CHAPTER 6

The Statistical Analysis of Dose–Effect Relationships

C. C. BROWN

Biometry Branch, National Cancer Institute, National Institute of Health, Bethesda, Maryland 20014, U.S.A.

6.1 INTRODUCTION 115
6.2 EXPERIMENTAL DESIGN 117
6.3 QUANTAL RESPONSES 120
6.4 MATHEMATICAL MODELS OF TOLERANCE DISTRIBUTIONS 122
6.5 ESTIMATION OF A QUANTAL DOSE–RESPONSE CURVE 126
6.6 QUANTITATIVE RESPONSES 136
6.7 TIME-TO-OCCURRENCE MODELS 136
6.8 APPLICATION TO ECOLOGICAL RISK ASSESSMENT 140
6.9 CONCLUSIONS 141
6.10 REFERENCES 142
6.11 APPENDIX: THE MAXIMUM-LIKELIHOOD METHOD OF FITTING DOSE–RESPONSE MODELS TO QUANTAL DATA 145

6.1. INTRODUCTION

The relation of responses to doses of environmental toxicants is an important element in the control and prevention of ecological problems. In general terms, dose–response is the relation between any measurable stimulus, physical, chemical, or biological, and the response of living matter in terms of the reactions the stimulus produces over some range of the amount of stimulus. In toxicological situations, there is normally, though not always, a monotone relation between the intensity of the stimulus and the particular response it elicits.

The reactions to any one stimulus may be multiple in nature, e.g. loss of weight, decrease in blood sugar, central nervous system disorders, decrease in organ function, or even death. Each reaction will have its own unique relation with the degree of the stimulus. In addition, the measurement of any specific reaction can be made in terms either of the magnitude of the effect produced, including whether the effect is produced or not, or of the time required for the appearance of a specific effect. These responses may be acute reactions, sometimes occurring within minutes of the stimulus, or they may be long-delayed effects such as cancer, which may not appear clinically until most of the animal's normal lifespan has elapsed.

Other responses may not even appear in the exposed subject, but may become manifest in some later generation.

The degree of stimulus, or in general terms, the dose level, may be measured in different ways. For example, consider some animal that is exposed to a chemical toxicant in the environment, either through the air breathed, the food eaten, or through some other external exposure. The magnitude of the stimulus, or exposure level, may be quantified as parts per million in the air or food, or may be measured as the quantity of the substance actually reaching the target receptor, some internal organ or other tissue. The former quantity can be thought of as the 'actual' exposure, or dose level, while the latter may be termed the 'effective' exposure level. The actual level may be modified by absorption, distribution, metabolism, detoxification, and excretion of the chemical substance. Therefore, the effective level may well be some complex function of the actual level along with the biochemical and physiological dimensions of the host resulting in a potentially quite different relation between the levels of the stimulus and the magnitude of response, depending upon the manner in which the stimulus is measured. Swartz and Spear (1975) recommend relating the time-integrated internal exposure to the observed response in carcinogenesis studies since mechanistic conclusions, which are dependent upon this relation, may be obscured by the relation of external to internal exposure.

The relation between the dose level of a toxicant and the resulting reaction is often impossible to estimate by direct measurements of the stimulus itself unless its mechanism of action is known. A toxic element in different chemical constitutions may produce quite different biological effects in the same host due to different modes of interaction with the organism and its tissues. Therefore, lacking complete mechanistic knowledge, the relation between the toxicant and its effects on a biological system must be either observed in nature or tested in the laboratory or field. Experimentation under laboratory conditions is ideal for the control of extraneous variation or possible bias. The experimental conditions may be rigidly controlled and the results are subject to considerations of reproducibility. However, because of potential physical limitations of the laboratory, the experimental conditions may well be unrealistic images of the natural environment and hence, extrapolation to the real world may be difficult and sometimes unwarranted. Experiments on plants or animals may also be conducted in the field. Here, the idea is to reproduce the natural environment as closely as possible, but with this type of experiment one cannot easily control the many factors which may have an influence on the results of the test.

This section is concerned with the statistical techniques used for the estimation of dose–response relations. It will be assumed that the particular agent in question is known to be generally toxic and that the purpose of the following statistical analyses is to obtain an indication of the relation between dose level and the toxic response. The general methods are applicable to any experimental situation, be it in a highly controlled laboratory environment, in the field, or gradations in between.

One feature common to all experiments in any field, biological or other, is the variability in the measured effects from a given stimulus. In experiments with living matter this variability will usually be much greater than in the common chemical or physical measurements. In addition to the simple variation inherent in the measuring device, such as a scale to measure weight or a more complex assay of the amount of a certain chemical in the blood, the response of the experimental subjects, be they plants or animals, may also be influenced by biological and physiological factors such as sex, age, or some other physical conditions. The test subjects themselves will not be a completely homogeneous group with respect to all important factors which may affect the stimulus–response reaction. The susceptibilities of the subjects to the stimulus may well be dependent upon genetic differences which, even if known, cannot be completely controlled. In any experiments used to estimate a dose–response relation, the results of the experiment and its analysis must include some measures of the variability of the results, for such results to be properly interpretable.

6.2. EXPERIMENTAL DESIGN

The assessment of any stimulus–response relation will depend upon the data on which it is based. Inadequate data, whether experimental or observational, will not permit estimation of this relation. As an extreme example, when the effect is measured in terms of the per cent incidence of a particular response, any experiment in which the dose levels are either too low to show any response, or so high that they all show 100% response, can obviously give little information on the dose–effect relation. Any study, properly designed to elicit adequate information on the relation between dose and response, will entail the consideration of many factors (Emmens, 1948; Jerne and Wood, 1949; Finney, 1964).

The initial step is selection of the species of test subjects. Ideally, the subjects should be that species to which the results of the experiment are to be applied, or a closely comparable species. If one wishes to estimate the toxicological effect of some chemical pollutant in the environment, then representative species in the environment, either terrestrial or aquatic or both, should be tested. However, this approach is often not feasible for a variety of reasons. The species to be tested should be chosen on the basis of its susceptibility to the induction of the response of interest and on the basis of its biochemical and physiological similarity to the species to which the experimental results are to be applied. Similar dose–effect relations for many species will produce invaluable information on the general applicability of the dose–effect relation. Dissimilarities, which can be explained by known physiological differences between the species, may also be useful for extrapolating from one species to another.

The next step in the experimental design is the selection of the route and duration of the exposure which should be comparable to those occurring in nature. An inhalation study cannot be extrapolated to oral exposure without making a

number of assumptions concerning the fate of the toxic agent between initial exposure and its reaching the target tissue. Metabolic pathways or physiological barriers may vary with different exposure routes. The results of single exposure studies cannot always be applied to chronic exposures in the environment; a single dose of 100 units of some toxicant may be either more or less effective than 100 fractionated one-unit doses of the same agent distributed over a period of time. For example, Matsumura (1972) showed that the ratio of the chronic to acute oral dosage for mallards required to produce 50% mortality is 50 for DDT and 1 for dieldrin and 'Zectran', with other insecticides falling within this range. In addition, single exposure situations may depend on physiological factors such as the age of the subject. Huggins *et al.* (1961) have shown that the rate of induction of mammary cancer by 3-methycholanthrene in rats is strongly affected by the age of the animal at exposure.

The selection of dose levels for an experimental bioassay is a critical step in the design of the study and will depend upon the purpose of the study. If the primary purpose is to show that a certain effect can be produced by the test agent, then the ideal design would have one treated group at the highest dose level that can be tolerated by the test subjects, i.e. a dose that will not produce other toxic effects that may obscure the response of interest. To guard against the possibility of incorrectly using too high a level of the stimulus, a second, lower level is also commonly incorporated in the design. If the response under consideration may have a spontaneous occurrence, then an untreated control group must also be included. This type of design will not, however, produce much information about the shape of the dose–response relationship. An efficient design to ascertain this shape should consist of a number of dose levels selected to produce a range of response rates between 10% and 90%. If little or no prior information is available on the expected response rates at various dose levels, then some type of pre-experiment should be performed. Dixon and Mood (1948) proposed what has been termed an up-and-down method, using single-test subjects, to estimate the dose level required to produce a specific response rate of some acute effect. Their technique has been generalized and extended by Robbins and Munro (1951) and Hsi (1969) to estimate the dose levels that produce 10%, 50%, and 90% responses; the final dose–response bioassay study can then be designed to use dose levels within this range. Bartlett (1946) suggested the use of an 'inverse sampling rule' for this problem. Ideally, the more dose levels used, the better the dose–response relationship can be estimated, but the cost of the study will normally determine the total number of subjects in the experiment. Therefore there is some trade-off between having many dose levels and having adequate numbers of test subjects at each dose.

The optimal spacing between the chosen number of dose levels is unknown, but a common approach is to choose equal spacing on the dose–level scale that is to be used in the analysis. For example, if it is planned to use a probit or log-logistic model for the analysis, then the doses should be selected to have equal spacing on a

logarithmic scale. Hoel and Levine (1964) have given an optimal spacing solution to the general polynomial regression problem when the purpose is to estimate a value outside the observation range. Wetherill (1963) gives an optimal spacing for use in a logistic model.

Once the total number of test subjects has been decided, a decision must be made as to their allocation over the dose levels; this will depend upon the purpose of the study. If the design calls for k dose-level groups, each to be compared to a control group simply to find if an effect of treatment exists at some dose, then an optimal design is to allocate an equal number, say N_t, to each treated group, and place $\sqrt{k}N_t$ subjects in the control group. However, if the purpose is to estimate the response at each dose level equally well, then the number of subjects allocated to the ith level should be proportional to $p_i(1 - p_i)$, where p_i is the expected response rate. This means that one should allocate progressively less subjects to the extreme dose levels, both low and high. However, this does not imply that the number of control subjects should be zero unless the unexposed response rate is known to be zero. If no prior information exists on the expected response rates, then equal allocation should prove a reasonable strategy.

More complex bioassays involving combinations of toxic agents or the examination of different factors that modify the effects of a single toxicant may also be designed. The combined exposure to different toxicants may produce independent, additive, or synergistic effects, or they may be antagonistic to one another. Definitions of these actions, and the construction of theoretical models to explain them have been proposed by many authors; Plackett and Hewlett (1967), Hewlett and Plackett (1959, 1964), and Ashford and Smith (1964, 1965) are among them. Street *et al.* (1970) discuss the ecological significance of such interactive effects and the complexities inherent in their measurement. The subject is also discussed in Chapter 9.

A single experimental bioassay can measure the effect of a stimulus under only one fixed set of experimental conditions. However, nature presents more than a single face, and the ability to extrapolate experimental results to the natural environment will depend upon the generality of the bioassay design. Muirhead-Thompson (1971) discusses the influence of physical factors, such as temperature, water hardness, and pH, on the pesticide impact on fresh water. Matsumura (1972) showed that the mortality rate of brine shrimp exposed to various chlorinated hydrocarbon insecticides was dependent upon the salt concentration, the effect being greatest at either extremely low or high concentrations. Therefore, since we do not know whether these potential modifying factors exert independent or interactive effects, multifactorial studies should be designed in such a manner as to allow for the measurement of all possible joint effects. A complete multifactorial experiment will normally require a sizeable study. For example, a study with 3 factors having 4, 3, and 2 levels respectively along with 5 dose levels would require $120 = (4 \times 3 \times 2 \times 5)$ groups of test subjects. At twenty subjects per group, we would be faced with an experiment containing 2,400 subjects. However, using

experimental design techniques as described by Kempthorne (1952) and Cochran and Cox (1957) for general statistical problems and Finney (1964) and Das and Kulkarni (1966) for bioassays, the size of these studies may be reduced if one is willing to assume that certain interactions between the factors, the dose and the response are negligible. However, this reduction in experimental units produces greater complexity in the analysis of such studies, so great care should be exercised in their design.

6.3. QUANTAL RESPONSES

One type of response commonly measured in toxicological studies is the quantal, or all-or-nothing, response, e.g. death. Measurements of degree of effect will normally provide a more refined measure of the response to a stimulus, but quantification is often very difficult or, for some responses, impossible.

When the response is quantal, its occurrence, for any particular subject, will normally depend upon the degree of the stimulus. For this subject, under constant environmental conditions, there will usually be some level of the stimulus below which the response will not occur and above which it will. This level is referred to as the subject's tolerance. Because of the biological variability among the population of individuals, their tolerance levels will also vary, sometimes within quite wide limits.

For quantal responses it is therefore natural to consider the distribution of tolerances over the population in question. If D represents the dose level of a particular stimulus, then the distribution of tolerances may be mathematically expressed as $f(D)\mathrm{d}D$ which represents the proportion of individuals having tolerances between D and $D + \mathrm{d}D$, where $\mathrm{d}D$ is small. If we are willing to assume that all members of the population will respond to a sufficiently high level of the stimulus, then the sum of these proportions should equal unity, or

$$\int_0^\infty f(D)\mathrm{d}D = 1. \tag{6.1}$$

If a population is exposed to a dose of D_0, then all members with tolerances less than or equal to D_0 will respond, and the proportion they represent of the total population can be calculated as,

$$P(D_0) = \int_0^{D_0} f(D)\mathrm{d}D. \tag{6.2}$$

Figure 6.1 shows a hypothetical tolerance distribution, $f(D)\mathrm{d}D$, along with its corresponding cumulative distribution, $P(D)$, as defined in equation (6.2). The function $P(D)$ represents the dose–response relationship for the population when the response is quantal in nature. For any individual, the dose–response curve would be a step function, zero less than its tolerance and unity greater than its tolerance. However, for a population of individuals, the response can be measured

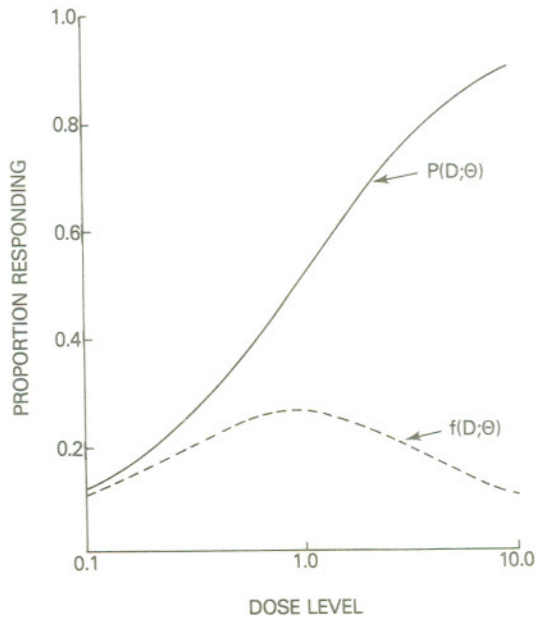

Figure 6.1 Example of relation between threshold distribution $f(D)$ and dose–reponse curve $P(D)$

as the proportion responding. The curve defined by $P(D)$ can also be considered as the probability that one individual, selected at random from the population, will respond to a dose level D. This particular distribution in Figure 6.1 assumes that $P(0) = 0$ (no responders for a zero dose) and $P(\infty) = 1$ (all will respond to some high dose). Either or both of these two assumptions may be untrue. The first assumes no spontaneous occurrence of the particular response which is false for a number of responses, while the second may be false if either an immune group exists within the population or the particular response in question becomes overwhelmed at high dose levels by a different response and does not have a chance to become manifest.

If a group of N test subjects were chosen at random from some population having a tolerance distribution given by $f(D)\mathrm{d}D$, and each subject was exposed to the same dose level D, then the number of subjects showing the response would be a random variable having a binomial probability distribution. In mathematical terms, the probability of R responders out of N subjects each given a dose of D, is given by,

$$\mathrm{Prob}(R) = \binom{N}{R} P(D)^R Q(D)^{N-R}, \tag{6.3}$$

where $Q(D) = 1 - P(D)$ and $P(D)$ is the probability of response for one randomly chosen test subject as defined in Equation 6.2. The observed proportion of responders in the test sample, $p(D) = R/N$, is then an estimate of the true proportion in the population, $P(D)$. This observed proportion can be either larger or smaller than the population value because of sampling variation, but the variation is centered around the value $P(D)$ and decreases as the size of the test sample increases.

An estimate of the dose–response relation can be obtained by testing various groups of subjects at different dose levels. Each value of the observed proportion of responders, $p(D)$, is an estimate of its corresponding $P(D)$, and from these quantities, the population cumulative tolerance distribution can be estimated. In general, $P(D)$ will increase with the dose D, but if the number of test subjects at each dose level is small, then sampling variation may interfere with the regularity of trend in the observed proportions $p(D)$.

The preceding discussion has assumed that the subjects chosen to be tested at each dose level have been randomly selected from some larger group of subjects and that the experimental conditions are the same for each dose. Deviations from these assumptions, either by choice or by chance, may result in the binomial model equation (6.3) being inapplicable. For example, non-random selection of test subjects, such as different age groups at the different dose levels or the selection of a group of subjects from the same parent to be tested at the same dose, would cause the ratio of the variation among dose groups to the variation within groups to be larger than expected under the binomial model. In addition, differences between the dose groups with respect to factors known to be associated with the response, such as age, sex, weight, or some conditions of the experiment itself, could produce an undesired bias in the observed proportions. In any experimental situation, care must be given to select the groups at random and to control other variables of the experiment.

6.4. MATHEMATICAL MODELS OF TOLERANCE DISTRIBUTIONS

A toxicological dose–response experiment, or bioassay, will result in a series of dose levels, D_i, $i = 1, \ldots, k$, along with their corresponding observed proportions responding, $p_i = p(D_i)$. These pairs of values (D_i, p_i) provide an estimate of the dose–response relation for only a limited number, k, of dose levels. An estimate of the entire dose–response curve can be made only by assuming some general functional form relating dose to response, i.e. $P(D) = g(D; \theta)$, where the function g represents some particular class, the member of the class being defined by the unknown constant, or constants, θ. For example, g may be a simple linear function, $g(D; \theta) = \theta_0 + \theta_1 D$, or a more complex function such as $g(D; \theta) = 1/2 + (1/\pi)\tan^{-1}(\theta_0 + \theta_1 \log(D))$.

The results of toxicity tests often show that the observed proportion of responders monotonically increases with dose and shows a sigmoid relation with

some function of the exposure level. This led to the development of the normal, or probit, model of the dose–tolerance distribution. This model assumes that the population distribution of tolerances is given by the normal probability model,

$$f(D;\theta) = (2\pi\theta_1^2)^{-1/2} \exp\left[-\frac{1}{2}\left[\frac{x(D)-\theta_0}{\theta_1}\right]^2\right], \theta_1 > 0 \tag{6.4}$$

or

$$P(D;\theta) = \int_{-\infty}^{\frac{x(D)-\theta_0}{\theta_1}} (2\pi)^{-1/2} \exp(-\tfrac{1}{2}t^2)\, dt,$$

where $x(D) = x$ is some transformed value of the dose level D. Some transformations commonly used in practice are

$$x = \log_{10}(D),$$

and, more generally

$$x = D^a,$$

where $a \leqslant 1$. The validity of any transformation will depend upon the mechanism of the response to the stimulus in question and, as such, is beyond the scope of this discussion. We, as Finney (1949), propose to use any transformation that appears to fit the observational data, but any additional mechanistic knowledge for a particular problem should be incorporated into the model. The normal model in equation (6.4) has commonly been used with the logarithmic dose transformation. A history of the development of this model is given by Finney (1952).

Other mathematical models of tolerance distributions which lead to the sigmoid appearance of their corresponding dose–response curves are:

1. The logistic curve,

$$P(D;\theta) = \{1 + \exp[\log(\theta_0) - \theta_1 \log(D)]\}^{-1}, \theta_0, \theta_1 > 0$$
$$= \frac{D^{\theta_1}}{\theta_0 + D^{\theta_1}} \tag{6.5}$$

which is derived from chemical kinetic theory and was proposed by Wilson and Worcester (1943) and Berkson (1944) for bioassay analyses, and

2. The sine curve,

$$P(D;\theta) = \tfrac{1}{2}\{1 + \sin[\theta_0 + \theta_1 \log(D)]\} \tag{6.6}$$

which is applicable only over a limited range of doses, $-\pi/2 \leqslant \theta_0 + \theta_1 \log(D) \leqslant \pi/2$, and has no theoretical justification other than computational simplicity (Knudsen and Curtis, 1947). Other dose–response models have been proposed on the basis of what has been called 'hit theory'. These models do not start with an assumed dose–tolerance distribution to produce a dose–response curve, but are derived on

general mechanistic dose–response assumptions. Turner (1975) summarizes all these models. The more commonly used models are the single hit model,

$$P(D;\theta) = 1 - \exp(-\theta D), \quad \theta > 0 \tag{6.7}$$

and the multi-hit model,

$$P(D;\theta) = 1 - \sum_{h=0}^{m-1} \frac{1}{h!} (\theta D)^h \exp(-\theta D), \quad \theta > 0 \tag{6.8}$$

where m is the minimum of 'hits' on a receptor required to obtain a response. Another model, which has been proposed as a mechanism in carcinogenesis, has been termed the multi-stage model. One derivation of this general process, due to Armitage and Doll (1961) and extended by Peto (1975), leads to the mathematical relation,

$$P(D;\theta) = 1 - \exp\left(-\sum_{h=1}^{m} \theta_h D^h\right), \quad \theta_h \geqslant 0, \quad h = 1, \ldots, m \tag{6.9}$$

where m is the number of stages in the process affected by the agent. A dose–tolerance distribution may be obtained from these models by inverting equation (6.2), i.e.

$$f(D;\theta) = \frac{\mathrm{d}P(D;\theta)}{\mathrm{d}D} \tag{6.10}$$

Therefore, the tolerance distribution corresponding to the multi-hit model (6.8) is the gamma distribution

$$f(D;\theta) = \frac{\theta^m}{\Gamma(m)} D^{m-1} \exp(-\theta D) \tag{6.11}$$

which looks similar to both the log-normal and log-logistic tolerance distributions.

Table 6.1 compares the dose–response relations of three models. It might be thought that the selection of one particular model over the others would be obvious from inspection of the calculated responses but this table shows that three of the most commonly used models, log-normal, log-logistic, and single-hit give results that differ by little over a 256-fold dosage range (FDA Advisory Committee on Protocols for Safety Evaluation, 1971). It would take an inordinately large experiment to be able to conclude which of the three models best described the observational data.

If the calculated dose–response curve is to be used to estimate the response rate that would be expected from an exposure level within this range of observable responses, then all three models will give comparable results. Interpolation between observed data points within the range of approximately 5%–95% response rates will not be greatly affected by the mathematical model selected. However, extrapola-

Table 6.1 Expected Per Cent Responding for Various Models over a Range of Dose Levels

Dose level	Log-normal model	Log-logistic model	Single-hit model
16	98	96	100
8	93	92	99
4	84	84	94
2	69	70	75
1	50	50	50
$\frac{1}{2}$	31	30	29
$\frac{1}{4}$	16	16	16
$\frac{1}{8}$	7	8	8
$\frac{1}{16}$	2	4	4

tion to exposure levels expected to give very low response rates is highly dependent upon the choice of mathematical models, which is shown in Table 6.2 extending the previous table to much smaller doses. It can be seen that the further one extrapolates from the observed response range, the more divergent the various models become. At a dose level that is 1/1000 of the 50% response dose, the single-hit model gives an estimated response rate 200 times as large as the log-normal model. The fact that a moderate-sized experiment conducted at dose levels high enough to give observable response rates cannot discriminate among these various models, and the fact that these same models show a substantial divergence at small dose levels present major difficulties for low dose extrapolation problems. Brown (1976a) has suggested the use of a multi-stage model (equation 6.9) along with an estimate of both sampling and model variability for this problem, since the multi-stage model has the extrapolation characteristics of most other models, depending upon the number of stages used.

It should be noted that all the mathematical dose–response models presented have the feature that for any dose $D > 0$, $P(D) > 0$, i.e. there is no absolute threshold level below which the probability of response is zero. A common

Table 6.2 Expected Per Cent Responding at Low Doses for Models Describing Observed Responses in the 5%–95% Range Equally Well

Dose level	Log-normal model	Log-logistic model	Single-hit model
0.01	0.05	0.4	0.7
0.001	0.00035	0.026	0.07
0.0001	0.0000001	0.0016	0.007

toxicological problem is whether or not a threshold level actually exists. Thresholds should, however, be considered from two different viewpoints: a 'theoretical' level below which a toxic response is impossible; and a 'practical' level below which the chance of response is highly unlikely or unobservable. Theoretical thresholds could vary substantially in an outbred population due to genetic differences, and their existence cannot be proven by statistical arguments; this proof would have to come from complete knowledge of the mechanism of toxicological action.

Experimental or observational evidence for the existence of a threshold is commonly presented in the form of a dose–response graph in which the response rate is plotted against dose level. Either the existence of doses not showing an increase in response over controls or the extrapolation of such curves to low doses which apparently would result in no increased response are cited as indications of the existence of some threshold below which no response is possible. This type of evidence is of little value. In the first situation, the observation of no responders does not guarantee that the probability of response is actually zero. From a statistical viewpoint, zero responders out of N at risk is consistent at the 5% significance level, with an actual response rate between zero and approximately $3/N$.

In the second case, when a graph of observed dose against responses is extrapolated downward to a no-effect level, the observed dose–response relation, often linear, is assumed to persist throughout the entire range of dose levels. This assumption can easily lead to an erroneous conclusion when the true dose–response curve has a rising slope, i.e. is convex. Brown (1976b) discusses this problem in detail when the response is carcinogenic in nature. He shows that statistical analyses of bioassay results cannot discriminate between mathematical models which assume the existence or non-existence of an actual threshold. Therefore, without a knowledge of the mechanism producing the response, when extrapolating below the observable response range, it would be prudent to assume that no threshold exists.

6.5. ESTIMATION OF A QUANTAL DOSE–RESPONSE CURVE

In this section we shall assume that we have concluded a typical quantal dose–response experiment, or bioassay, and, having observed the proportions responding at various dose levels, we wish to estimate the population dose–response relation.

First we have to select one of the mathematical models with which we shall perform the analysis. For the sake of simplicity, we shall use the log-logistic model (equation 6.5), though the general technique to be described is applicable to any model. The logistic model can be written as,

$$P(x; a, b) = \{1 + \exp[-(a + bx)]\}^{-1}, \; b \geqslant 0 \tag{6.12}$$

where P is the probability of a randomly selected individual from the population responding to an exposure of x = log(dose). The series of log dose levels are denoted

as $x_1, x_2, \ldots, x_k$ and their corresponding observed proportions responding as $p_i = p(x_i) = r_i/n_i$, $i = 1,2, \ldots, k$, where r_i is the number of test subjects responding out of n_i exposed at the ith dose level. The estimation of the dose–response curve consists of estimating the two unknown parameters a and b, which can be accomplished in a variety of ways, ranging from simple graphical techniques (DeBeer, 1945; Litchfield and Wilcoxon, 1949), to more sophisticated methods such as maximum likelihood or minimum chi-square. The maximum-likelihood method is an extremely general, fully efficient estimation technique but is computationally difficult (Cornfield and Mantel, 1950). Bliss (1935) and Finney (1952) give its application to the probit model and the details of this method are given for any general quantal response model in the Appendix to this chapter. A simpler computational approach has been given by Grizzle *et al.* (1969) for the general logistic model and by Berkson (1949) for bioassay data.

The logistic model in (6.12) can be rewritten as,

$$\log\left[\frac{P(x;a,b)}{Q(x;a,b)}\right] = a + bx \tag{6.13}$$

where $Q(x;a,b) = 1 - P(x;a,b)$. The transformation $\log(P/1-P)$ has been termed the logit transformation by Berkson (1944). This has reduced the problem from a non-linear to a simpler linear problem which can be solved by the method of least squares. The linear model in equation (6.13) relates the response to the dose for the ith experimental group as,

$$z_i = a + bx_i$$

where z_i is the logit of the response rate, and x_i is logarithm of the dose level.

Since the variances of the observed logits are not necessarily equal, we should properly use weighted, as opposed to unweighted, least squares. The variance of the ith response rate is P_iQ_i/n_i, where $Q_i = 1 - P_i$ and P_i is the population response rate. From asymptotic statistical theory, the variance of the logit of the response rate is $1/n_iP_iQ_i$, which is an approximate variance for finite samples. Therefore, the weighted least squares method uses $W_i = n_iP_iQ_i$ as the weights, which can be approximated by the observed response rates $w_i = n_ip_iq_i$. In the event that $p_i = 0$ or 1, which would result in the weighting factor being zero, Berkson (1953) suggests using $p_i = 1/2n_i$ in place of zero, and $p_i = 1 - 1/2n_i$ in place of unity. The weighted least squares technique produces the following estimates for a and b,

$$\hat{a} = \frac{\Sigma w_i x_i^2 \Sigma w_i z_i - \Sigma w_i x_i \Sigma w_i x_i z_i}{\Sigma w_i \Sigma w_i x_i^2 - (\Sigma w_i x_i)^2} = \bar{z} - \hat{b}\bar{x},$$

and (6.14)

$$\hat{b} = \frac{\Sigma w_i \Sigma w_i x_i z_i - \Sigma w_i z_i \Sigma w_i x_i}{\Sigma w_i \Sigma w_i x_i^2 - (\Sigma w_i x_i)^2} = \frac{\Sigma w_i (x_i - \bar{x})(z_i - \bar{z})}{\Sigma w_i (x_i - \bar{x})^2},$$

where $\bar{x} = \Sigma w_i x_i / \Sigma w_i$ and $\bar{z} = \Sigma w_i z_i / \Sigma w_i$. Once a and b are estimated, the population response rate can then be estimated for any dose level D in the following manner. First estimate the logit of the response rate using equation (6.13),

$$\hat{z} = \hat{a} + \hat{b} \log(D),$$

and then transform the logit to the response probability using equation (6.12),

$$\hat{P}(D) = (1 + e^{-\hat{z}})^{-1}$$

The estimated variances of these parameter estimates are given by,

$$S_a^2 = \frac{\Sigma w_i x_i^2}{\Sigma w_i \Sigma w_i x_i^2 - (\Sigma w_i x_i)^2} = \frac{\Sigma w_i x_i^2 / \Sigma w_i}{\Sigma w_i (x_i - \bar{x})^2},$$

and (6.15)

$$S_b^2 = \frac{\Sigma w_i}{\Sigma w_i \Sigma w_i x_i^2 - (\Sigma w_i x_i)^2} = \frac{1}{\Sigma w_i (x_i - \bar{x})^2}$$

The variance of the estimated logit z of the response rate for any value of the log dose, $X = \log(D)$, can be obtained from the relation,

$$\mathrm{Var}(z) = \mathrm{Var}(a + bX) = S_a^2 + X^2 S_b^2 + 2X S_{ab}^2,$$

where $S_{ab}^2 = -\bar{x} / \Sigma w_i (x_i - \bar{x})^2$ is the covariance between the two parameter estimates, $\hat{a}$ and $\hat{b}$. This can be simplified to become,

$$\mathrm{Var}(z) = S_z^2 = \frac{1}{\Sigma w_i} + \frac{(X - \bar{x})^2}{\Sigma w_i (x_i - \bar{x})^2} \qquad (6.16)$$

This variance can be used to place statistical confidence limits on the estimated logit,

$$z \pm Z_{\alpha/2} S_z \qquad (6.17)$$

where $Z_{\alpha/2}$ is a standard normal deviation corresponding to a total tail area of α. For 95% confidence limits, $\alpha = 0.05$ and $Z_{\alpha/2} = 1.96$. Once these limits are placed on the estimated logit, they can be transformed by equation (6.12) to give confidence limits on the estimated population response,

$$\{1 + \exp[-(z - Z_{\alpha/2} S_z)]\}^{-1} \leqslant P(D) \leqslant \{1 + \exp[-(z + Z_{\alpha/2} S_z)]\}^{-1} \qquad (6.18)$$

The regression equation (6.13) can also be used in reverse to find that dose level which is expected to produce a certain population response rate. A common method of characterizing the toxicity of a stimulus is by means of the median effective dose. This value is defined as the dose which will produce a response in half the population, and thus is sometimes called the median tolerance. This median

effective dose is commonly denoted by ED_{50}. When the response in question is death, then the term LD_{50}, the median lethal dose is used. Analogous symbols may be denoted dose levels which affect other proportions of the population, such as the ED_{10}, the dose which causes 10% of the population to respond.

In general, an estimate of the ED_p, the dose resulting in a proportion p of responders, can be obtained from equation (6.13) as,

$$\hat{X}_p = \log(D_p) = (\log(p/q) - \hat{a})/\hat{b} \tag{6.19}$$

The approximate variance of this log dose is given by,

$$S^2_{\hat{X}_p} = \left(\frac{1}{\hat{b}}\right)^2 \left(\frac{1}{\Sigma w_i} + \frac{(\hat{X}_p - \bar{x})^2}{\Sigma w_i (x_i - \bar{x})^2}\right). \tag{6.20}$$

As can be seen from this expression, the variance of this log dose increases as the estimated log dose moves away from the average log dose of the experiment. As before, confidence limits for the dose D_p giving the desired response rate p can be obtained from equations (6.19) and (6.20) as,

$$\exp(\hat{X}_p - Z_{\alpha/2} S_{X_p}) \leqslant D_p \leqslant \exp(\hat{X}_p + Z_{\alpha/2} S_{X_p}) \tag{6.21}$$

The following is an example of the concepts and calculations of these statistical procedures. The observed data come from an experiment designed to measure the lethal effect of rotenone sprayed on the chrysanthemum aphis. The data are given in Table 6.3, reproduced from Finney (1952). The proportions responding to treatment, (response here is death) are shown in column 5 for each of the five concentrations used in the experiment. The next five columns are quantities used in the calculation of the parameters a and b and their variances in the log-logistic model. The calculation of these parameters is shown below the table. The log dose associated with 50% mortality is estimated from equation (6.19) where $\log(P/Q) = \log(0.5/0.5) = 0$ resulting in the estimate being simply, $-\hat{a}/\hat{b}$. The last two columns of the table give the estimated proportion of responders at each dose level, calculated from equation (6.12), and the contributions to a chi-square test for comparing the fitted logistic model with the observed data. Figure 6.2 shows the observed proportions responding at each dose level and a graph of the log-logistic model fitted to these data along with 95% confidence limits on the fitted curve. The limits were obtained from equation (6.18). The figure shows the sigmoid nature of the log-logistic model and that it appears to describe adequately the observed dose–response relationship. One should, however, use a statistical test to evaluate the fit of the model to the data.

The Pearson chi-square statistic, denoted X^2, is commonly used to test whether or not the assumed model, here a log-logistic, fits the observed data. The statistic X^2 is given by,

$$X^2 = \Sigma \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

Table 6.3 Example of Log-logistic Model Applied to Quantal Response Data. Toxicity of Rotenone. (Reproduced by permission of Cambridge Univ. Press from Finney, 1952)

Concentration (mg/l) D	Log dose x	Responders r	Number at risk n	Proportion responding p	$w = npq$	wx	wx^2	wz	wxz	Estimated proportion responding	Chi square
2.6	0.956	6	50	0.120	5.380	5.048	4.826	−10.518	−10.055	0.129	0.036
3.8	1.335	16	48	0.333	10.661	14.232	19.0	− 7.388	− 9.863	0.322	0.027
5.1	1.629	24	46	0.522	11.477	18.696	30.456	1.010	1.645	0.539	0.054
7.7	2.041	42	49	0.857	6.007	12.260	25.023	10.759	21.959	0.805	0.844
10.2	2.322	44	50	0.880	5.280	12.260	28.468	10.518	24.423	0.907	0.432
					38.705	62.496	107.773	4.381	28.109		

$$\bar{x} = \frac{\Sigma wx}{\Sigma w} = 1.615$$

$$\chi^2_{(3)} = 1.393$$

$$\bar{z} = \frac{\Sigma wz}{\Sigma w} = 0.113$$

$$S_a^2 = \frac{\Sigma wx^2}{(\Sigma w \Sigma wx^2 - (\Sigma wx)^2)} = 0.406$$

$$\hat{a} = \bar{z} - \hat{b}\bar{x} = -4.838$$

$$S_b^2 = \frac{\Sigma w}{(\Sigma w \Sigma wx^2 - (\Sigma wx)^2)} = 0.146$$

$$\hat{b} = \frac{(\Sigma w \Sigma wxz - \Sigma wz \Sigma wx)}{(\Sigma w \Sigma wx^2 - (\Sigma wx)^2)} = 3.065$$

$$\hat{x}_{50} = \frac{-\hat{a}}{\hat{b}} = 1.578$$

$$\hat{LD}_{50} = 4.85$$

95% confidence limits on LD_{50}: $4.37 \leqslant LD_{50} \leqslant 5.37$

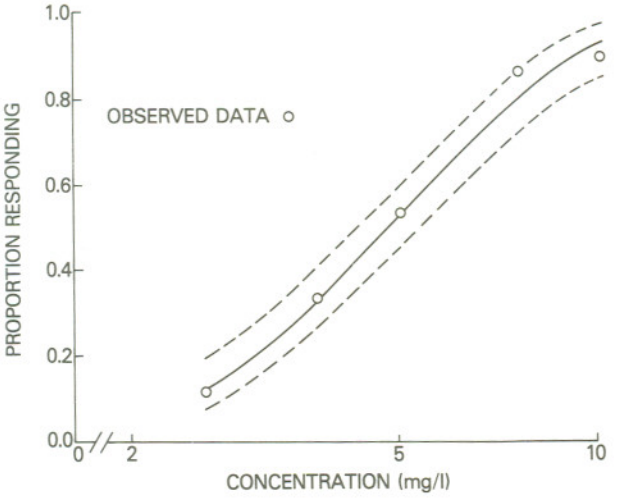

Figure 6.2 Estimated dose–response curve with 95% confidence limits (rotenone toxicity example). (Reproduced by permission of Cambridge Univ. Press from Finney, 1952)

which, for this type of bioassay experiment, is equal to,

$$X^2 = \Sigma_i \frac{n_i}{\hat{p}_i \hat{q}_i} (p_i - \hat{p}_i)^2 \tag{6.22}$$

where $\hat{p}_i$ is the estimated response probability for the ith dose level, and $\hat{q}_i = 1 - \hat{p}_i$. The degrees of freedom for this statistic are given by the number of dose levels minus 2, the number of parameters in the model that were estimated from the data. For this example, the tables show that $X^2 = 1.39$ with three degrees of freedom, which is not statistically significant. Therefore, we may conclude that the model adequately fits the observed data.

This is not always the case, however, as shown in the following example. The data are from an experiment by Sinnhuber *et al.* (1968) in which Rainbow trout, held in 200-gallon tanks, were fed with different commercial diets plus additives. The amount of aflatoxin in the diet and additives was measured and the response of interest was the incidence of hepatomas. The fish were sampled at 6, 9, and 12 months. Table 6.4 shows the results of the 12 month sample.

The last column of the table, the chi-square calculation to test the adequacy of the log-logistic model, yields a chi square of 21.9 with 8 degrees of freedom which is statistically significant, $P < 0.01$. An examination of the standardized differences between the observed and expected proportions responding, given by

$$R_i = \frac{\hat{p}_i - p_i}{\sqrt{\hat{p}_i \hat{q}_i / n_i}} \tag{6.23}$$

Table 6.4 Example of Log-logistic Model Applied to Heterogeneous Quantal Data. Incidence of Hepatomas in Rainbow Trout Fed Diets Containing Aflatoxin for 12 Months. (Reproduced by permission of the authors from Sinnhuber *et al.*, 1968)

Concentration (ppb) D	Log dose x	Responders r	Number at risk n	Proportion responding p	$w = npq$	wx	wz	wx^2	wxz	Estimated proportion responding	Chi square
0.8[a]	−0.223	0	12	0.0	0.444	−.099	−1.429	.022	0.319	0.104	1.393
3.7	1.308	5	10	0.5	2.5	3.27	0.0	4.277	0.0	0.380	0.611
4.0	1.386	2	13	0.154	1.907	2.643	−2.910	3.663	−4.033	0.401	3.302
5.0	1.609	9	18	0.5	4.5	7.241	0.0	11.651	0.0	0.460	0.116
7.9	2.067	5	12	0.417	2.929	6.054	−0.908	12.514	−1.877	0.584	1.378
8.0[a]	2.079	20	30	0.667	6.720	13.970	4.495	29.044	9.346	0.587	0.792
15.3	2.728	62	82	0.756	15.251	41.604	17.005	113.496	46.388	0.742	0.084
19.0	2.944	34	39	0.872	4.625	13.616	8.492	40.086	25.0	0.785	1.749
36.5	3.597	31	40	0.775	7.121	25.613	8.538	92.130	30.710	0.881	4.287
42.0	3.738	89	90	0.988	1.459	5.454	5.966	20.387	22.301	0.896	8.175
					47.456	119.366	39.249	327.270	128.154		

[a]Fed for 13 months.

$\chi^2_{(8)} = 21.887$

$$\bar{x} = \frac{\Sigma wx}{\Sigma w} = 2.515 \qquad \bar{z} = \frac{\Sigma wz}{\Sigma w} = 0.827$$

$$a = \frac{\Sigma wx^2 \Sigma wz - \Sigma wx \Sigma wxz)}{(\Sigma w \Sigma wx^2 - (\Sigma wx)^2)} = -1.912$$

$$S_a^2 = \frac{\Sigma wx^2}{(\Sigma w \Sigma wx^2 - (\Sigma wx)^2)} = 0.255$$

$$b = \frac{(\Sigma w \Sigma wxz - \Sigma wz \Sigma wx)}{(\Sigma w \Sigma wx^2 - (\Sigma wx)^2)} = 1.089$$

$$S_b^2 = \frac{\Sigma w}{(\Sigma w \Sigma wx^2 - (\Sigma wx)^2)} = 0.037$$

may sometimes give a clue as to where the assumed model fails. Draper and Smith (1966) give an excellent discussion of residual analysis. In this situation, there appear to be no systematic deviations related to dose level nor to response rate, so we would have to conclude that the assumption of binomial sampling may be in error. The variation in the data is larger than would be expected under the binomial assumption. The original experiment consisted of 15 groups being fed various diets containing different amounts of aflatoxin. This variation in diets and additives may well have created the extra sampling variation. Kleinman (1973) and Cochran (1943) discuss this problem of proportions with extraneous variation from the viewpoint of statistical tests in the analysis of variance. They consider the extra variation to be additive to the binomial sampling variation. Finney (1952) suggests assuming that the true variation is simply a multiple of the binomial variation, and he proposes multiplying each of the variance estimates in equations (6.15), (6.16), and (6.20) by a heterogeneity factor, X^2/k, where k is the number of degrees of freedom for the chi-square statistic X^2. In addition, in order to allow for the uncertaintly in estimating this heterogeneity factor, the normal deviates, $Z_{\alpha/2}$, used in calculating confidence limits, equations (6.17) and (6.21), should be replaced by t values, $t_{k,\alpha/2}$ where t has k degrees of freedom. Since these t values are larger than the corresponding normal deviations, this has the effect of producing wider confidence intervals. In the previous example with heterogeneous data, the 95% confidence limits on the probability of response at a concentration of 5.0 p.p.b. of aflatoxin in the diet would be, using no heterogeneity factor and using normal deviations,

$$0.35 \leqslant P(5 \text{ ppb}) \leqslant 0.571$$

However, after multiplying the variance in equation (6.16) by the heterogeneity factor $21.887/8 = 2.736$, and using a t value with 8 degrees of freedom, 2.306, in equation (6.18), the proper 95% confidence limits become,

$$0.264 \leqslant P(5 \text{ ppb}) \leqslant 0.669$$

which are substantially wider than those calculated assuming no heterogeneity. It should be noted, however, that this correction procedure is only an approximation and should not be used if the Pearson X^2 statistic is not statistically significant.

The preceding results have been obtained by tacitly assuming that no response was possible at a zero dose, i.e. $P(0) = 0$. This is seen to be true for the log-logistic model defined in equation (6.12). When the dose is zero, the log dose, x, is minus infinity, and since the parameter b is restricted to be non-negative, the probability of response becomes zero. This assumption of no response for no stimulus will not necessarily be true. In the two previous examples, both experimental designs contained a control, non-treated, group which produced no responders, so there was no apparent reason to consider the possibility of spontaneous, non-dose-related, response. However, if the response under consideration is one which either appears among a group of untreated subjects, or if such controls are not available, it

may be expected to have a spontaneous occurrence, then any mathematical dose–response model should properly allow for this possibility.

Two methods have been proposed to incorporate the possibility of response due to factors other than the stimulus in question. The first is known as 'Abbott's correction', attributed to W. S. Abbott (1925), and is based on the assumption of an independent action between the stimulus and the background, or spontaneous response. If a proportion C of the subjects would respond in the absence of any stimulus, then the total response rate at a dose level D, assuming independent actions, would be,

$$P'(D) = C + (1 - C)P(D) \tag{6.24}$$

From this equation it follows that the proportion responding due to the stimulus alone is,

$$P(D) = \frac{(P'(D) - C)}{(1 - C)} \tag{6.25}$$

If the spontaneous response rate C were known exactly, then the previous log-logistic model could be used with only minor modifications. The observed response rates p_i' should be transformed to their corresponding dose-related response rates p_i by use of equation (6.25). These p_i would be transformed to logits, $\log(p/1 - p)$. The weights w_i used for the calculation of the parameters a and b, and their variances, become,

$$w_i' = \frac{n_i p_i (1 - p_i)}{1 + \dfrac{C}{p_i (1 - C)}} \tag{6.26}$$

which are equal to w_i when $C = 0$. These modifications should not add undue complexities to the estimation of a dose–response curve. However, the spontaneous response rate is usually unknown, and even the knowledge of the proportion responding in some control group will only provide a range of plausible spontaneous rates. In this common situation of uncertainty, more complex methods of estimation, such as maximum likelihood, will be required and the general technique described in the Appendix to this chapter can be applied.

The second method of allowing for the spontaneous occurrence of response was proposed by Albert and Altshuler (1973) in which they suggested that the natural environment contains some additive background level of the stimulus, D_0, so that the response rate for a dose D would be,

$$P'(D) = P(D + D_0) \tag{6.27}$$

Abbott's correction factor would then be given as $C = P'(0) = P(D_0)$. Therefore, if C and the true dose–response curve were known, the background level D_0 could also be computed. This method, which will give results similar to the previous approach,

can only be employed using more complex estimation procedures. The maximum-likelihood method, assuming that D_0 is an unknown parameter, can be used.

In general, when the response under consideration may have a spontaneous occurrence independent of the stimulus, one should take care when using a model that assumes no such possibility. Figure 6.3 shows the effect of a spontaneous occurrence on the log-logistic dose–response function. The probability of response is given by equation (6.24) where $P(D)$ is the log-logistic model. The figure shows that, on a logit scale, whereas the log-logistic model is a linear function of the log dose (equation 6.13), the addition of a spontaneous response (here $C = 0.05$) induces a curvature at the low response rates. This could produce a bias in estimating the parameters of the log-logistic model assuming no spontaneous occurrence. The model from which the 'true' curve was produced has parameters $a = -5$ and $b = 3$, but the straight line fitted by eye through this 'true' curve would estimate them as $\hat{a} = -3.2$ and $\hat{b} = 2$. In general, when the 'true' dose–response curve contains a spontaneous element, then any model which assumes no such possibility will lead to an underestimate of the slope and an overestimate of the intercept. Looking at the difference between the estimated and 'true' curves in Figure 6.3 shows this difference to be negative for low dose levels, positive for moderate dose levels, and negative for high dose levels. This pattern provides a useful clue when examining the standardized residuals from the fit of a model to experimental data.

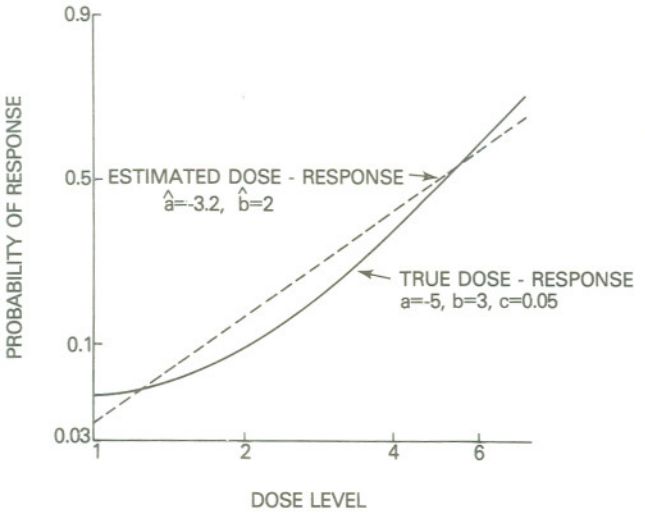

Figure 6.3 Comparison of true dose–response curve having spontaneous occurrence with estimated curve assuming no spontaneous occurrence

6.6. QUANTITATIVE RESPONSES

The preceding discussion has been concerned only with quantal responses. In many biological investigations, however, it is possible to quantify the magnitude of the response. Such data could be reduced to the quantal form by a simple dichotomy of the measurements into those greater than or less than some selected value. Since this procedure is wasteful of information, it would be better to use the actual measurements to construct a dose–response relation.

Dose–response data obtained over a limited range of dose levels may be analysed by general statistical regression techniques. The estimated regression equation will however, not be valid outside this range; the magnitude of the response is often limited to be non-negative and to have a maximum possible response at high doses. The general techniques of the previous sections can be applied to these quantitative data by transforming the responses to lie between zero and one which can be done by dividing each one by the maximum response (if unknown it is treated as an unknown parameter to be estimated from the data). Therefore, quantitative responses can be thought of as a special case of quantal responses. The statistical treatment of such data is similar to, but somewhat different from, that for quantal responses. Finney (1952, 1964) provides a discussion of these analyses.

6.7. TIME-TO-OCCURRENCE MODELS

The estimation and analysis of the dose–response models discussed in the previous section are based on observational data that are quantal in nature, i.e. the subject either responds or not. However, for many experimental situations, there is an additional piece of information that has been infrequently used in the past, viz. the time since initiation of treatment at which the subject responded, or in the case of no response, the total length of time the subject was observed without a response. This additional information may be of benefit in two ways. It should add data to define more clearly the dose–response relation, especially in those situations for which the response rates at the high dose levels are close to 100%. Those experiments for which most dose levels produce 100% response will not provide much information on the quantal dose–response relation, but upon examination of the times to response, a monotone relationship between dose levels and the mean, or median, time to occurrence may be revealed, Brown (1973). The further advantage of using these occurrence times is that it allows for the construction of mathematical models which relate the dose level to the expected time of response. These models can then be used to estimate the number of responders in some population at risk at various points in time. A third potential advantage will be important in some studies but not in others. This relates to the problem of competing risks. If an agent produces two or more toxic responses, then relating dose to the incidence of a late-occurring response may be obscured by a different, earlier toxic response such as death.

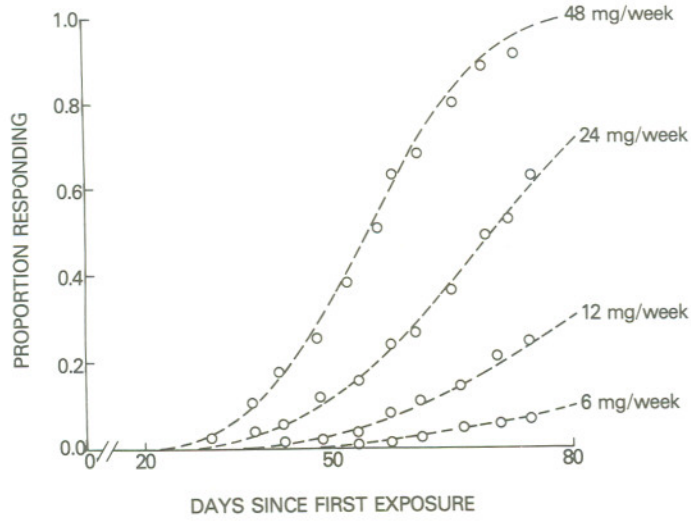

Figure 6.4 Comparison of estimated (———) and observed (○) proportions of animals with skin tumours at four dose levels. (Reproduced by permission of H. K. Lewis and Co. Ltd., from Lee and O'Neill, 1971)

Another aspect of time-related mathematical models which has received little attention is the effect of chronic exposure upon later generations of the organism in question. Brown (1958) discusses two possible changes in the dose–response relation from one generation to the next:

1. A reduced slope of the dose–response curve with a corresponding increase in the LD_{50} as the more susceptible phenotypes are eliminated from the population. This can be followed by a steepening of the slope of the dose–response curve as the population becomes more homogeneous for resistance.
2. Increases in the LD_{50} may also occur without changes in the slope due to an increased vigour of the strain rather than elimination of specific phenotypes.

A number of experiments have submitted colonies of insects to selection pressure from a specific insecticide, and when the LD_{50} levels are plotted against the generation number, they are often found to constitute a sigmoid curve. In the first few generations there is little increase in the LD_{50}, then a sharp increase followed by a flattening out, implying that a maximum resistance has been reached. Such increased resistance may be expected to develop faster when the selection pressure is high rather than when only a small proportion is killed in each generation. Once resistance has been increased and the selection pressure removed,

it has often been found that the strain shows a reversion toward the original susceptibility.

This process of adaptation to a toxicant has also been found with phytoplankton. The period of adaptation before resumption of normal exponential growth will depend upon the level of exposure. Stockner and Antia (1976) found that this adaptation period for the marine diatom *Skeletonema costatum* in various concentrations (10%–30%) of kraft pulpmill effluent ranged from 2 to 12 days. No mathematical models have been proposed to explain either of these phenomena.

6.8. APPLICATION TO ECOLOGICAL RISK ASSESSMENT

Once the proper experimental or observational data have been obtained in the laboratory and one can estimate the relation between exposure level and response, the impact of an exposure upon the total ecosystem or its individual components can in theory be evaluated. The changes to be produced within the entire ecosystem will entail knowledge of the complex interactions among the component parts, which is beyond the scope of this chapter. We shall consider only the assessment of risk to the one individual component which relates to the data in hand.

This risk assessment can be based on a variety of measures and responses: increased incidence of some undesirable response in the exposed population, e.g. death, decreased reproductive capacity, or other response; life-shortening due to one or more toxicological responses; or a manifestation of these responses passed through genetic mutations from the exposed population to their offspring. The selection of particular responses and their measurement may depend upon practical considerations but a variety of such measurements should be made to obtain a more complete assessment of the total risk.

A common problem in the ecological assessment of risk is that of extrapolating from the results of experiments on laboratory animals which are generally conducted under highly controlled conditions with genetically homogeneous animals, to animals of different species not genetically homogeneous, living under diverse environmental conditions, and exposed to a variety of other toxicological agents. Nutritional differences and the physical environment can affect the response to many stimuli. In addition, the natural chemical environment with its great variety of substances provides the possibility for either synergistic or antagonistic activity. Genetic differences can affect many aspects of toxicological susceptibility. Therefore, the environment and genetic variability of the target population should, whenever possible, be considered in the extrapolation process. (In general, this heterogeneity should reduce the slope of any experimentally derived dose–response curve.)

The animal species used in the laboratory experiment is often different from the species in the ecosystem to which the experimental results are to be extrapolated. Many simplistic methods for extrapolating from one species to another have been proposed. A useful first approximation is provided by the surface area rule. The

The construction of a mathematical time-to-occurrence model for dose and response is made up of two parts. The first is the mathematical form for the probability distribution of the response-time random variable t,

$$\text{Probability } (t \leqslant T) = F(T; \theta_1, \ldots, \theta_k) \tag{6.28}$$

where $F(\cdot)$ is some cumulative distribution function having probability function $f(\cdot) = \mathrm{d}F(\cdot)/\mathrm{d}t$, and $\theta_1, \ldots, \theta_k$ are unknown parameters. It is assumed that the mathematical form of F is the same for each dose level, and that one, or more, of the parameters θ_i are functions of dose. It is also assumed that all subjects will eventually respond, i.e. $F(\infty) = 1$, but because of competing risks such as death without evidence of the desired response, the subject may be removed from observation before the response can occur. This assumption is probably more valid for chronic exposure than acute exposure situations.

The second assumption concerns the relation between the dose level D and the parameters in equation (6.28). A general empirical relationship that has been proposed by Busvine (1938) and others is,

$$\theta = \alpha D^{\beta}$$

or

$$\log(\theta) = \log(\alpha) + \beta \log(D) \tag{6.29}$$

There is no presumed biological basis for this relationship, but when θ is the median time to response, a linear relation between the logarithm of θ and the logarithm of dose has often been observed.

Many mathematical time-to-occurrence models have been proposed and all have a direct correspondence with quantal dose–response models (Chand and Hoel, 1974). One of the first models was proposed by Druckrey (1967) in which he assumed that the probability distribution of the occurrence times was log normal,

$$f(t; \theta_1, \theta_2) = (\sqrt{2\pi}\theta_2 t)^{-1} \exp\left[-\frac{1}{2}\left(\frac{\log(t) - \log(\theta_1)}{\theta_2}\right)^2\right] \tag{6.30}$$

where θ_1 is the median time to occurrence and θ_2 is the standard deviation of the log response times. The value of θ_1 is assumed to be related to dose through equation (6.29) while θ_2 is assumed to be independent of the dose. The probability of a response at or before time T can be written as,

$$\begin{aligned} P(D) = \int_0^T f(t; \theta_1, \theta_2)\mathrm{d}t &= \Phi[(\log(T) - \log(\theta_1))/\theta_2] \\ &= \Phi[(\log(T) - \log(\alpha) - \beta\log(D))/\theta^2] \\ &= \Phi(a + b \log(D)) \end{aligned}$$

where $\Phi(\cdot)$ is the cumulative normal distribution function, $a = (\log T - \log \alpha)/\theta_2$, and $b = \beta/\theta_2$. This shows that the log normal time-to-occurrence model produces the normal, or probit, quantal response model.

Other time-to-occurrence models include the Weibull model proposed by Pike (1966) and Peto *et al.* (1972),

$$f(t;\theta_1,\theta_2) = \theta_1\theta_2 t^{\theta_2 - 1} \exp(-\theta_1 t^{\theta_2}) \tag{6.31}$$

where θ_1 is assumed to be related to dose and θ_2 is independent of dose to give,

$$\begin{aligned} P(D) &= \int_0^T f(t;\theta_1,\theta_2)\mathrm{d}t \\ &= 1 - \exp(-\alpha D^{\beta} T^{\theta_2}) \\ &= 1 - \exp\{-\exp[a + \beta\log(D)]\} \end{aligned}$$

which corresponds to the one-hit model when $\beta = 1$. The compound Weibull, or generalized Pareto model,

$$f(t;\theta_1,\theta_2,\theta_3) = \frac{\theta_1\theta_2 t^{\theta_2 - 1}}{(1 + \theta_1 t^{\theta_2}/\theta_3)^{\theta_3 + 1}} \tag{6.32}$$

leads to the log-logistic quantal response model if θ_1 is assumed to be related to dose through equation (6.29) and θ_3 is assumed to be unity,

$$\begin{aligned} P(D) &= \int_0^T f(t;\theta_1,\theta_2,\theta_3 = 1)\mathrm{d}t \\ &= (1 + \alpha D^{\beta} T^{\theta_2})^{-1} \\ &= \{1 + \exp[a + \beta\log(D)]\}^{-1} \end{aligned}$$

These and other models have been studied by Shortley (1965) and Gart (1965). The estimation of the parameters for any of these models from observed data cannot be done in a simple straightforward manner. Methods such as the iterative maximum-likelihood procedure previously discussed will have to be used. Figure 6.4 shows the results of fitting a Weibull time-to-response model (equation 6.31) to the results of an experiment in which benzopyrene was chronically painted on the backs of mice and the time until appearance of a skin tumour was noted. Lee and O'Neill (1971) found that, using equation (6.29) to describe the relation between induction time and dose, the parameter β was approximately equal to 2 indicating a relationship of time to response to the square of the dose level. This is an excellent example of the degree to which time-to-response models can describe observed data.

When such models are used to estimate risks from pollution of the natural environment, it should be noted that the estimated median response time, from equation (6.29), may often be greater than the natural lifespan of the species under consideration. This does not mean that the pollutant is without observable hazard. Due to the variation in response times around this median, some proportion of the subjects at risk may respond within their lifespan.

basic assumption is that the locus of action of any chemical is on some surface area; which particular surface may be unknown. If we assume an essential similarity, except size, between different mammalian species and assume about equal densities, then any surface area in an organism will be approximately proportional to the $\frac{2}{3}$ power of its weight. This surface area extrapolation rule is, however, only an approximation. After an animal is exposed to a chemical toxicant, a number of events may occur which can influence the observed toxic effect. These events include: absorption, distribution and storage, metabolism, excretion and re-absorption. Comparisons of the similarity of these events should be made among various animal species to improve the extrapolation of toxic effects between the different species. Absorption rates of chemicals through the gastrointestinal tract, the lung, and the skin are measurable in animals. The surface area-to-volume ratio in the gastrointestinal tract often differs among species. The presence of bacteria in the gastrointestinal tract may indirectly affect absorption. Once a chemical is absorbed, it is distributed through, and stored in, various parts of the body. The toxicant must pass through a variety of barriers before reaching its site of action. Intra-species variation in this distribution system should be taken into account when extrapolating from one species to another. Since a metabolite of the chemical to which an animal is exposed may be the toxic agent, rather than the original compound, metabolic differences among species will also enter the extrapolation equation. Differences in excretory rates of compounds through the kidneys, liver, and intestines may also be important.

These intra-species comparisons should provide for an 'equivalent dose' rule among species. For example, suppose we have two species, one of which is the experimental test animal denoted species T, and the other is the species in the ecosystem, denoted species E, for which we wish to make a risk assessment. Then we can relate the ecological exposure to species E, d_E, to the equivalent dose for the test species, $d_T = H(d_E)$, where the function H will depend upon differences in the biochemical and physiological properties between the two species. However, since this extrapolation rule between species will not be known with certainty, some measure of the error in estimating the species' properties should be incorporated into the final extrapolation. Therefore, the estimation of risk to any population in the ecosystem will have some measure of uncertainty which is a combination of sampling errors in the data, choice of a particular mathematical model among the many possible models when extrapolating outside the observable range, and the uncertainties inherent in extrapolating between different animal species. All these sources of variation can lead to large potential errors in the final assessment of risk.

6.9. CONCLUSIONS

In the past, research on the analysis of dose–response relations has been limited to describing the results of an experiment conducted on a single species under

controlled conditions. Due to the magnitude of interactions inherent in any ecosystem, these techniques will not be generally applicable for predicting the effect of pollutants upon an entire system or even subsystems. Future work on doses and responses for estimating risks to ecosystems should examine the following problems.

1. Time-dependent models of ecosystems, with as many interactions as feasible, should be designed and tested. This will entail, as a first step, definitions of ecosystem response variables. These responses can range from a simple measure of the population size for a particular species, to a measure of both population size and mix of a number of species. The time-dependent nature of the model would allow for the estimation of these response variables over an extended time frame.
2. With respect to estimation of the effects on single species, more work should be done on modelling the changes over time in resistance or susceptibility to chronic exposure to toxicants.
3. Increased effort should be devoted to study both the interactive effects of combined toxicants and the modifying effects of other non-toxic environmental conditions since extrapolation of experimental results to a natural ecosystem will depend upon the generality of the bioassay.

In general, the weakness of current techniques used to measure the relations of doses and responses is that they are aimed at single species under controlled conditions. We must begin to consider an ecosystem as a single entity, albeit a complex one, and formulate new methods of estimating dose response of the system as a whole.

6.10. REFERENCES

Abbott, W. S., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18**, 265–7.

Albert, R. E. and Altshuler, B., 1973. Considerations relating to the formulation of limits for unavoidable population exposures to environmental carcinogens. In *Radionuclide Carcinogenesis* (Ed. J. E. Ballou), AEC Symposium Series CONF-72050, Springfield, Virginia NTIS.

Armitage, P. and Doll, R., 1961. Stochastic models for carcinogenesis. *Fourth Berkeley Symposium on Mathematical Statistics and Probability*, University of California Press, pp. 19–38.

Ashford, J. R. and Smith, C. S., 1964. General models for quantal response to the joint action of a mixture of drugs. *Biometrika*, **51**, 413–28.

Ashford, J. R. and Smith, C. S., 1965. An alternative system for the classification of mathematical models for quantal responses to mixtures of drugs in biological assay. *Biometrics*, **21**, 181–8.

Bartlett, M. S., 1946. A modified probit technique for small probabilities. *J. Roy. Stat. Soc. Suppl.*, **8**, 113–7.

Berkson, J., 1944. Application of the logistic function to bioassay. *J. Am. Stat. Assoc.*, **39**, 357–65.

Berkson, J., 1949. Minimum chi-square and maximum likelihood solution in terms of a linear transform, with particular reference to bioassay. *J. Am. Stat. Assoc.*, **44**, 273–8.

Berkson, J., 1953. A statistically precise and relatively simple method of estimating the bioassay with quantal response, based on the logistic function. *J. Am. Stat. Assoc.*, **48**, 565–99.

Bliss, C. I., 1935. The calculation of the dosage–mortality curve. *Ann. Appl. Biol.*, **22**, 134–67.

Brown, A. W. A., 1958. *Insecticide Resistance in Arthropods*, World Health Organization Monograph Series No. 38, Geneva.

Brown, C. C., 1976a. Variability of risk estimation in dose–response experiments. Presented at NIEHS conference on problems of extrapolating the results of laboratory animal data to man.

Brown, C. C., 1976b. Mathematical aspects of dose–response studies in carcinogenesis–the concept of thresholds. *Oncology*, **33**, 62–5.

Brown, V. M., 1973. Concepts and outlook in testing the toxicity of substances to fish. In *Bioassay Techniques and Environmental Chemistry* (Ed. G. E. Glass) Ann Arbor Science, Ann Arbor.

Busvine, J. R., 1938. The toxicity of ethylene oxide to *Calandra oryzae* C. *granaria*, *Tribolium castaneum*, and *Cimex lectularis*. *Ann. Appl. Biol.*, **25**, 605–32.

Chand, N. and Hoel, D. G., 1974. A comparison of models for determining safe levels of environmental agents. In *Reliability and Biometry SIAM*, Philadelphia, pp. 681–700.

Cochran, W. G., 1943. Analysis of variance for percentages based on unequal numbers. *J. Am. Stat. Assoc.*, **38**, 287–301.

Cochran, W. G. and Cox, G. M., 1957. *Experimental Designs*, 2nd ed., Wiley and Sons, New York.

Cornfield, J. and Mantel, N., 1950. Some new aspects of the application of maximum likelihood to the calculation of the dosage response curve. *J. Am. Stat. Assoc.*, **45**, 181–210.

Das, M. N. and Kulkarni, G. A., 1966. Incomplete block designs for bioassays. *Biometrics*, **22**, 706–29.

DeBeer, E. J., 1945. The calculation of biological assay results by graphic methods. The all-or-none type of response. *J. Pharmacol. Exp. Ther.*, **85**, 1–13.

Dixon, W. J. and Mood, A. M., 1948. A method for obtaining and analyzing sensitive data. *J. Am. Stat. Assoc.*, **43**, 109–26.

Draper, N. R. and Smith, H., 1966. *Applied Regression Analysis*, Wiley and Sons, New York.

Druckrey, H., 1967. Quantitative aspects of chemical carcinogenesis. In *Potential Carcinogenic Hazards from Drugs (Evaluation of Risks)* (Ed. R. Truhaut), Springer-Verlag, New York.

Emmens, C. W., 1948. *Principles of Biological Assay*, Chapman and Hall, London.

Finney, D. J., 1949. The choice of a response metameter in bioassay. *Biometrics*, **5**, 261–72.

Finney, D. J., 1952. *Probit Analysis*, 2nd ed., University Press, Cambridge.

Finney, D. J., 1964. *Statistical Methods in Biological Assays*, 2nd ed., Griffin, London.

Food and Drug Administration Advisory Committee on Protocols for Safety Evaluation, 1971. Panel on carcinogenesis report on cancer testing in the safety

evaluation of food additives and pesticides. *Toxicol. Appl. Pharmacol.*, **20**, 419–38.
Gart, J. J., 1965. Some stochastic models relating time and dosage in response curves. *Biometrics*, **21**, 583–99.
Grizzle, J. E., Starmer, C. F., and Koch, G. G., 1969. Analysis of categorical data by linear models. *Biometrics*, **25**, 489–504.
Hewlett, P. S. and Plackett, R. L., 1959. A unified theory for quantal responses to mixtures of drugs: non-interactive action. *Biometrics*, **15**, 591–610.
Hewlett, P. S. and Plackett, R.L., 1964. A unified theory for quantal responses to mixtures of drugs: competitive action. *Biometrics*, **20**, 566–75.
Hoel, P. G. and Levine, A., 1964. Optimal spacing and weighting in polynomial prediction. *Ann. Math. Stat.*, **35**, 1553–60.
Hsi, B. P., 1969. The multiple sample up-and-down method in bioassay. *J. Am. Stat. Assoc.*, **64**, 147–62.
Huggins, C., Grand, L. C., and Brillantes, F. P., 1961. Mammary cancer induced by a single feeding of polynuclear hydrocarbons, and its suppression. *Nature*, **189**, 204–7.
Jerne, N. K. and Wood, E. C., 1949. The validity and meaning of the results of biological assays. *Biometrics*, **5**, 273–99.
Kempthorne, O., 1952. *The Design and Analysis of Experiments*, Wiley and Sons, New York.
Kleinman, J. C., 1973. Proportions with extraneous variance: single and independent samples. *J. Am. Stat. Assoc.*, **68**, 46–54.
Knudsen, L. F. and Curtis, J. M., 1947. The use of the angular transformation in biological assays. *J. Am. Stat. Assoc.*, **42**, 282–96.
Lee, P. N. and O'Neill, J. A., 1971. The effect of both time and dose applied on tumour incidence rate in benzopyrene skin painting experiments. *Br. J. Cancer*, **25**, 759–70.
Litchfield, J. T. and Wilcoxon, F., 1949. A simplified method of evaluating dose effect experiments. *J. Pharmacol. Exp. Ther.*, **96**, 99–113.
Matsumara, F., 1972. Biological effects of toxic pesticidal contaminants and terminal residues. In *Environmental Toxicology of Pesticides* (Eds. F. Matsumura, G. M. Boush, and T. Misato), Academic Press, New York and London.
Muirhead-Thompson, R. C., 1971. *Pesticides and Freshwater Fauna*, Academic Press, London and New York.
Peto, R., 1975. Presentation to the NIEHS conference on extrapolation of risks to man from environmental toxicants on the basis of animal experiments.
Peto, R., Lee, P. N., and Paige, W. S., 1972. Statistical analysis of the bioassay of continuous carcinogens. *Br. J. Cancer*, **26**, 258–61.
Pike, M. C., 1966. A method of analysis of a certain class of experiments in carcinogenesis. *Biometrics*, **22**, 142–61.
Plackett, R. L. and Hewlett, P. S., 1967. A comparison of two approaches to the construction of models for quantal responses to mixtures of drugs. *Biometrics*, **23**, 27–44.
Robbins, H. and Munro, S., 1951. A stochastic approximation method. *Ann. Math. Stat.*, **22**, 400–7.
Shortley, G., 1965. A stochastic model for distributions of biological response times. *Biometrics*, **21**, 562–82.
Sinnhuber, R. O., Wales, J. H., Ayres, J. L., Engebrecht, R. H., and Amend, D. L., 1968. Dietary factors and hepatoma in rainbow trout (*Salmo gairdneri*). I. Aflatoxins in vegetable protein foodstuffs. *J. Natl. Cancer Inst.*, **41**, 711–8.

Stockner, J. G. and Antia, N. J., 1976. Phytoplankton adaptation to environmental stresses from toxicants, nutrients, and pollutants – a warning. *J. Fish. Res. Board Can.*, **33**, 2089–96.

Street, J. C., Mayer, F. L., and Wagstaff, D. J., 1970. Ecological significance of pesticide interactions. In *Pesticides Symposia* (Ed. W. B. Deichman), Halos, Miami.

Swartz, J. and Spear, R. C., 1975. Dynamic model for studying the relationship between dose and exposure in carcinogenesis. *Math. Biosc.*, **26**, 19–39.

Turner, M. E., 1975. Some classes of hit-theory models. *Math. Biosc.*, **23**, 219–35.

Wetherill, G. B., 1963. Sequential estimation of quantal response curves. *J. Roy. Stat. Soc.*, **B25**, 1–48.

Wilson, E. B. and Worcester, J., 1943. The determination of LD_{50} and its sampling error in bioassay. *Proc. U.S. Natl. Acad. Sci.*, **29**, 79–85.

6.11. APPENDIX: THE MAXIMUM-LIKELIHOOD METHOD OF FITTING DOSE–RESPONSE MODELS TO QUANTAL DATA

Assume that the data are of the following form: at each of m dose levels, denoted $d_1, d_2, \ldots, d_m$, we have observed r_i responders out of a total of n_i subjects at risk, $i = 1, \ldots, m$. Assume further that we wish to fit some mathematical model relating dose level to the probability of response which takes the general form,

$$\text{Probability of response at dose } d_i = P(d_i; \theta_1, \ldots, \theta_k) = P_i \tag{6.33}$$

where $\theta_1, \ldots, \theta_k$ are unknown parameters to be estimated from the observed data. The method of maximum likelihood chooses those values of the θ_i that maximize the likelihood of the observed data.

Assuming that the binomial model holds for each dose level, then the likelihood of observing r_i responders out of n_i subjects at the ith dose level is simply the binomial probability,

$$L(r_i) = \text{Prob}(r_i \text{ out of } n_i) = \binom{n_i}{r_i} P_i^{r_i} (1 - P_i)^{n_i - r_i} \tag{6.34}$$

where P_i is the unknown response probability at the ith dose. Since the result at any dose level is independent, in the statistical sense, of the results at each of the other doses, the likelihood of the entire set of data can be written as the product of the individual likelihoods,

$$L = L(r_1, \ldots, r_m) = \pi_i L(r_i) = \pi_i \binom{n_i}{r_i} P_i^{r_i} (1 - P_i)^{n_i - r_i} \tag{6.35}$$

Inserting the dose–response model (equation 6.33) into this likelihood expression gives,

$$L = \pi_i \binom{n_i}{r_i} P(d_i; \theta_1, \ldots, \theta_k)^{r_i} [1 - P(d_i; \theta_1, \ldots, \theta_k)]^{n_i - r_i}$$

The estimates $\hat{\theta}_i$ of the unknown parameters θ_i are obtained by maximizing this expression over all possible values of the θ_i. It is often easier to maximize the logarithm of the likelihood, since maximizing one will maximize the other.

$$l = \log(L) = \Sigma_i \{\log\binom{n_i}{r_i} + r_i \log[P(d_i; \theta_1, \ldots, \theta_k)] + (n_i - r_i) \log[1 - P(d_i; \theta_1, \ldots, \theta_k)]\} \quad (6.36)$$

One method of obtaining the maximum of a function is to find the values of θ_i such that the first partial derivatives, with respect to the θ_i, are all equal to zero. These partial derivatives are given by

$$\frac{\partial l}{\partial \theta_j} = \Sigma_i \frac{(r_i - n_i P_i)}{P_i Q_i} \frac{\partial P_i}{\partial \theta_j}, j = 1, \ldots, k \quad (6.37)$$

where $P_i = P(d_i; \theta_1, \ldots, \theta_k)$ and $Q_i = 1 - P_i$.

Solving this set of non-linear equations is often computationally difficult and must be accomplished by iteration. The Newton–Raphson iteration technique is one such method that can be used. It can be described as follows:

(1) start with a set of initial estimates of the $\hat{\theta}_j$ denoted by $\hat{\theta}_j^{(0)}$;

(2) for each $\hat{\theta}_j$, find its correction factor denoted by $\Delta_j^{(0)}$;

(3) the new estimates are then given by $\hat{\theta}_j^{(1)} = \hat{\theta}_j^{(0)} + \Delta_j^{(0)}$;

(4) continue this iteration procedure, steps (2) and (3), until the equations (equation 6.37) are all equal or close to zero; in general, the new parameter estimates at the $(i + 1)$st stage are obtained from those at the ith stage by the relation, $\hat{\theta}_j^{(i+1)} = \hat{\theta}_j^{(i)} + \Delta_j^{(i)}$.

For the Newton–Raphson iteration method, the correction factors $\Delta_j^{(i)}$ are computed from the second partial derivatives of the log likelihood. These second derivatives are,

$$\frac{\partial^2 l}{\partial \theta_s \partial \theta_t} = \Sigma_i \left[\frac{(r_i - n_i P_i)}{P_i Q_i} \frac{\partial^2 P_i}{\partial \theta_s \partial \theta_t} + \frac{((r_i - n_i P_i) P_i - r_i Q_i)}{P_i^2 Q_i^2} \left(\frac{\partial P_i}{\partial \theta_s}\right) \left(\frac{\partial P_i}{\partial \theta_t}\right) \right]$$
$$s, t = 1, \ldots, k \quad (6.38)$$

Using matrix notation and denoting the vector of first derivatives in equation (6.37) by $M_1^{(i)} = (\partial l/\partial \theta_1, \ldots, \partial l/\partial \theta_k)'$ evaluated at the $\hat{\theta}_j^{(i)}$, $j = 1, \ldots, k$, and denoting the matrix of the second derivatives in equation (6.38) by $M_2^{(i)} = (\partial^2 l/\partial \theta_s \partial \theta_t)$ also evaluated at the $\hat{\theta}_j^{(i)}$, $j = 1, \ldots, k$, then the vector of the ith correction factors, $\Delta^{(i)} = (\Delta_1^{(i)}, \ldots, \Delta_i^{(i)})'$ is obtained from the matrix equation,

$$\Delta^{(i)} = -(M_2^{(i)})^{-1} M_1^{(i)} \quad (6.39)$$

The set of $\hat{\theta}_j$ which solve the equations equation (6.37) are the maximum-likelihood estimates of the parameters. The variances and covariances of these estimates are given by the negative inverse of the matrix of second derivatives

(equation 6.38) evaluated at the maximum-likelihood estimates $\hat{\theta}_j, j = 1, \ldots, k$. In matrix notation,

$$\Sigma = (\sigma_{st}^2) = -M_2^{-1}, \tag{6.40}$$

where σ_{ss}^2 is the estimated variance of $\hat{\theta}_s$ and σ_{st}^2 is the estimated covariance of $\hat{\theta}_s$ and $\hat{\theta}_t$.

A specific example of this general technique for the log-logistic model follows. The probability of response is given by,

$$P(x_i; \theta_1, \theta_2) = \{1 + \exp[-(\theta_1 + \theta_2 x_i)]\}^{-1},$$

where $x_i = \log(d_i)$. The first and second derivatives of the log likelihood in equations (6.37) and (6.38) are easily obtained by using the relations,

$$\frac{\partial P_i}{\partial \theta_1} = P_i Q_i \text{ and } \frac{\partial P_i}{\partial \theta_2} = P_i Q_i x_i$$

These derivatives turn out to be,

$$\frac{\partial l}{\partial \theta_1} = \Sigma_i (r_i - n_i P_i), \frac{\partial l}{\partial \theta_2} = \Sigma_i (r_i - n_i P_i) x_i$$

$$\frac{\partial^2 l}{\partial \theta_1 \partial \theta_1} = -\Sigma_i n_i P_i Q_i$$

$$\frac{\partial^2 l}{\partial \theta_1 \partial \theta_2} = \frac{\partial^2 l}{\partial \theta_2 \partial \theta_1} = -\Sigma_i n_i x_i P_i Q_i$$

$$\frac{\partial^2 l}{\partial \theta_2 \partial \theta_2} = -\Sigma_i n_i x_i^2 P_i Q_i$$

Table 6.5 Maximum-likelihood Iterative Computation for Rotenone Toxicity Example

Iteration i	Parameter estimates		First derivatives		Correction factors	
	$\hat{\theta}_1^{(i)}$	$\hat{\theta}_2^{(i)}$	$\partial l/\partial \theta_1$	$\partial l/\partial \theta_2$	$\Delta_1^{(i)}$	$\Delta_2^{(i)}$
0	0	1	−69.474	−88.064	−5.362	2.180
1	−5.362	3.180	13.540	21.742	0.606	−0.152
2	−4.754	3.028	− 0.434	− 0.175	−0.133	0.076
3	−4.887	3.104	− 0.005	0.0002	−0.0021	0.0012
4	−4.889	3.105	0.000	0.000		

The correction factors become

$$\Delta_1 = -[(\Sigma n_i x_i P_i Q_i)(\Sigma(r_i - n_i P_i)x_i) - (\Sigma n_i x_i^2 P_i Q_i)(\Sigma(r_i - n_i P_i))]/D$$

$$\Delta_2 = -[(\Sigma n_i x_i P_i Q_i)(\Sigma(r_i - n_i P_i)) - (\Sigma n_i P_i Q_i)(\Sigma(r_i - n_i P_i)x_i)]/D$$

where $D = (\Sigma n_i P_i Q_i)(\Sigma n_i x_i^2 P_i Q_i) - (\Sigma n_i x_i P_i Q_i)^2$.

The P_i and Q_i are calculated using the current values of $\hat{\theta}_1$ and $\hat{\theta}_2$. Convergence of this iterative procedure depends upon the initial estimates $\hat{\theta}_1^{(0)}$ and $\hat{\theta}_2^{(0)}$, but is normally quite rapid. Table 6.5 shows the computational steps to obtain maximum-likelihood estimates for the rotenone example in Table 6.3. The initial estimates were $\hat{\theta}_1^{(0)} = 0$ and $\hat{\theta}_2^{(1)} = 1$, far from the final estimates, yet only 4 iterations were required. It should also be noted that the estimates obtained from the previous least squares solution agree closely with these maximum-likelihood estimates. This will generally be true for moderately large sized experiments.