poison concentration to give a toxicity curve (Figure 4.2). It is customary to use logarithmic scales on both axes, since the ranges of survival times and poison concentrations to be displayed frequently span several orders of magnitude. This transformation does not, of course, alter the shape of the curve.

An alternative method of plotting the raw data is widely used. This method produces essentially the same toxicity curve but it is arrived at by a different route. For each observation time, a graph is plotted of percentage mortality (transformed to probits) against poison concentration (transformed to log concentration). Instead of a series of survival time-mortality curves (Figure 4.1) we now have a series of concentration-mortality curves (Figure 4.3). For each observation time, the median lethal concentration or LC50 (sometimes termed median tolerance limit, TL_M) can be read off. This value is defined as the concentration causing half the animals to die *within a specified period of time*. Its confidence limits can be estimated by a procedure analogous to that described above (Litchfield and Wilcoxon, 1949), based on the original method of Bliss (1935). The resulting toxicity curve (Figure 4.4) of LC50 against observation time differs only in that confidence limits are expressed in terms of concentration rather than time. For certain applications this approach has some advantages; for example, where the test forms part of a research programme designed to establish water quality standards, it is obviously preferable to estimate errors associated with lethal concentrations rather than with survival times. However, the computation of the results is more complex since, among other difficulties, the number of points on each probit line is small. Unless the chosen range of concentrations is narrow, most groups of animals in the test will show either zero or 100% mortality at any single observation time. Thus the lines of best fit must be calculated rather than fitted by eye (Litchfield and Wilcoxon, 1949).

Since these calculations can be tedious, there are obvious advantages in using a computer. Probit analysis is available on several commercially-available statistical software packages, and a purpose-designed program in fact requires a microcomputer of very modest capacity. Inexperienced users should, however, beware of some major problems which can arise with commercial software packages; since they are designed for general purposes, difficulties can arise with the mathematical notations, the format of the input data and in the interpretation of the output, which can lead to erroneous results. The use of 'home-made' programs must be particularly strongly discouraged. Unless they are written with a full understanding of the mathematical and toxicological principles involved, they almost invariably produce ridiculous results. Buhagiar and Abel (1991) provide a full discussion of the subject, and introduced the Toxicologist System, a dedicated and efficient program which carries out probit analysis for toxicity tests with aquatic organisms, plots the graphs and assists in the interpretation of

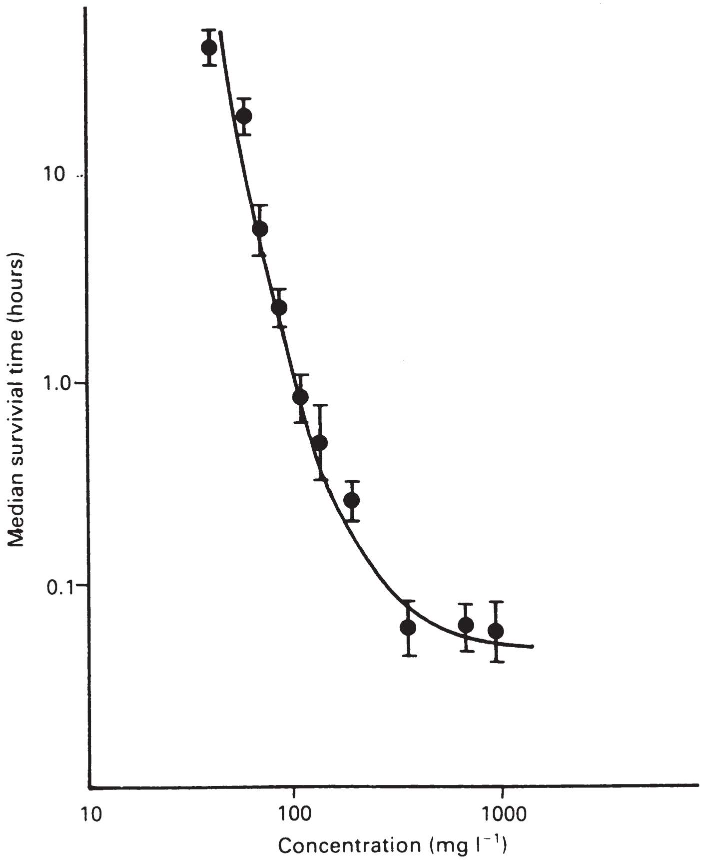

Figure 4.2 Toxicity curve relating median survival time to poison concentration; rainbow trout (*Salmo gairdneri*) exposed to sodium lauryl sulphate (Abel, 1978). Vertical bars represent 95% confidence limits

unusual probit lines. It also takes advantage of the computer to produce a mathematically exact solution rather than an approximation, while to the operator it appears to follow the familiar graphical procedure.

We may now consider the interpretation of lethal toxicity test results.

4.1.3 Probit Lines

The theoretical basis of the log-probit transformation of mortality data is given by Bliss (1935, 1937) and Finney (1971). This technique of data analysis is widely used in many types of toxicological and pharmacological research. Hewlett and Plackett (1979) provide a lucid and succinct account of the general theory and practice of probit analysis, and Sprague (1969) discusses its application to toxicity tests involving aquatic animals. In practical terms, the

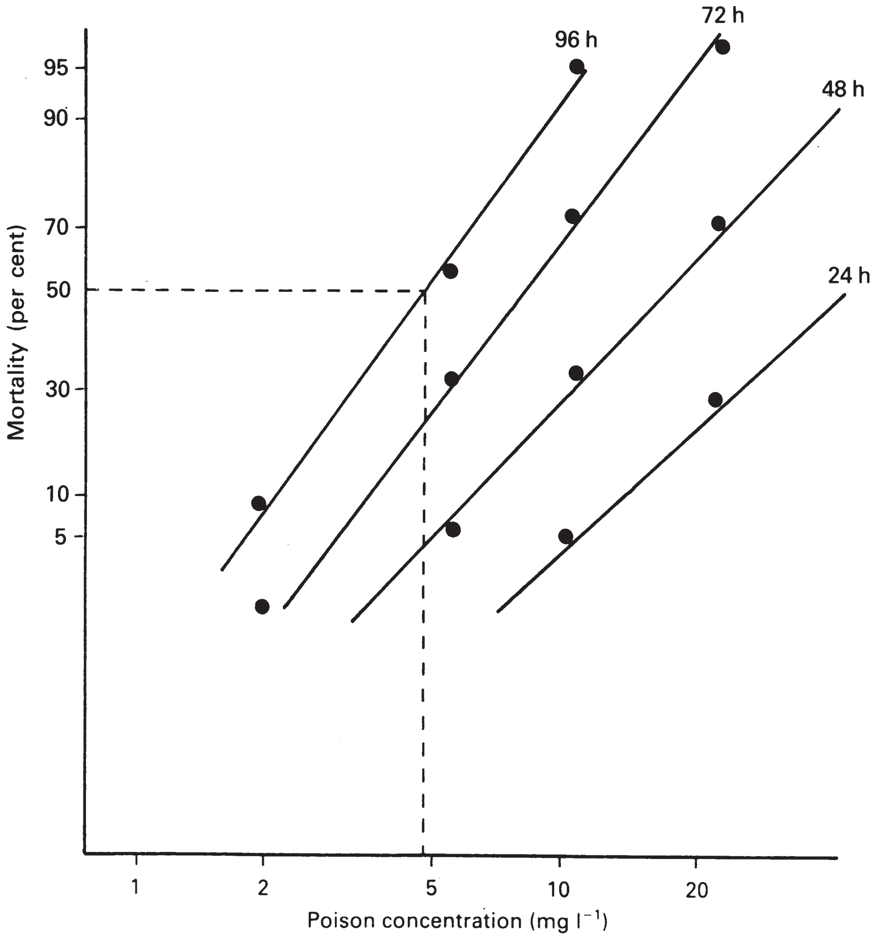

Figure 4.3 Probit lines resulting from plots of percentage mortality at specified observation times (probability scale) against poison concentration (log scale). The dotted line shows how the 96 h LC50 value may be read off

purpose of the transformation is to allow the estimation of median survival times and/or median lethal concentrations, and their confidence limits, from a relatively small sample. Other transformations have occa sionally been used (Sprague, 1969) and some have been discussed in detail by Stephan (1977), who argues that the log-probit transformation is not necessarily the best. However, it is by far the most widely used, and in practice all appropriate transformations appear to produce very similar results.

Because the probit lines are an essential intermediate stage in the construction of the final toxicity curve, it is often overlooked that they can provide other information of toxicological interest. Useful indications are

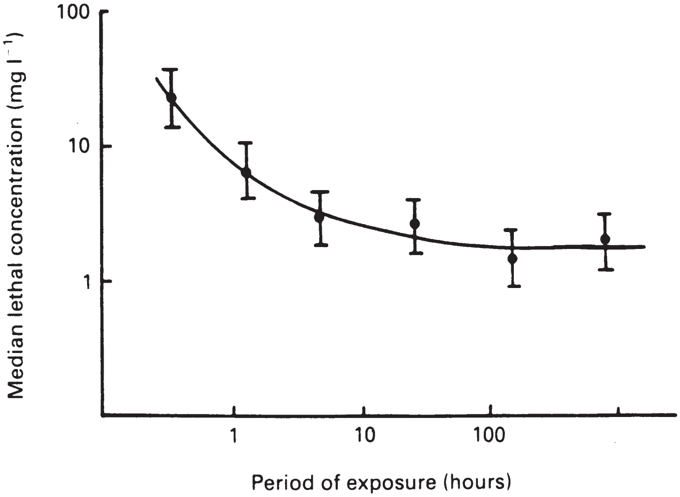

Figure 4.4 A toxicity curve in which median lethal concentrations and their confidence limits have been plotted against time. See Figure 4.15 for other examples

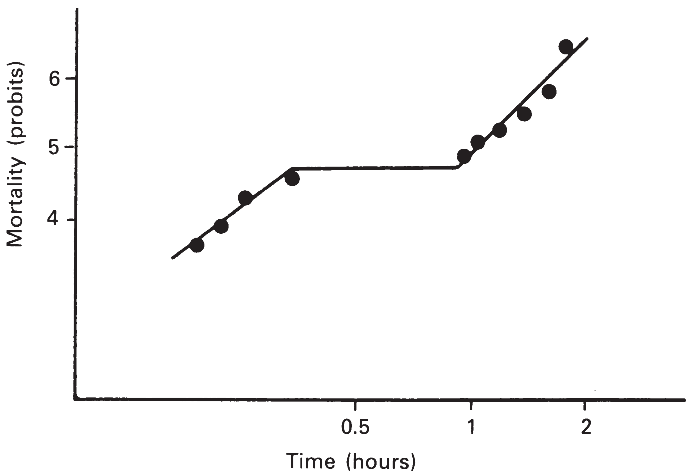

Figure 4.5 A 'split' probit line from an experiment to determine the lethal toxicity of the detergent sodium lauryl sulphate to rainbow trout, *Salmo gairdneri*. The discontinuity indicated a heterogeneity in the population of test animals, in this case due to the existence of two separate mechanisms of toxic action (Abel, 1978)

provided by examination of probit lines for irregularities of slope or the presence of inflections. 'Split' probit lines (Figure 4.5) occasionally occur, which indicates a heterogeneity of the population from which the sample was drawn (Hewlett and Plackett, 1979). Such heterogeneity may be due to intra-specific variability in susceptibility to the poison between sexes or age classes, or may indicate the development of resistant strains within the population.

Another possible explanation is that the poison may have two (or more) mechanisms of action, and that animals which are resistant to one mechanism may subsequently succumb to another. Tyler (1965) concluded on the basis of split probit lines that high temperature had three separate mechanisms of lethal action in fish. Confirmation that such an interpretation can be correct is given by studies on the toxicity and toxic effects of an anionic detergent (Abel, 1976, 1978). A concentration-dependent change in the mode of lethal action of the detergent was found to be associated with the occurrence of split probit lines in toxicity tests.

Where the heterogeneity in susceptibility to the poison is not pronounced, it may be manifested by an alteration in the slope of the probit lines rather than in the occurrence of inflections (Hewlett and Plackett, 1979). Simple tests for significant differences in the slope of probit lines are available (e.g. Litchfield, 1949) and have been used to demonstrate the occurrence of more than one mode of lethal action in fish exposed to toxic pollutants (Abel, 1978; Burton *et al.,* 1972).

Ball (1967a) showed that the slope of the probit lines was an important datum in comparing the relative susceptibilities of species to poisons. Ammonia was equally toxic, in terms of the 5-day LC50 value, to roach *(Rutilus rutilus)* and rudd *(Scardinius erythrophthalamus).* However, there was a considerable difference in the slope of the concentration-mortality curves. In practical terms, this may be important in the application of the toxicological data to water quality standards. For example, an ammonia concentration equivalent to two-thirds of the 5-day LC50 may be expected to kill less than 1% of rudd, but 16% of roach.

These examples illustrate the potential importance of a full analysis of probit lines in the investigation of lethal toxicity. Such detailed consideration is rarely given, and it would appear that much useful information from toxicity tests may thereby be lost. Where irregularities of the kind described above are found to occur, they may indicate promising lines of further investigation.

4.1.4 Toxicity Curves

The toxicity curve describes the empirical relationship between poison concentration and survival time of the animals. Some representative curves are shown in Figures 4.2–4.9. There have been several attempts at mathematical descriptions of toxicity curves (reviewed by Sprague, 1969), but the validity and usefulness of this are questionable (Brown, 1973), as is speculation concerning the shape of the curve, and such practices have long been discontinued. As Brown (1973) points out, for any poison there will be a concentration so low that it will never cause the death of half the animals.