3. The program output includes the following (see Figure 9):
   a. A table of the concentrations tested, number of organisms exposed, and mortalities.
   b. The amount of trim used in the calculation.
   c. The estimated LC50 and the associated 95% confidence interval.

4. The analysis results for this example are as follows:
   a. The observed proportion mortalities smoothed and adjusted for mortality in the control.
   b. The amount of trim used to calculate the estimate:

$$\text{trim} = \max\{0.00, 0.204\} = 0.204.$$

   c. The estimate of the LC50 is 77.3%, with a 95% confidence interval of (73.6%, 81.2%).

11.2.5 THE PROBIT METHOD

11.2.5.1 Description

1. The Probit Method is a parametric statistical procedure for estimating the LC50 and the associated 95% confidence interval (Finney, 1978).

2. The analysis consists of transforming the observed proportion mortalities with a probit transformation, and transforming the effluent concentrations to $\log_{10}$.

3. Given the assumption of normality for the $\log_{10}$ of the tolerances, the relationship between the transformed variables mentioned above is approximately linear.

4. This relationship allows estimation of linear regression parameters, using an iterative approach.

5. The estimated LC50 and associated confidence interval are calculated from the estimated linear regression parameters.

11.2.5.2 Requirements

1. To obtain a reasonably precise estimate of the LC50 with the Probit Method, the observed proportion mortalities must bracket 0.5.

2. The $\log_{10}$ of the tolerance is assumed to be normally distributed.

3. To calculate the LC50 estimate and associated 95% confidence interval, two or more of the observed proportion mortalities must be between zero and one.

11.2.5.3 General Procedure

1. Due to the intensive nature of the calculations for the estimated LC50 and associated 95% confidence interval using the Probit Method, it is recommended that the data be analyzed by a computer program.

2. A computer program to estimate the LC50 and associated 95% confidence intervals using the Probit Method was developed by EMSL-Cincinnati. The program was written in IBM PC Basic for the IBM compatible PC by Computer Sciences Corporation, 26 W. Martin Luther King Drive, Cincinnati, Ohio 45268. A full listing and a machine-readable, compiled, version of the program can be obtained from EMSL-Cincinnati by sending a diskette with a written request to the Quality Assurance Research Division, Environmental Monitoring Systems Laboratory, at the above address.

11.2.5.4 Example Using the Computer Program

1. Data from Table 18 are used to illustrate the operation of the Probit program for calculating the LC50 and the associated 95% confidence interval.

2. The program begins with a request for the following initial input (see Figure 10):
   a. Output designation (P = printer, D = disk file).
   b. Title for the output.
   c. Control data.
   d. Toxicant concentration data.

3. The program output includes the following (see Figure 11):
   a. A table of the observed proportion mortality, the adjusted observed proportion mortality, and the predicted proportion mortality for each effluent concentration.
   b. The calculated chi-squared statistic for heterogeneity and the tabular value. This test is one indicator of how well the data fit the model. The program will issue a warning when the test indicates that the data do not fit the model.
   c. Estimates of the mean (mu) and the standard deviation (sigma) of the underlying tolerance distribution.
   d. Estimates and standard errors of the intercept and slope of the fitted probit regression line.
   e. The estimated LC50 and 95% confidence limits.
   f. A plot of the fitted regression line with observed data overlaid on the plot (see Figure 12).

4. The results of the data analysis for this example are as follows:
   a. The observed proportion mortalities were not adjusted for mortality in the control.
   b. The test for heterogeneity was not significant (the calculated Chi-square was less than the tabular value), thus the Probit Method appears to be appropriate for this data.
   c. The estimate of the LC50 is 22.9% with a 95% confidence interval of (18.8%, 27.8%).

11.3 DETERMINATION OF NO-OBSERVED-ADVERSE-EFFECT CONCENTRATION (NOAEC) FROM MULTI-CONCENTRATION TESTS, AND DETERMINATION OF PASS OR FAIL (PASS/FAIL) FOR SINGLE-CONCENTRATION (PAIRED) TESTS

11.3.1 Determination of the No-Observed-Adverse-Effect Concentration (NOAEC), for multi-concentration toxicity tests, and pass or fail (Pass/Fail) for single-concentration toxicity tests is accomplished using hypothesis testing. The NOAEC is the lowest concentration at which survival is not significantly different from the control. In Pass/Fail tests, the objective is to determine if the survival in the single treatment (effluent or receiving water) is significantly different from the control survival.

11.3.2 The first step in these analyses is to transform the responses, expressed as the proportion surviving, by the arc-sine-square-root transformation (Figures 13 and 14). The arc-sine-square-root transformation is commonly used on proportionality data to stabilize the variance and satisfy the normality requirement. Shapiro Wilk's test may be used to test the normality assumption.

11.3.3 If the data do not meet the assumption of normality and there are four or more replicates per group, then the non-parametric test, Wilcoxon Rank Sum Test, can be used to analyze the data.

11.3.4 If the data meet the assumption of normality, the F test for equality of variances is used to test the homogeneity of variance assumption. Failure of the homogeneity of variance assumption leads to the use of a modified t test, where the pooled variance estimate is adjusted for unequal variance, and the degrees of freedom for the test are adjusted.

```
EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING LC/EC VALUES
Version 1.5

Do you wish abbreviated (A) of full (F) output? A
Output to printer or disk file (P / D)? P
Title ? PROBIT EXAMPLE

Number of responders in the control group = ? 0
Number of exposure concentrations, exclusive of controls ? 5

Input data starting with the lowest exposure concentration

Concentration = ? 6.25
Number responding = ? 0
Number exposed = ? 20

Concentration = ? 12.5
Number responding = ? 3
Number exposed = ? 20

Concentration = ? 25
Number responding = ? 9
Number exposed = ? 20

Concentration = ? 50
Number responding = ? 20
Number exposed = ? 20

Concentration = ? 100
Number responding = ? 20
Number exposed = ? 20

                              Number     Number
  Number          Conc.       Resp.      Exposed
  ______        ________      _____      _______
    1             6.2500        0          20
    2            12.5000        3          20
    3            25.0000        9          20
    4            50.0000       20          20
    5           100.0000       20          20

Do you wish to modify your data ? n
The control response rate =  0
Do you wish to modify it? n
```

Figure 10. Example of input for computer program for Probit Method.

EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING LC/EC VALUES
Version 1.5

PROBIT EXAMPLE

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Proportion Responding Adjusted for Controls
6.2500	20	0	0.000	0.000
12.5000	20	3	0.1500	0.1500
25.0000	20	9	0.4500	0.4500
50.0000	20	20	1.0000	1.0000
100.0000	20	20	1.0000	1.0000

Chi - Square for Heterogeneity (calculated) = 3.076
Chi - Square for Heterogeneity
(tabular value at 0.05 level) = 7.815

PROBIT EXAMPLE

Estimated LC/EC Values and Confidence Limits

Point	Exposure Conc.	Lower 95% Confidence	Upper Limits
LC/EC 1.00	7.924	4.147	10.959
LC/EC 50.00	22.872	18.787	27.846

Figure 11. Example of output for computer program for Probit Method.

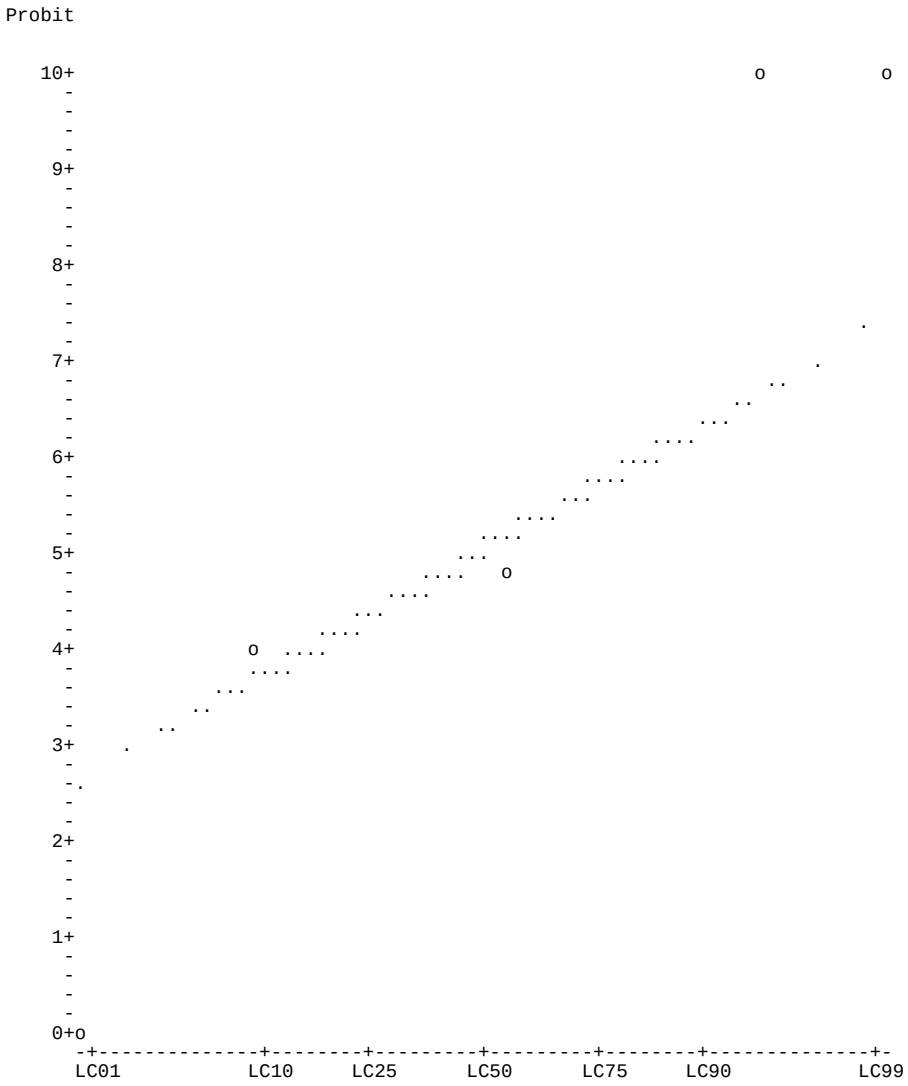

Figure 12. Plot of adjusted Probits and predicted regression line.