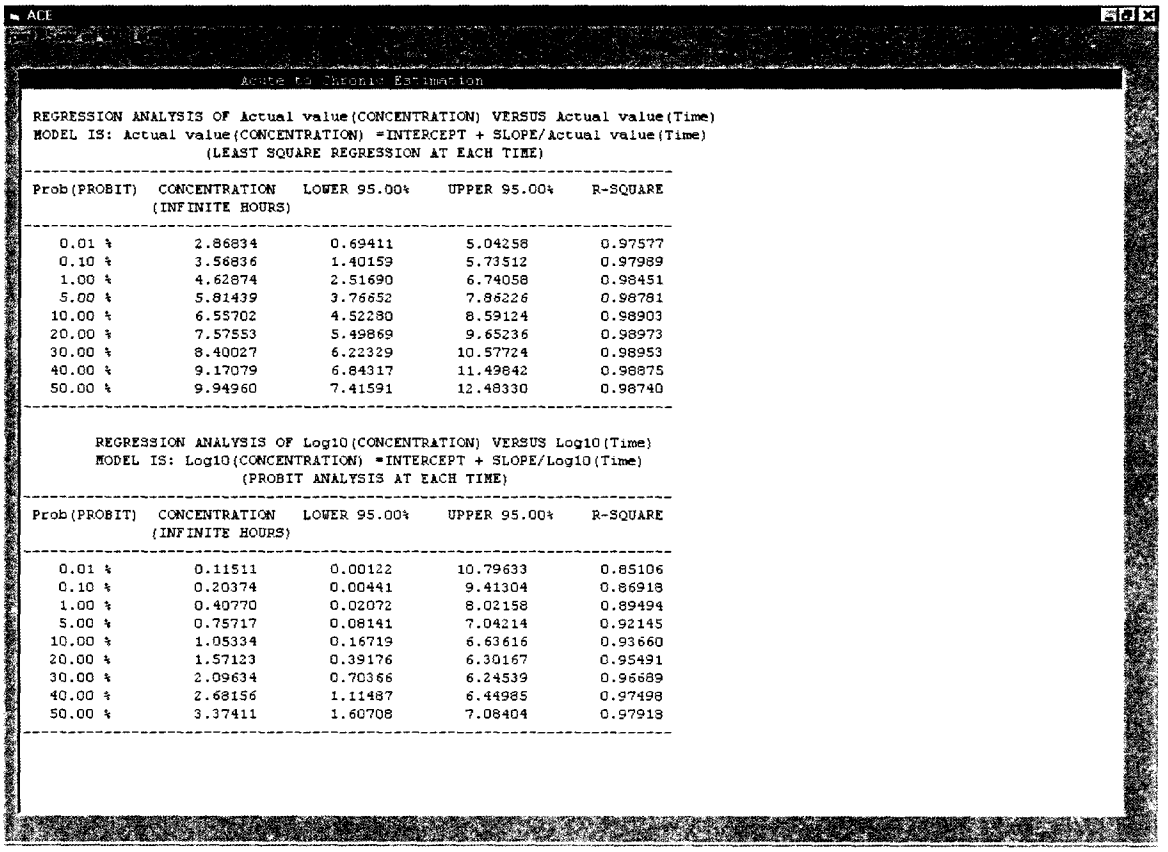
Calculations are based on a two-stage regression analysis, and two analyses will appear; one based on linear regression analysis and another based on probit analysis in stage 1. The following values are given: percent effect (0.01 – 50%; 0.01% is recommended for LRA), estimated chronic no-effect concentration at infinite hours, 95% confidence limits, and r^2. Select the chronic no-effect concentration for 0.01% with the largest r^2 value. Six models are used in the analyses, but only the best stage 1 linear regression and probit analyses (highest r^2) appear in Fig. 7. Click on **Log** to see analyses for all six models.

Note: If percent effects are selected above low percentages (0.01 – 1.0%), abberant values may be apparent when slopes among observation times in stage 1 are very unparallel.

LRA does not require partial responses. If no partial responses are present in the acute toxicity data, LRA uses the highest concentration having 0% (i.e., 0.01%) response at each time period for stage 1, and in stage 2, only the least square analysis is performed.

The only change that can be made for LRA is the *alpha* level for confidence limits (see **Options**); time in hours is set at infinity.

Figure 7 – LRA Full Screen



Options

A number of options are available for controlling the output of each of the ACE models. The options screen is obtained from the main ACE screen. Click **Options** located in the upper left-hand corner of the main ACE window and the following screen will appear (Fig. 8). Once an *alpha* for confidence limits, chronic exposure time, MPA model, and/or statistical output title are changed, click **Save Options**. These changes will remain for present and future analyses. If **Save Options** is not selected, the changes will only remain for the current analysis and then return to default values the next time ACE is used. Click **Restore defaoul options** at the bottom right of the Options window to return to default values.

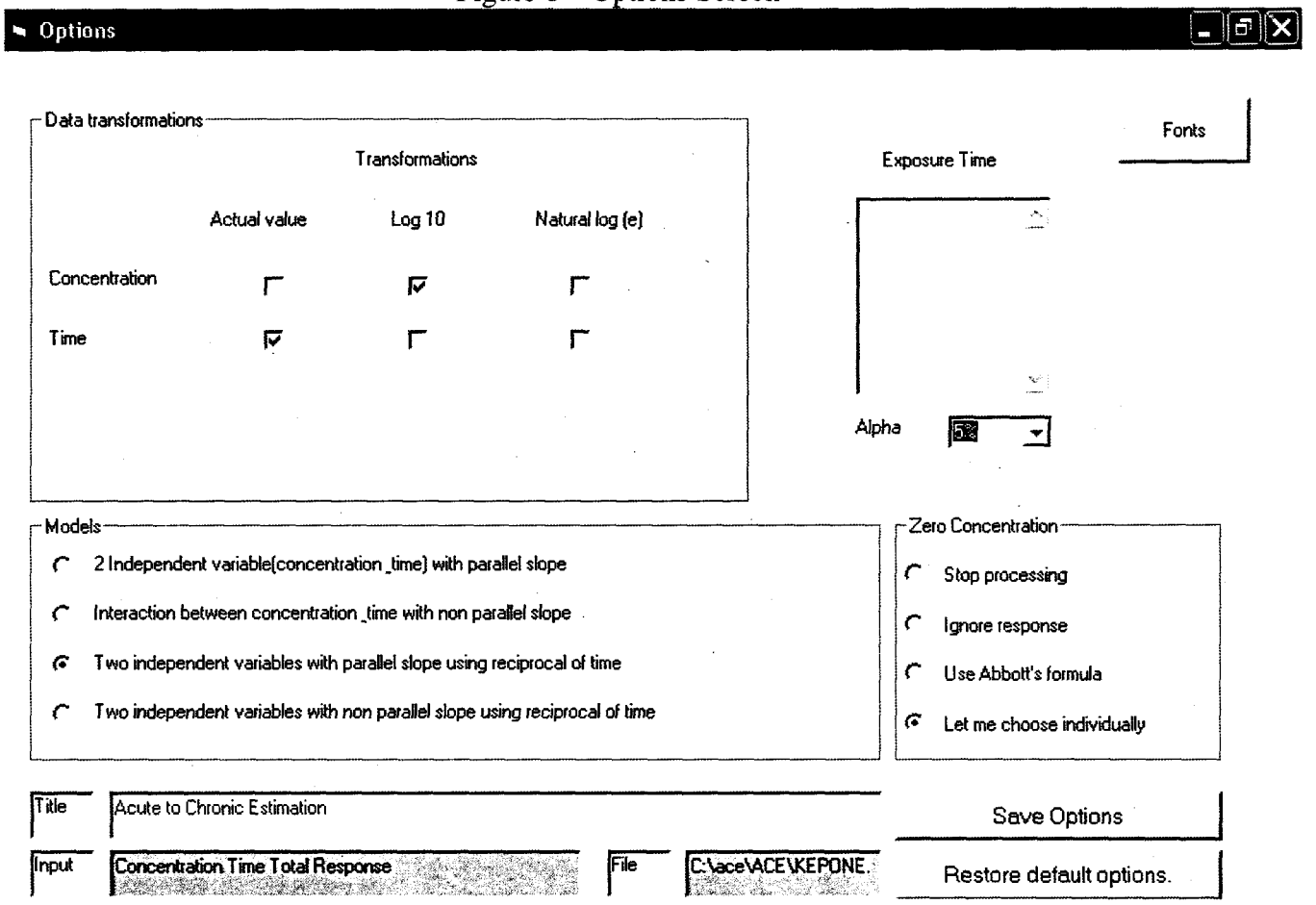
Font

Select **Font** (upper right-hand corner) to change font style of statistical output. Two font styles are presented; fixed font styles should be selected in the left-hand box. The font size may also be changed to fill the data output screen.

Alpha

To change *alpha* levels, click on the arrow associated with **Alpha** located on the upper right side of the **Options** screen; choose the desired *alpha* percent. The *alpha* controls the *t*, z, or chi-square values for producing confidence limits; the *alpha* default value is 5%.

Figure 8 – Options Screen



Exposure Time

In order to change to a different time, go to options window as described previously. To the right is a white box with the header **Exposure Time**. A time change can be accomplished in a number of ways. Type in a number (in hours) in the white box. If a number already exists in the box, write over it or add a number below the existing numbers. No time definition is needed if the number is in terms of hours. However, if one wants to enter days, just type the number of days desired and type "days" after the number and days will be converted to hours by the program. Weeks, months or years can be used as well, by typing in the appropriate time description. Two of these time descriptions (eg., days and months) cannot appear together on the same line. The default for the ALT is 30, 60 and 90 days. The default for MPA is infinite time if the model is based on the reciprocal of time. If the models are not based on the reciprocal of time, a number has to be placed in the **Exposure Time** box in order for the program to calculate NOEC values. The LRA procedure only calculates for infinite time.

Zero Concentration

This section applies only to the MPA and LRA. Abbott's formula (Finney 1978) is used to adjust data if control mortality (zero concentration) exists when probit analysis is performed. The default is **Let me**

choose individually. If control mortality exists, the MPA or LRA will present a message box that allows the user to choose Abbott"s correction. If only one control mortality is present, the message box will appear only once. If control mortality appears more than once, the message box will appear for each one. If **Stop processing** is selected, MPA and LRA will not run if control mortality is present. The **Ignore response** option does not apply Abbott's correction. The **Use Abbott's formula** applies Abbott's correction to all control mortalities.

Title

The title of the statistical output can be changed; click on **Title** and type in a new title. The default title is "Acute to Chronic Estimation".

Selecting MPA Models

The basic Multifactor Probit Analysis equation has a general form in which LC% = Intercept + b_1(Exposure Concentration) + b_2(Time) where b_1 and b_2 are partial regressions for exposure concentration and time, respectively. An additional b_3 [interaction of (exposure concentration)(time)] is added if the slopes among probits are not parallel (see Lee et al. 1995, Mayer et al. 2002).

A number of statistics require evaluation to determine the MPA model of choice. If the chi-square statistic is $\leq$ the critical chi-square value, the data fit the model adequately. Should the other models provide a smaller chi-square statistic, that model is preferred.

To change to one of the other three basic MPA models, exit the MPA program by clicking the **X** in the upper right corner, and then click on **Options** in the upper left-hand corner of the main ACE screen; select **Models** and the four models listed below will appear. The model parameters can be changed to actual values or log values of time and concentration within **Data Transformation** located in the upper left portion of the **Options** screen. This procedure takes much more manipulation to determine the best model. The combination of model choice and actual or log values of concentration and time that gives the lowest chi-square statistics is the best model.

The four models are as follow (1.281 = probit value for 0.01%):

Model 1: Chronic exposure time is specified and equal slopes among observation times are assumed.

Exposure Concentration – Time – Response relationship is defined as:

$$Probit_P = \alpha + \beta(Concentration) + \Upsilon(Time)$$

Chronic no-effect concentration (NOEC) at specified T hours is:

$$NOEC_T = \frac{1.281 - \alpha - \gamma * T}{\beta}$$

Model 2: Chronic exposure time is unknown and equal slopes among observation times are assumed.

Exposure Concentration – Time – Response relationship is defined as:

$$Probit_p = \alpha + \beta(Concentration) + \Upsilon/Time$$

NOEC at infinite time is:

$$NOEC_T = \frac{1.281 - \alpha - \gamma * T}{\beta + \delta + T}$$

Model 3: Chronic exposure time is specified and it is assumed that the slope changes with constant rate as observation times increase.

Exposure Concentration – Time - Response relationship is defined as:

$$Probit_p = \alpha + \beta(Concentration) + \Upsilon(Time) + \delta(Concentration)(Time)$$

NOEC at T hours is:

$$NOEC = \frac{1.281 - \alpha}{\beta}$$

Note: This is the default model in ACE; actual value of time and the log10 of concentration.

Model 4: Chronic exposure time is unknown and and it is assumed that the slope changes with constant rate as observation times increase.

Exposure Concentration – Time – Response relationship is defined as:

$$Probit_p = \alpha + \beta(Concentration) + \Upsilon/Time + \delta\ (Concentration)/(Time)$$

NOEC at infinite time is:

$$NOEC = \frac{1.281 - \alpha}{\beta}$$

Note: Chronic times are necessary for Models 1 and 2; default chronic time is infinity for Models 3 and 4, but additional chronic times may be added.

Estimating Sublethal Effects

Raw data for sublethal endpoints are seldom available under acute exposure conditions for modeling chronic no-effect concentrations. Sublethal endpoints are also difficult to estimate from chronic lethality data. Conservative chronic no-effect concentrations for sublethal endpoints may be estimated by multiplying the predicted NOEC for lethality by 0.2 for growth and other sublethal endpoints and 0.1 for reproductive endpoints. This is based on the analysis of differences among endpoints in chronic toxicity tests (Table 1). However, it must be understood that these estimates of chronic sublethal effects are extremely conservative; note that the median values (that value where 50% of the observations are above or below it) are approximately 1.0 for growth and reproduction and only slightly below 1.0 for "other" sublethal endpoints. In addition, the NOECs for lethality were exactly the same or less than those for weight, length, reproduction, and "other" endpoints 59, 58, 56, and 41% of the time, respectively. Based on the extreme variation of ratios, and the fact that no central tendency exists within the distribution of ratios, **the authors do not recommend using factors to estimate sublethal endpoints at this time**. The data (see table below) are based on hypothesis testing, and using regression analysis to estimate no-effect concentrations for lethal and sublethal endpoints might provide an improved comparison and deserves further investigation.

Univariate analyses for the ratios of growth, reproduction, or other sublethal endpoint chronic no-effect concentrations (NOEC) to that for survival (sublethal NOEC/survival NOEC)[1].

Univariate parameter	Growth		Reproduction	Other[2]
	Weight	Length		
n	46	62	18	22
Mean	0.96	0.90	1.13	0.76
Median	1.0	1.0	1.0	0.6
Range	0.10-4.4	0.16-2.3	0.12-4.5	0.06-2.0
95% CL	0.7-1.2	0.8-1.1	0.6-1.7	0.5-1.0
$\pm$1 SD	0.2-1.8	0.3-1.5	0.1-2.2	0.2-1.3
95th percentile	2.3	2.2	4.5	2.0
Median	1.0	1.0	1.0	0.6
5th Percentile	0.1	0.2	0.1	0.2

[1]Data are from Mayer et al. (1986) and the USEPA Gulf Ecology Division (ORD/NHEERL), Gulf Breeze, FL.
[2]Sublethal endpoints deemed detrimental to survival and/or ability to contribute to population success were cataracts, disease susceptibility, severe fin erosion, severe organ pathology, and spinal curvature.

Additional Model Documentation

Details regarding each model and validation of those models using paired acute and chronic toxicity data are published (Lee et al. 1992, Lee et al. 1995, Mayer 1990, Mayer 1991, Mayer et al. 1992a, Mayer et al. 1992b, Mayer et al. 1994, Mayer et al. 1999, Mayer et al. 2002, Sun et al. 1992, Sun et al. 1994, Sun et al. 1995a, Sun et al. 1995b).

ALT

The ALT procedure uses a Quasi-Newton method to find the maximum likelihood estimates of parameters. Confidence limits for parameters are based on Normal approximations to distributions of the maximum likelihood estimates. The parameter estimates given in Fig. 3 are used in the following model to obtain predicted chronic no-effect concentrations for a particular percent effect and exposure time in days.

$$\text{No-effect concentration} = \text{Exp}[(\ln(-\ln(1-p))-\ln(A) - C*\ln(\text{days}*0.24))/B]$$

A, B, and C are parameter estimates and p is the percent effect, ranging from 0.01 to 10% (see **ALT – Accelerated Life Testing Model**).

MPA

The MPA method uses all time and concentration data simultaneously to produce a multiple regression probit equation to predict chronic no-effect values for specified times.

If the chi-square statistic is $\leq$ the critical chi-square value, a variance-covariance matrix is produced and is necessary to calculate confidence limits. If the chi-square statistic is not $\leq$ the critical chi-square value, the variance-covariance matrix is adjusted by a heterogeneity factor to produce an adjusted variance-covariance matrix. The heterogeneity factor (HF) is given in the statistical output and is equal to the chi-square statistic divided by the degrees of freedom (n – 1 of data used; Finney 1978).

The assumptions of independence may be violated with typical acute toxicity data using MPA. The procedure is appropriate if observations at one time are not the same experimental units at another time. Regardless of the issue of independence, MPA does provide acceptable acute and predicted no-effect chronic concentrations when adequate partial responses are present in the acute data.

LRA

Calculations are based on a two-stage regression analysis. Stage 1 performs two types of analyses. The first type is a simple linear regression at each observation time in which the X axis is log10 concentration and the Y axis is the probit transformation of proportion responding (dead). The second type is a probit analysis at each observation time (Finney 1978). Following these two types of analyses, no-effect concentration values are estimated at different percent response levels. The concentrations are transferred to the stage 2 simple linear regression in which the X axis is the reciprocal of time (1/t) and the Y axis is the concentration at each observation time for a specific percentage value. The equation is:

$c = a + b/t$ where c = chronic no-effect concentration
a = Y intercept
b = regression coefficient
t = time

There are three possible transformations that are made in the stage 2 regression: 1) actual values of concentration and time, 2) log10 of concentration and actual value of time, and 3) log10 of both concentration and time. Thus, six analyses occur due to two types of analyses in stage 1 and three transformations of data in stage 2. As time goes to infinity, the term b/t goes to zero; thus, the concentration at infinite time is the intercept (a), or the chronic no-effect concentration for lethality.

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