METHODS OF ESTIMATING THE LD 50 IN QUANTAL RESPONSE DATA

By P. ARMITAGE and IRENE ALLEN

From the Medical Research Council Statistical Research Unit, London School of Hygiene and Tropical Medicine

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INTRODUCTION

1. A quantal response is one in which a certain event either happens or does not happen. If, in an animal experiment, we record merely whether or not the animal dies, we are measuring a quantal response. The type of data with which we are concerned here is familiar to all workers in biological assay, and occurs constantly in bacteriological and immunological experiments. A number of animals is divided randomly into several groups, and all the animals in each group are treated with the same dose of a certain substance. The doses differ from group to group, and are frequently arranged so that successive doses differ by a common dilution factor. At each dose the numbers of animals which respond positively and negatively are recorded. The potency of the substance may be measured by that dose which would in the long run produce a positive response in exactly 50 % of the animals, and the main statistical problem is how to estimate this dose (the LD 50) from the available data. It is assumed that any inaccuracies in measuring the doses are negligible in comparison with the sampling errors due to the inevitable differences between experimental animals.

2. This problem has been solved to the satisfaction of most statisticians by the technique known as 'probit analysis', a full account of which is given by Finney (1947*a*). The extent of the computation required for a probit analysis is frequently exaggerated, but the experimental worker with no great computing facilities at hand is clearly justified in pressing the statistician to invent simpler methods of analysis. In recent years a number of such simplified methods have been suggested. but no very definite guidance has been given for choosing between them.

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3. Methods of estimating the LD 50 may be divided into two broad classes: curve-fitting methods, and methods of interpolation. In the first method it is assumed that each animal has some dose, which may be called an individual tolerance, below which it will respond negatively and above which it will respond positively. The population of experimental animals thus forms a 'tolerance distribution', which may be assumed to be of some particular form. The LD 50 is the median of this distribution. In probit analysis the logarithms of the tolerances are assumed to be normally distributed, and the mean and standard deviation of this normal distribution are estimated by the method of maximum likelihood. Maximum likelihood solutions for other types of tolerance distribution are discussed by Finney (1947*b*); they are all obtained by iterative processes similar to that used in probit analysis.

Two simpler methods of curve fitting may be mentioned here. Berkson (1944) has pointed out that if the logarithms of the tolerances are assumed to be distributed in a logistic* form, and if, instead of a maximum likelihood solution we seek a minimum χ^2 solution, a good approximation is available which requires only one cycle of computation. The *exact* minimum χ^2 solution is no more simple than the maximum likelihood solution (cf. §§8 and 10, below).

Knudsen & Curtis (1947) have suggested a method based on the angular transformation

$$y = \sin^{-1}\sqrt{p},$$

which is a familiar statistical tool for analysing data expressed in terms of proportions. It is a single-cycle method of fitting a particular type of tolerance distribution, approximates to the maximum likelihood solution, and has the advantage that the weights used in the regression analysis depend only on the number of animals used at each dose.

4. The two best-known methods of interpolation are those of Kärber and Reed & Muench, a number of references to which are given in §§14 and 15. These two methods make no assumption about the nature of the underlying tolerance distribution, although Cornfield & Mantel (1948) have pointed out that for a log-logistic tolerance distribution, Kärber's method is, under certain restrictions on the design of the experiment, a good approximation to the maximum likelihood solution.

The Reed-Muench and Kärber methods unfortunately lead to a bias in the estimate of the LD 50 if the logarithms of the doses are not spaced symmetrically about the true log LD 50, a situation which is at times unavoidable. Reed & Muench suggested a modification by which this bias could be effectively removed, and a similar modification is available in Kärber's method. Alternatively, one could use the methods of interpolation proposed by Thompson (1947), based on moving averages.

5. In the next two sections we discuss a number of the questions which arise

* The term 'logistic' is commonly used to refer to the *cumulative* probability distribution, whereas a 'normal' distribution is usually thought of as the bell-shaped frequency distribution. For convenience we have extended the term 'logistic' to describe also the frequency distribution.

when an attempt is made to assess the relative merits of the different methods. Ideally, we should like to know the sampling distributions of the different estimates of the LD 50 under various hypotheses, and the correlations between them. This is, unfortunately, a quite impracticable project. Some of the questions may be at least tentatively answered by an extension of the method of comparison used by Irwin & Cheeseman (1939*a*, *b*) and by Thompson (1947) for Topley's data. We have therefore chosen twelve sets of data (all of which have already been discussed in the literature) and for each set estimated the LD 50 by a number of different methods. In addition, for each of the curve-fitting methods, the χ^2 index has been calculated. This is given by

$$\chi^2 = S\frac{n(p-P)^2}{PQ},$$

where n is the number of animals used at any dose, p the observed proportion of positive responses, P the expected proportion calculated from the fitted curve, $Q = 1 - P$, and the summation is taken over all the doses. This quantity is usually taken as being approximately distributed as χ^2 on $k-2$ degrees of freedom (k being the number of doses), but no assumption need be made here about its distribution. $(\chi^2 - k + 2)/Sn$ may be regarded as a measure of the goodness of fit of any assumed tolerance distribution.

CURVE-FITTING METHODS

6. The following curve-fitting methods have been used in the present study:

(*a*) Normal distribution of log tolerances. Maximum likelihood estimation. (The usual probit method.)
(*b*) Normal distribution. Minimum χ^2.
(*c*) Logistic distribution of log tolerances. Maximum likelihood.
(*d*) Logistic distribution. Minimum χ^2.
(*e*) Berkson's approximation to (*d*).
(*f*) Knudsen & Curtis's angular transformation.

7. *Normal. Maximum likelihood.* This is the usual method of probit analysis, involving an iterative process of successive approximation to the final result. At each stage a computation similar to that of regression analysis is performed. The method is described in detail by Finney (1947*a*). The rule used for deciding when to stop the computation was that the expected probits obtained at the final stage should differ by not more than one unit in the second decimal place from those obtained at the previous stage. The probit transformation was taken from Table IX in Fisher & Yates (1948), and the working probits and weighting coefficients from some unpublished tables prepared by the U.S. Food and Drug Administration. Occasionally, when the range of the latter tables was exceeded, interpolation in Fisher & Yates's Table XI was necessary. Other useful tables are given by Finney (1947*a*).

As an indication of the sampling error to which the estimate of the LD 50 is subject, the approximate 95 % fiducial range for the true value was obtained. This

was calculated by placing fiducial limits at a distance $\pm 1{\cdot}96$ S.E. (log LD 50) about the estimated log LD 50, the standard error being calculated by the formula

$$\text{S.E.}(M) = \frac{1}{b}\sqrt{\left\{\frac{1}{Snw} + \frac{(M-\bar{x})^2}{Snw(x-\bar{x})^2}\right\}}, \tag{1}$$

where M is the logarithm of the estimated LD 50, b is the final slope, w is the weighting coefficient, x is the log dose, and $\bar{x}$ is the weighted mean, $Snwx/Snw$.

8. *Normal. Minimum* χ^2. The minimum χ^2 solution for any postulated form of tolerance distribution may be obtained by an iterative process very similar to that used for the maximum likelihood solution. When the tolerance distribution is assumed to be log-normal, the procedure is exactly the same as the standard probit technique, except that the working probit is

$$Y + \frac{(p-P)}{2Z}\left\{\frac{q}{Q} + \frac{p}{P}\right\},$$

instead of

$$Y + \frac{p-P}{Z}.$$

A slightly different iterative process is given by Berkson (1949). In Berkson's method the working probit is the same as in the maximum likelihood solution, namely,

$$Y + \frac{(p-P)}{Z},$$

but the weighting coefficient is (apart from a factor $\frac{1}{2}$, which has been inserted here in order that the two coefficients should be almost equal)

$$\frac{Z^2}{2PQ}\left\{\frac{q}{Q} + \frac{p}{P}\right\},$$

instead of

$$\frac{Z^2}{PQ}.$$

The relation between the two methods of arriving at the minimum χ^2 solution is discussed in Appendix I. The first method was used in this study not because it was thought to be quicker than Berkson's, but merely because the work was started before Berkson's paper was published.

Whichever procedure is used, the minimum χ^2 solution is clearly rather more laborious than the maximum likelihood. The same criterion for stopping was used as in the maximum likelihood method.

9. *Logistic. Maximum likelihood.* The probability of a positive response is assumed to be given by an equation of the form

$$P = \frac{1}{1 + e^{-2(\alpha' + \beta x)}},$$

where x is the logarithm of the dose, and α' and β are unknown parameters defining

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the tolerance distribution. By analogy with the definition of *probit*, Finney defines Y, the *logit* corresponding to P, to be*

$$Y = 5 + \tfrac{1}{2}\log_e(P/Q). \tag{2}$$

Y is then linearly related to x.

The procedure for obtaining the maximum likelihood estimates of the parameters is analogous to that used for a normal distribution. The weighting coefficient is

$$w = 4PQ,$$

and the working logit is $$Y + \frac{p-P}{2PQ}.$$

The weighting coefficients and working logits may be obtained by interpolation in Finney's Table 4 (1947*b*), and were taken to 3 and 2 places of decimals, respectively. For the transformation from percentages to logits, and back again, the nomograph given by Berkson (1944) was used, remembering that Finney's logit Y is related to Berkson's logit l by the relation

$$Y = 5 - \tfrac{1}{2}l.$$

For very high or very low percentages, the logits were calculated directly from equation (2). The computing stopped when none of the expected logits differed from those obtained at the previous stage by more than 1 unit in the second decimal place.

10. *Logistic. Minimum* χ^2. The procedure is exactly the same as in § 9, except that the working logit is

$$Y + \frac{p-P}{4PQ}\left\{\frac{q}{Q} + \frac{p}{P}\right\} = Y + \frac{p-P}{w}\left\{\frac{q}{Q} + \frac{p}{P}\right\}.$$

These values had to be calculated directly. The remarks made in § 8 about Berkson's alternative method of successive approximation apply also in this case.

11. *Berkson's approximation to the solution of* § 10. We have defined χ^2 as

$$\chi^2 = S\frac{n(p-P)^2}{PQ}.$$

Now, from equation (2),

$$Y = 5 + \tfrac{1}{2}\log_e\{P/(1-P)\},$$

and

$$P = 1/\{1 + e^{-2(Y-5)}\}.$$

Hence

$$\frac{dP}{dY} = 2PQ.$$

If y is the logit corresponding to the *observed* proportion, p, we have

$$p - P = (y - Y)\left[\frac{dP}{dY}\right]_{P'}$$

$$= 2(y - Y)\,P'Q',$$

* This differs from Berkson's original definition (1944) of a *logit* as $l = -\log_e(P/Q)$, and also from his revised definition (1949), whereby $l = +\log_e(P/Q)$. This ambiguity in the definition of a *logit* is most unfortunate. We use Finney's definition throughout this paper.

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where the differential coefficient is evaluated at some point P' between p and P. Writing approximately,

$$P'Q' \doteq \sqrt{(pP)}\,\sqrt{(qQ)},$$

we have

$$(p-P)^2 \doteq (y-Y)^2\,4PQpq,$$

and

$$\chi^2 \doteq S4npq(y-Y)^2$$

$$= Snw'(y-Y)^2,$$

where the weighting coefficient w' is given by

$$w' = 4pq, \tag{3}$$

and is independent of the population values P and Q.

The procedure for minimizing χ^2 is therefore approximately equivalent to fitting a weighted regression line of y (observed logit) on x (log dose); the weight w' is given by equation (3) and depends only on the *observed* proportion p. Unlike the methods of §§7–10, this involves no process of successive approximation.

In discussing Berkson's method it is legitimate to ask whether there is any disadvantage in choosing a logistic rather than a normal curve for the tolerance distribution, and the minimum χ^2 method of estimation rather than maximum likelihood. As far as we are aware, there are no theoretical reasons for preferring one method of estimation to the other. As we show in Appendix I the two methods tend to give the same result, as the numbers of animals at each dose increase indefinitely, provided the assumed type of tolerance distribution is the true one. Berkson (1949) has pointed out that if this condition is not satisfied—and in practice it will not hold exactly—the two solutions may not converge.

Theoretical reasons have occasionally been adduced for one type of tolerance distribution rather than another. Berkson (1944) attempted to show that the logistic curve was a more realistic model than the normal by fitting a normal curve by maximum likelihood and a logistic curve by his minimum χ^2 approximation to seven sets of data, and comparing the values of χ^2 obtained in each case. This is not a fair comparison, since any type of curve fitted by minimum χ^2 will always yield a smaller χ^2 than the same type fitted by maximum likelihood. The results of the present study do not support Berkson's conclusions.

The case for Berkson's approximation clearly lies in the avoidance of the iterative process. The main point at issue is whether it does, in fact, provide a close approximation to the exact minimum χ^2 solution. This is discussed later.

One difficulty in applying Berkson's method is that when $p = 0$ or 1 the weight vanishes and the logit becomes infinite. Berkson suggests that in such cases a preliminary fit should be made 'omitting the observation in question, and a substitute observation used, half way between the estimate given by this fit and the actual observation'. It is not clear whether Berkson would advocate an extra cycle of computation for the preliminary fit, or whether this would be done by eye. In the present study the preliminary fit was made by eye, the observations in question being taken into account as far as possible.

12. *Knudsen & Curtis's angular transformation.* A familiar procedure in the statistical treatment of proportions, p, is to apply the transformation

$$p = \sin^2 y,$$

or

$$y = \sin^{-1} \sqrt{p}.$$

If y is measured in radians, the variance of y is approximately $1/4n$, independently of the population proportion P, n being the size of the sample from which p is calculated. If y is measured in degrees, the variance of y is approximately $820{\cdot}7/n$. Tables facilitating the transformation are given by Snedecor (1946, Table 16·8) and Fisher & Yates (1948, Tables XII–XIV).

Knudsen & Curtis (1947) suggest that the weighted regression of y on x should be calculated, the weight at each dose being $n/820{\cdot}7$, and the LD 50 estimated from the fitted line as the antilog of the value of x corresponding to $y = 45°$.

This is equivalent to the first cycle of a maximum likelihood or minimum χ^2 solution, on the assumption that the underlying tolerance distribution is of the form

$$P = \sin^2(\alpha + \beta x) \quad (0 \leqslant \alpha + \beta x \leqslant 90), \tag{4}$$

where $x =$ log dose and α and βx are measured in degrees. This differs from the normal and logistic curves in having a finite range. It is assumed that there is some dose below which no animals will respond positively, and some other dose above which all animals will respond positively. It is not clear on *a priori* grounds whether or not this is more realistic than an assumption of a tolerance distribution with infinite range. The method is, however, open to the objection that the expected values of Y, as given by the fitted line, may for some doses be greater than 90° or less than 0°. Superficially, it would appear that the fitted values of P may increase to unity as x increases, and then decrease with further increases in x. This anomaly is removed if the exact maximum likelihood solution is obtained, but this is an iterative process no less laborious than the maximum likelihood solution for the normal curve.

If this difficulty is ignored an estimate of the LD 50 may be obtained from the fitted line, and compared with those obtained by other methods. Knudsen & Curtis used the angular transformation to obtain an estimate of the relative potency of a test preparation in terms of a standard, and gave approximate fiducial limits for this estimate. In several cases the fiducial range thus calculated was shorter than that given by probit analysis. This was taken to indicate that the angular transformation provided at least as accurate a method as probit analysis. This is a rather dubious conclusion, for Knudsen & Curtis's fiducial limits are based on the assumption that equation (4) is true. If the true tolerance distribution is somewhat different from equation (4), the fiducial limits will not be strictly valid.

In the present study, the χ^2 index has been calculated wherever possible. When the fitted line gives values of Y outside the range (0, 90), the value of χ^2 is infinite. In such cases, χ^2 has been replaced by the weighted sum of squares of y about the regression line; this quantity is known to be equal to χ^2 for a maximum likelihood solution.

METHODS OF INTERPOLATION

13. The following methods of interpolation have been studied:

(*a*) Kärber's method, with and without modification.

(*b*) The Reed-Muench (or Behrens) method, with and without modification.

(*c*) Thompson's method, based on moving averages of three successive points.

For convenience we shall assume that the response with which we are concerned is the death of the animal, and that the death-rate increases as the dose increases.

14. *Kärber's method.* Kärber's method is described by Gaddum (1933), Irwin (1937) and Irwin & Cheeseman (1939*a*, *b*). Epstein & Churchman (1944) have pointed out that it was first suggested by Spearman (1908). When the log doses are equally spaced, it consists in calculating

$$M = x_r - d(S' - \tfrac{1}{2}), \tag{5}$$

where M is the estimated log LD 50, x_r is the logarithm of the first dose at, and above, which all the animals die, d is the common log-dose interval, and S' is the sum of the proportional mortalities at all doses up to and including the rth. If the highest dose used gives a mortality less than unity it is assumed that all the animals would have died at the next higher dose, and this is regarded as the rth.

Kärber's method becomes clearer if equation (5) is expressed in the form

$$M = \tfrac{1}{2}S(p_{i+1} - p_i)(x_{i+1} + x_i), \tag{6}$$

where p_i is the proportional mortality at the ith dose. The summation is taken over the whole dose range, $i = 1$ to $i = k - 1$, provided that the mortalities p_1 and p_k at the extreme doses are equal to 0 and 1 respectively. If this is not so, it is assumed that the next lower or next higher doses would give $p_0 = 0$ or $p_{k+1} = 1$, respectively, and the summation is taken from $i = 0$ to $i = k$. It is clear from equation (6) that Kärber's method yields an approximately unbiased estimate of the *mean* log tolerance.

Cornfield & Mantel (1948) have shown that Kärber's method provides the maximum likelihood solution when the following conditions are satisfied:

(*a*) Equal numbers of animals at each dose level.

(*b*) Equally spaced log-dose intervals.

(*c*) An underlying log-logistic tolerance distribution.

(*d*) The mean and standard deviation of the tolerance distribution are defined in terms of a frequency distribution with finite class intervals rather than an integral with infinitesimal class intervals.

(*e*) The whole range of response between 0 and 100 % is covered.

Equation (6) can be used when the log-dose intervals are unequal. A difficulty arises, however, when it is necessary to extend the actual range of doses in order to obtain x_0 or x_{k+1}. Two methods have been used in the present work:

(*a*) The interval $x_1 - x_0$ is made equal to $x_2 - x_1$, and $x_{k+1} - x_k$ equal to $x_k - x_{k-1}$.

(*b*) The intervals $x_1 - x_0$ and $x_{k+1} - x_k$ are both made equal to the arithmetic mean of the intervals actually used.

In practice the two rules give almost the same results.

The most serious bias* arises when the log doses are asymmetrically placed about the true log LD 50, especially if the range of mortalities from 0 to 1 is far from being covered. The effect is to bias the estimate of the LD 50 towards the middle of the dose range. The bias will, in general, be reduced if, after a preliminary estimate has been obtained, the extreme doses are omitted until there are equal numbers of doses on each side of the dose whose logarithm is nearest the estimated log LD 50. A revised estimate may then be obtained from the reduced data. This modification of Kärber's method is essentially the same as that proposed for their method by Reed & Muench, as described in §15 below.†

Each set of data has been analysed by Kärber's method; where extension of the dose range was necessary and the log-dose interval varied, both of methods (*a*) and (*b*) were used. Where necessary, a further estimate of the LD 50 has been obtained by the modified method. An example of the calculations will be found in Appendix II.

15. *The Reed-Muench method.* This method is described by Reed & Muench (1938), and is essentially the same as that due to Behrens, which has been discussed by Gaddum (1933) and Irwin (1937). We assume that any animal which dies at a given dose would also die at any higher dose, and that any animal which survives at a given dose would also survive at a lower dose. A quotient is then calculated at each dose, in which the numerator is equal to the total number of animals which die at that dose or a lower one, and the denominator is obtained by adding to the numerator the total number of animals which survive at that dose or a higher one. If one of these quotients is equal to 0·5 the corresponding dose is taken as the LD 50. If not, the LD 50 is obtained by linear interpolation between two values of the quotient, with respect to the logarithm of the dose.

If the distribution of the logarithms of the tolerances is symmetrical, this method will yield an approximately unbiased estimate of the log LD 50 if equal numbers of animals are tested at each dose, if the log-dose interval is constant, and if the log doses are spaced approximately symmetrically about the true log LD 50. Thompson (1947) has pointed out that the bias due to unequal numbers is removed if, before summation, the numbers of deaths and survivals are replaced by the proportions or percentages of deaths and survivals.

The more serious bias due to asymmetrical choice of the dose levels with respect to the true LD 50 was noted by Reed & Muench. The effect is to bias the estimate towards the middle of the dose range. Reed & Muench suggested that this bias could be reduced if the estimation were done in two stages. After the first estimate is obtained in the ordinary way, extreme doses are omitted in such a way that there are equal numbers of doses left on either side of the dose whose logarithm is nearest the estimated log LD 50. The process is then repeated with the reduced dose range.

In a private communication, Dr J. O. Irwin has suggested a generalization of the Reed-Muench method, to be used when the log-dose intervals are unequal. In the

* Strictly speaking, the estimate of the log LD 50 should be described not only as *biased*, but also as *inconsistent*. This means broadly, that as the number of observations at each dose increases indefinitely, the estimate almost certainly tends to a value other than the true log LD 50.

† Different rules for reducing the dose range can easily be formulated, but we have used here that suggested by Reed & Muench.

simple case where the log-dose interval is constant and a wide range of mortalities is covered, the cumulative sum of deaths at and below the ith dose, when multiplied by the log-dose interval, provides an approximately unbiased estimate of the integral of the cumulative mortality curve up to the mid-point of the interval between the ith and $(i+1)$th log doses. When the log-dose interval is not constant, a better estimate of this integral is obtained by summing not the deaths, but the product of the number of deaths at the ith dose with half the difference between the $(i-1)$th and the $(i+1)$th log doses. Similarly for the survivors. This involves the extension of the dose range by one dose at each end, and, just as in § 14, the question arises, where to place the additional doses. We have used here only method (*a*) of § 14.

Each set of data has been analysed by the Reed-Muench method, using Thompson's correction for unequal numbers; where necessary, a further estimate of the LD 50 has been obtained by the modified method. In the series with unequal spacing, Dr Irwin's generalization of the Reed-Muench method has been used.

16. *Thompson's method of moving averages.* Thompson (1947) has suggested that sampling fluctuations of the points on the log dose-mortality curve should be smoothed out by taking moving averages of K successive points. For each set of K points, the mean mortality and the mean log dose are calculated. These moving averages of the mortalities will usually form an increasing sequence without any reversals, and the log LD 50 may be obtained by two-point interpolation or extrapolation. The choice of K is arbitrary, and Thompson appears to favour $K=3$. We have accordingly applied Thompson's method with $K=3$. An example will be found in Appendix II.

Thompson showed that Kärber's method could be regarded as a degenerate form of the moving average procedure. Another particular case of Thompson's method is very similar to the Reed-Muench method modified by reduction of the dose range.

DESCRIPTION OF THE DATA

17. Table 1 forms a summary of the twelve sets of data which have been used in this comparative study. Each set has been quoted or referred to in at least one other paper on quantal response analysis, and many have by now become almost 'classic' examples. The third and fourth columns of Table 1 list respectively the authors who have quoted each set of data in full, and those who have made some reference to the data, without quoting the figures in full.

The last column contains the range of doses used in each experiment. The words 'equal' and 'unequal' refer to the spacing of the logarithms of the doses, not to the doses themselves. For example, in Wilson Smith's data, the doses range from 0·000625 to 0·01 c.c., adjacent doses differing by a factor 2. The log doses are therefore equally spaced. Fisher & Yates (1948) merely state that the doses are in geometrical progression, and use as working units for x the integers 3–9. We have assumed that these are logarithms to base 10.

It should be noted that in three sets of data the original dose range has been curtailed by previous authors. Murray's data for female flies had originally the same dose range as for males, but in order to obtain a satisfactory fit by probits he

Table 1. *Description of data*

Data	Original publication	Data also given by	Data also discussed by	Description	Response	No. of doses	Total no. of animals	Dose range and spacing
Murray (male)	1938	Berkson (1944)	—	Pyrethrins on male flies	Death	11	5495	40–300 mg./100 c.c., unequal
Murray (female)	1938	—	Berkson (1944)	Pyrethrins on female flies	Death	9	3121	120–300 mg./100 c.c., unequal
Wilson Smith	1932	Irwin & Cheeseman (1939*b*), Garwood (1941)	Irwin (1937), Berkson (1944)	Antipneumococcus serum on mice	Survival	5	200	0·000625–0·01 c.c., equal
Strand I	1930	Bliss (1935), Irwin (1937)	Berkson (1944)	Carbon disulphide on flour beetles	Death	6	175	56·91–76·54 mg./l., unequal
Strand II	1930	Bliss (1935), Irwin (1937)	Berkson (1944)	Carbon disulphide on flour beetles	Death	6	187	56·91–76·54 mg./l., unequal
Chen *et al.*	1938	Bliss (1938)	Berkson (1944)	Gelsemicine hydrochloride on rabbits	Death	8	80	0·06–0·13 mg./kg., unequal
Woodard *et al.*	1941	—	Berkson (1944)	Dichloracetic acid on mice	Death	8	80	3·000–8·913 mg./kg., unequal
Fisher & Yates	1938/48	Garwood (1941), Cornfield & Mantel (1948)	—	Arsenic on brine-shrimp	Death	7	56	Antilog 3-Antilog 9, equal
J. Wilson & Topley, A	Irwin & Cheeseman (1939*a*, *b*)	Garwood (1941), Thompson (1947)	Cornfield & Mantel (1948)	Toxic fraction from *Bact. typhi murium* on mice	Death	7	35	0·0625–4·0 mg., equal
J. Wilson & Topley, B	Irwin & Cheeseman (1939*a*, *b*)	Thompson (1947)	Cornfield & Mantel (1948)	Toxic fraction from *Bact. typhi murium* on mice	Death	7	35	0·0625–4·0 mg., equal
J. Wilson & Topley, E	Irwin & Cheeseman (1939*a*, *b*)	Thompson (1947)	Cornfield & Mantel (1948)	Toxic fraction from *Bact. typhi murium* on mice	Death	7	35	0·0625–4·0 mg., equal
J. Wilson & Topley, F	Irwin & Cheeseman (1939*a*, *b*)	Thompson (1947)	Cornfield & Mantel (1948)	Toxic fraction from *Bact. typhi murium* on mice	Death	7	35	0·0625–4·0 mg., equal

Table 2. *Table of results*

Data	No. of doses	Total no. of animals	D.F. for χ^2	Value of $\chi^2 = Sn(p-P)^2/PQ$: Normal: Max. lik.	Normal: Min. χ^2	Logistic: Max. lik.	Logistic: Min. χ^2	Logistic: Berkson's approx.	Angular transf. approx.	Estimate of LD50 by curve-fitting methods: Normal: Max. lik.†	Normal: Min. χ^2	Logistic: Max. lik.	Logistic: Min. χ^2	Logistic: Berkson's approx.	Angular transf. approx.	Estimate of LD50 by interpolation: Kärber‡ (a)	Kärber‡ (b)	Reed-Muench‡	Thompson (3-span)	LD50 to be multiplied by
Murray (male)	11	5495	9	11·9	11·9	6·4	6·4	6·3	73·8	66·52 (64·01–69·12)	66·50	66·79	66·78	66·86	65·01	70·13 (65·85)	71·86 (67·44)	70·84 (67·31)	66·65	
Murray (female)	7	3121	5	3·37	3·37	2·48	2·46	2·43	5·57	215·7 (209·9–221·7)	215·7	216·0	216·0	215·9	215·3	208·1 (219·6)	207·3 (217·6)	205·3 (216·6)	218·6	
Wilson Smith	5	200	3	2·69	2·65	1·75	1·73	1·73	5·28	1·42 (1·13–1·77)	1·42	1·40	1·40	1·40	1·45	1·49 (1·32)		1·49 (1·35)	1·35	10^{-3}
Strand I	6	175	4	0·70	0·68	1·10	1·09	1·09	**1·14***	60·03 (58·39–61·71)	60·02	60·02	60·01	60·00	59·90	60·59 (60·29)	60·67 (60·32)	60·60 (60·30)	60·10	
Strand II	6	187	4	5·15	4·63	5·37	4·77	4·84	**7·28***	60·31 (58·96–61·70)	60·43	60·27	60·45	60·37	60·18	60·72 (60·23)	60·76 (60·26)	60·84 (60·37)	59·95	
Chen *et al.*	8	80	6	7·98	7·37	8·44	7·73	7·76	8·54	9·13 (8·48–9·85)	9·24	9·12	9·23	9·19	9·07	9·07 (9·07)	(9·08)	9·00	8·66	10^{-3}
Woodard *et al.*	8	80	6	3·04	2·99	2·86	2·77	2·77	3·55	5·53 (4·79–6·38)	5·50	5·56	5·54	5·54	5·48	5·55 (5·56)	5·51 (5·59)	5·62 (5·62)	5·80	
Fisher & Yates	7	56	5	3·79	3·51	4·13	3·75	3·86	**4·33***	4·07 (0·97–17·0)	4·24	4·41	4·53	4·49	3·03	4·22		5·30	6·31	10^{6}
J. Wilson & Topley, A	7	35	5	1·08	0·96	1·27	1·14	1·37	**5·26***	0·148 (0·088–0·248)	0·153	0·149	0·154	0·163	0·129	0·154		0·157	0·162	
J. Wilson & Topley, B	7	35	5	5·01	4·77	5·12	4·86	5·15	**9·23***	0·219 (0·115–0·419)	0·235	0·220	0·239	0·259	0·196	0·233		0·272	0·229	
J. Wilson & Topley, E	7	35	5	1·31	1·19	1·49	1·33	2·70	**7·71***	0·274 (0·187–0·402)	0·267	0·268	0·263	0·222	0·338	0·268		0·250	0·268	
J. Wilson & Topley, F.	7	35	5	5·11	4·74	5·67	5·01	7·85	**9·02***	0·453 (0·293–0·701)	0·462	0·463	0·470	0·631	0·446	0·467		0·545	0·466	

* Calculated as the weighted sum of squares about the regression line. † Figures in brackets are approximate 95 % fiducial limits.
‡ Figures in brackets are results after modification by omitting extreme dose(s).

omitted the four lowest doses. Similarly, in dealing with Strand's two series, Bliss omitted the two lowest doses in each case. In each of these three sets of data we have worked with the *reduced* range.

In quoting Strand's Series I, Bliss copied an error of Strand's, the percentage mortality corresponding to a dose of 56·91 mg./l. being given as 32·9 % instead of 32·14 %. This may have misled Berkson (1944), who obtained $\chi^2 = 1{\cdot}12$ from a normal maximum likelihood solution with this data, as compared with our value of 0·70 (see Table 2).

18. The results of the analyses are to be found in Table 2. The left-hand section of the table contains the values of χ^2 obtained for each of the curve-fitting methods, and the right-hand section gives the estimates of the LD 50 obtained by the curve-fitting and interpolation methods. (The LD 50 should in some cases be multiplied by a power of 10, as shown in the last column of Table 2.)

The values of χ^2 for the angular transformation shown in heavy type indicate that the fitted straight line gave values of y, the transformed variate, either greater than 90° or less than 0°. As explained in § 12, χ^2 has been replaced here by the weighted sum of squares about the regression line.

The approximate 95 % fiducial range for the LD 50, calculated from the normal maximum likelihood solution as indicated in § 7, is given in brackets under the estimate from this solution.

The column for Kärber's method is divided into sections marked (*a*) and (*b*), according to the two methods of extending the range, which are explained in § 14. The figures in brackets under some of the Kärber and Reed-Muench estimates refer to the modified method of making the dose range approximately symmetrical about the LD 50.

19. The results in Table 2 are believed to be accurate to within a few units in the last decimal place quoted. The greatest inaccuracy in the χ^2 values occurs with the two Murray series. Since, in these series, the number of animals at each dose is large (over 400), the inaccuracy involved in stopping an iterative method when the second decimal place of the expected probits or logits are approximately stable has a much larger effect on the χ^2 values than it has in the other series. For these two series, the values of χ^2 quoted for Berkson's method are actually slightly smaller than those for the true minimum χ^2 solution, an anomaly clearly due to the difficulty just mentioned. In Murray's series for male flies it was only found possible to give χ^2 values to one decimal place, if near accuracy in the last place was to be achieved.

DISCUSSION OF RESULTS

20. *Logistic versus normal.* Berkson (1944) examined seven of our twelve sets of data (the first seven in Tables 1 and 2) by fitting a normal curve by maximum likelihood, and a logistic curve by his approximation to minimum χ^2. He found that the values of χ^2 for the logistic curve were in each case smaller than the values of χ^2 for the normal curve, which suggested that the logistic curve provided a more realistic assumption than the normal.

It would have been more logical to compare the fits of the two curves by comparing the χ^2 values either from the two maximum likelihood solutions, or from the two

minimum χ^2 solutions. If we do this from Table 2, we find that in four cases (the two Murray series, Wilson Smith's data, and Woodard's data) the logistic gives the smaller χ^2, while in the other eight series the advantage lies with the normal curve. For Chen's series and Strand's second series, Berkson's method of comparison was definitely misleading. The other series for which our results differ appreciably from Berkson's is Strand I, where the discrepancy may be due to the error referred to in § 17.

There would thus appear to be no evidence that the logistic curve usually provides a better fit than the normal to quantal response data.

21. *Maximum likelihood and minimum* χ^2. The twelve series are arranged in Table 2 in order of total number of animals used, except for Strand's series I and II which are presented in that order for convenience. In Murray's two series, which have many more observations than any of the rest, the maximum likelihood solutions are almost indistinguishable from the corresponding minimum χ^2 solutions, either by the χ^2 values or by the LD 50 estimates. As the number of observations decreases the differences between the maximum likelihood and minimum χ^2 solutions become more marked; in the four Wilson & Topley series, in each of which only thirty-five animals were used, estimates of the LD 50 from the same curve by the two methods are no more alike than are estimates from normal and logistic curves by the same method.

22. *Berkson's method.* We have stated in § 11 that there is no convincing theoretical reason for preferring a maximum likelihood solution to a minimum χ^2, or vice versa. The results of § 20 provide no definite evidence as to which of the two curves, logistic or normal, is more likely to give a better fit to experimental data. Furthermore, it may be seen from Table 2 that the differences between the estimates of the LD 50 by the four iterative methods considered here—the normal and logistic maximum likelihood and minimum χ^2 solutions—are very small in comparison with the range of sampling error, and are also small in comparison with the range of estimates obtained by other methods. It would appear, therefore, that the decision between the four iterative processes should be taken on grounds of computational facility. The advantage clearly lies with the usual probit method for the normal maximum likelihood solution, since tables for this method are the most readily available.

It follows that Berkson's single-cycle approximation to the logistic minimum χ^2 solution may be regarded as a simple alternative to probit analysis, provided that it is, in fact, a good approximation to the exact solution. Table 2 shows that Berkson's method gives a very satisfactory approximation to the exact minimum χ^2 estimate of the LD 50, except for the four Wilson & Topley series. In three of these (A, B and E) the discrepancy is quite small in comparison with the sampling error, but the approximation is not as good as that provided by, for example, Kärber's method. In Wilson & Topley's series F, the estimate by Berkson's method is clearly unsatisfactory.

The partial failure of Berkson's method with Wilson & Topley's series is probably due to the difficulty of dealing with data in which observed mortalities of 0 and 100 % occur. In Wilson & Topley's series A, B, E and F, there were respectively

4, 3, 5 and 5 doses out of 7 with 0 or 100 % mortalities, and the preliminary fit by eye was particularly difficult with series F, in which the observed deaths out of 5 were 0, 0, 2, 1, 5, 5, 5.

A preliminary fit had also to be performed with Strand's series I and II, Chen's series and Fisher & Yates's series, but in these cases the result was more satisfactory. As the number of observations per dose increases there is, of course, less room for subjective judgement in the preliminary fit.

23. *The angular transformation.* In eight of the twelve sets of data, the expected value of the variate Y was, for one or more doses, outside the range (0, 90). Moreover, in each of the twelve series, the value of χ^2 (or the weighted sum of squares about the regression line, when this measure had to be used) was greater than that obtained by any of the other curve-fitting methods. In Murray's series for male flies, the angular transformation gave $\chi^2 = 73{\cdot}8$, a value highly significant on the customary test, using 9 degrees of freedom. The angular transformation cannot, therefore, be regarded as being founded on as sound a basis as the other curve-fitting methods.

Except for Wilson & Topley's series F, the estimates of the LD 50 by the angular transformation are not as close to the four iterative solutions as are those obtained by Berkson's method, and in this respect the method compares unfavourably with Kärber's method, at least for the six series with the smallest numbers of observations.

24. *Methods of interpolation.* The first point to be noted from Table 2 is that, for the Kärber and Reed-Muench methods, the modification based on the reduced range was required in most of the larger series. In almost every case the revised estimate is closer than the original to the values obtained by the iterative curve-fitting methods. For the two Murray series the original estimates by both methods are outside the fiducial range, but the estimates obtained after modification are quite satisfactory. It is worth remarking that Dr Irwin's adaptation of the Reed-Muench method for unequal spacing proved especially valuable in the two Murray series. If it had not been used the estimates for both series would have been outside the fiducial range, even after reduction of the dose range.

The Reed-Muench and Kärber methods give very similar results for each series. The Kärber estimates are, however, noticeably closer to the iterative solutions in the four Wilson & Topley series, which suggests that Kärber may be a more reliable method than Reed-Muench when the numbers of observations are small. The Reed-Muench method requires, if anything, rather more computation than Kärber. There is, incidentally, very little to choose between the two methods (*a*) and (*b*) of extending the dose range in Kärber's method, when the log doses are unequally spaced.

Thompson's method gives results well within the range of sampling variation of the probit estimate, except possibly for Chen's series. Apart from the first four series, Thompson's method does not give any results which are appreciably better than Kärber's (modified where necessary), and for Strand II, Chen, Woodard, Fisher & Yates and Wilson & Topley's A, the advantage is definitely with Kärber. Thompson's method is probably easier to perform than either Kärber or Reed-

Muench, especially when the log-dose interval varies, or when asymmetrical choice of doses necessitates a reduction of the dose range.

It should be noted that in none of the present series was it necessary to estimate the LD 50 by extrapolation beyond the given dose range. For data in which this is necessary, or where the LD 50 is very near the one end of the dose range, the modification of Kärber's method is inapplicable, and Thompson's is undoubtedly the more satisfactory. Thompson's method, in fact, involved extrapolation for the two Strand series, the moving averages of the mortalities being all greater than 0·5. The estimate, however, was still within the original dose range.

OTHER FACTORS INFLUENCING CHOICE OF METHOD

25. We have been concerned so far in assessing the advantages and disadvantages of the various methods of estimating the LD 50, which may be regarded as the most important characteristic of the tolerance distribution. Occasionally, however, we require from an analysis of quantal response data something more than a bare estimate of the LD 50. In particular, it is frequently useful to have an estimate of the sampling fluctuation to which the LD 50 estimate is subject. This is usually expressed by means of the standard error of the log LD 50, or by fiducial limits for the true LD 50. In addition, we may require an estimate of the standard deviation of the tolerance distribution, with an estimate of its error.

It may be useful to indicate briefly which of the methods we have considered allow estimates to be made of the sampling variation of the LD 50, and the standard deviation of the tolerance distribution. We shall not discuss the accuracy of these estimates.

26. *Sampling variation of the LD* 50. In each of the curve-fitting methods, the standard error of the log LD 50 may be estimated from equation (1). Fieller's (1944) method of obtaining more exact fiducial limits may be used when required.

The estimate of the log LD 50 by Kärber's method (whether modified or not) is a linear function of the observed mortalities, and an estimate of its sampling variance may be obtained if the population mortalities are replaced by the observed proportions (Epstein & Churchman, 1944), or, preferably by smoothed values (Irwin & Cheeseman, 1939*a*, *b*). The formula is very simple when the log doses are equally spaced, so that the log LD 50 is estimated by equation (5). The extension to the case of unequal spacing is quite straightforward.

Thompson (1947) has given approximate expressions for the standard error of the estimated log LD 50 by his method. These formulae might be improved if Irwin & Cheeseman's method of smoothing were adopted.

So far as we are aware, no proposal has yet been made for estimating the sampling error of the Reed-Muench method, except in the trivial case where the population standard deviation is known (Gaddum, 1933). As was stated in § 16, the modified Reed-Muench method may be regarded as very nearly a special case of Thompson's procedure. Thompson's expressions for the standard error of the log LD 50 would therefore provide an approximate result for the modified Reed-Muench method.

27. *Estimation of the standard deviation of the tolerance distribution.* In each of the curve-fitting methods considered in this paper, a consistent estimate of the standard

deviation of the tolerance distribution is given by C/b, where C is a constant for each type of curve, and b is the slope of the fitted regression line. For the normal curve, $C = 1$. For the logistic and angular transformations, the values of C are respectively $\pi/\sqrt{12} = 0{\cdot}9069$, and $45\sqrt{(1 - 8/\pi^2)} = 19{\cdot}586$ (see Appendix III). In each case fiducial limits for the standard deviation or variance of the tolerance distribution may be obtained by calculating the standard error of the slope by the standard method.

Epstein & Churchman (1944) have pointed out that Kärber's method may be extended to give estimates of the second and higher moments of the tolerance distribution. The problem of obtaining an unbiased estimate of the population variance is discussed at length by Cornfield & Mantel (1948). No satisfactory estimate is available unless the range of mortality from 0 to 100 % is almost completely covered. The problem of estimating the standard deviation or variance of the tolerance distribution by either the Reed-Muench method or Thompson's method does not appear to have been discussed in the literature.

THE χ^2 TEST

28. It was not our purpose in this investigation to consider sampling distributions of statistics obtained by different methods, and consequently we can make no detailed contribution to the question of the validity of the χ^2 test for goodness of fit of any assumed type of tolerance distribution. It is, however, noticeable from Table 2 that the χ^2 values from the iterative solutions tend to be lower than the expectation usually associated with the test, namely the number of dose levels minus 2. In fact, adding the values of χ^2 from the twelve series, we find for the four iterative solutions (in the order shown in Table 2) the following values for total χ^2: 51·1, 48·8, 46·1 and 43·0. There are in each case 62 degrees of freedom. These values, although not significant, are low enough to suggest that the χ^2 test often underestimates the significance of departures from the assumed mathematical model.

CONCLUSIONS AND RECOMMENDATIONS

29. Of the two single-cycle methods of approximation which have been considered in the present study—Berkson's method and Knudsen & Curtis's angular transformation—Berkson's method undoubtedly gives estimates of the LD 50 closer to those obtained by iterative processes. Furthermore, as judged by the χ^2 index, the angular transformation is based on a rather less satisfactory model. Berkson's method gives very satisfactory results, provided that the numbers of animals on each dose are not so small that responses of 0 or 100 % are frequent. In such cases, Berkson's method appears to be less satisfactory than the interpolation methods of Kärber or Thompson.

30. If an even less involved method than Berkson's is required, or if there are less than about eight animals per dose, any of the three interpolation methods considered here—Kärber, Reed-Muench, or Thompson—appear to be satisfactory. When the first estimate of the LD 50 by Kärber or Reed-Muench does not lie near the middle of the dose range, a further estimate should be obtained by omitting certain dose

levels. If the Reed-Muench method is to be applied when the log-dose interval is not constant, the generalized method described in §15 should be used.

Kärber's method is perhaps the most reliable when the number of observations is small, and it has the advantage that if a sufficiently wide range of mortality is covered by the dose range, an estimate of the standard deviation of the tolerance distribution may be obtained. Thompson's method is, however, preferable from a computational point of view when the other two methods have to be modified by reduction of the dose range.

APPENDIX I. MAXIMUM LIKELIHOOD AND MINIMUM χ^2

31. The theory of the maximum likelihood solution for quantal response data is now well known, and is summarized in Appendix II of Finney (1947*a*). More recently, Berkson (1949) has described an iterative method of obtaining the minimum χ^2 solution, which differs slightly from the method used in this investigation. The connexion between these two methods of obtaining the minimum χ^2 solution is discussed in §32. A further point, which it seems useful to discuss here, is the relation between the maximum likelihood and the minimum χ^2 solutions. In estimating parameters of a frequency distribution from a large sample, the two methods are known to be equivalent. Quantal response data constitute a rather different problem, an examination of which will be found below. The point is discussed in rather less detail by Berkson (1949).

32. Let P, the expected proportional response at any dose, be a function of a number of unknown parameters θ_j, and suppose p to be an estimate of P based on n observations. Let $Q = 1 - P$, $q = 1 - p$. By definition,

$$\chi^2 = S\frac{n(p-P)^2}{PQ},$$

the summation here, and later, being over the various doses. After some reduction, we have

$$\frac{\partial \chi^2}{\partial \theta_j} = -S\frac{n(p-P)}{P^2Q^2}(P - 2Pp + p)\frac{\partial P}{\partial \theta_j}. \tag{7}$$

An iterative solution to the equations $\partial\chi^2/\partial\theta_j = 0$ may be obtained by following the procedure given in Finney's Appendix II for the maximum likelihood solution. A slight difference, however, is that the expected values of the second-order derivatives $\partial^2\chi^2/\partial\theta_j^2$ and $\partial^2\chi^2/\partial\theta_j\partial\theta_k$ are not very simple expressions, but we may take as approximations to these expected values,

$$\left.\begin{aligned} E\left(\frac{\partial^2\chi^2}{\partial\theta_j^2}\right) &\simeq S\frac{2n}{PQ}\left(\frac{\partial P}{\partial\theta_j}\right)^2, \\ \text{and} \quad E\left(\frac{\partial^2\chi^2}{\partial\theta_j\partial\theta_k}\right) &\simeq S\frac{2n}{PQ}\frac{\partial P}{\partial\theta_j}\frac{\partial P}{\partial\theta_k}. \end{aligned}\right\} \tag{8}$$

(These will be seen to differ by a factor -2 from the expected values of the second derivatives of L, the log likelihood.)

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If there are two parameters θ, ϕ to be estimated, and preliminary estimates θ_1, ϕ_1 are available, adjustments $\delta\theta, \delta\phi$ to θ, ϕ are given by the equations

$$\left.\begin{aligned} \delta\theta\, S\frac{n}{PQ}\left(\frac{\partial P}{\partial\theta}\right)^2+\delta\phi\, S\frac{n}{PQ}\frac{\partial P}{\partial\theta}\frac{\partial P}{\partial\phi} &= S\frac{n(p-P)}{2P^2Q^2}(P-2Pp+p)\frac{\partial P}{\partial\theta},\\ \delta\theta\, S\frac{n}{PQ}\frac{\partial P}{\partial\theta}\frac{\partial P}{\partial\phi}+\delta\phi\, S\frac{n}{PQ}\left(\frac{\partial P}{\partial\phi}\right)^2 &= S\frac{n(p-P)}{2P^2Q^2}(P-2Pp+p)\frac{\partial P}{\partial\phi}. \end{aligned}\right\} \tag{9}$$

These equations are analogous to Finney's (II, 4), and, in fact, differ from the latter equations only in the right-hand members. P, Q, and the differential coefficients, are to be evaluated as functions of θ_1, ϕ_1.

It may now be seen that the procedure for obtaining the minimum χ^2 solution is exactly analogous to the standard technique for the maximum likelihood solution, the only difference being in the values given to the working probits (in the case of the normal distribution) or working logits (for the logistic). The weighting coefficients are unchanged. Thus, for the normal distribution, the weighting coefficient and working probit are respectively

$$w=\frac{Z^2}{PQ} \quad \text{and} \quad y=Y+\frac{(p-P)(Pq+pQ)}{2ZPQ},$$

where, as usual, Z denotes the ordinate and Y the probit obtained from the last approximation. Similarly, for the logistic distribution, we find (using the notation of Finney, 1947*b*)

$$w=4PQ \quad \text{and} \quad y=Y+\frac{p-P}{4PQ}\left\{\frac{q}{Q}+\frac{p}{P}\right\}.$$

Now if, instead of the approximations (equation (8)) to the expected values of the second derivatives, we use the slightly different approximations

$$E\left(\frac{\partial^2\chi^2}{\partial\theta_j^2}\right)\doteq S\frac{n}{P^2Q^2}(P-2Pp+p)\left(\frac{\partial P}{\partial\theta_j}\right)^2,$$

and

$$E\left(\frac{\partial^2\chi^2}{\partial\theta_j\partial\theta_k}\right)\doteq S\frac{n}{P^2Q^2}(P-2Pp+p)\frac{\partial P}{\partial\theta_j}\frac{\partial P}{\partial\theta_k},$$

we find that different weighting coefficients and working probits are required for the iterative solution. For the normal distribution these are

$$w=\frac{Z}{2P^2Q^2}(Pq+pQ) \quad \text{and} \quad y=Y+\frac{(p-P)}{Z},$$

and for the logistic

$$w=2(Pq+pQ) \quad \text{and} \quad y=Y+\frac{(p-P)}{2PQ}.$$

This is essentially the method of approximation proposed by Berkson (1949). The weighting coefficients differ from his by a factor $\frac{1}{2}$, as we have thought it desirable to use weighting coefficients which for large samples approach those used in the maximum likelihood solution; the expression for working probit for the logistic curve differs from that given in Berkson's Table 1 only in so far as Berkson defines his logit differently from Finney (see the footnote on p. 302 of the present paper).

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33. Let L be the logarithm of the likelihood of the results. Then, from Finney (1947*a*), equation (II, 2),

$$\frac{\partial L}{\partial \theta_j} = S\frac{n(p-P)}{PQ}\frac{\partial P}{\partial \theta_j}, \tag{10}$$

and from our equation (7),

$$\frac{\partial \chi^2}{\partial \theta_j} = -S\frac{n(p-P)}{P^2Q^2}(P-2Pp+p)\frac{\partial P}{\partial \theta_j}.$$

If we assume that n is a constant proportion of the total number of observations, N, then each of these two expressions, equations (10) and (7), is of order $N^{\frac{1}{2}}$ with probability 1, and it may be verified that

$$\frac{\partial L}{\partial \theta_j}+\frac{1}{2}\frac{\partial \chi^2}{\partial \theta_j} = -S\frac{n(p-P)^2}{2P^2Q^2}(1-2P)\frac{\partial P}{\partial \theta_j},$$

which is $O(1)$ with probability 1. In this sense the equations

$$\frac{\partial L}{\partial \theta_j} = 0 \quad \text{and} \quad \frac{\partial \chi^2}{\partial \theta_j} = 0$$

become equivalent in large samples.

We shall now examine the convergence of the two solutions more fully. Suppose there are, as is usual in quantal response analysis, only two parameters θ, ϕ. Let the maximum likelihood estimates be $\hat{\theta}$, $\hat{\phi}$. If these values are used as an approximation to the minimum χ^2 solution, the adjustments $\delta\theta$, $\delta\phi$ are given by equation (9), P, Q and the derivatives being evaluated as functions of $\hat{\theta}$, $\hat{\phi}$.

But, since $\hat{\theta}$, $\hat{\phi}$ are maximum likelihood estimates, it follows from Finney's (II, 2) that

$$S\frac{n(p-P)}{PQ}\frac{\partial P}{\partial \theta} = S\frac{n(p-P)}{PQ}\frac{\partial P}{\partial \phi} = 0.$$

The right-hand member of the first equation of (9) may therefore be written as

$$S\frac{n(p-P)}{2P^2Q^2}\{2PQ+(q-Q)(2P-1)\}\frac{\partial P}{\partial \theta} = S\frac{n(p-P)^2(1-2P)}{2P^2Q^2}\frac{\partial P}{\partial \theta},$$

which is $O(1)$ with probability 1. Similarly, for the second equation of (9). But the coefficients of $\delta\theta$ and $\delta\phi$ are $O(N)$ with probability 1, and the solutions $\delta\theta$ and $\delta\phi$ are consequently $O(N^{-1})$ with probability 1.

We have shown, then, that the difference between the maximum likelihood estimate and the minimum χ^2 estimate of either θ or ϕ is $O(N^{-1})$ with probability 1. Each of these estimates differs from the true value, θ, or ϕ, by $O(N^{-\frac{1}{2}})$ with probability 1. As N increases, therefore, the difference between the two methods of estimation becomes increasingly negligible in comparison with sampling fluctuations of the estimates from the true value.

It should again be emphasized that, as Berkson (1949) has pointed out, this result holds only under the condition that the assumed form of tolerance distribution is in fact true.

APPENDIX II. ILLUSTRATION OF METHODS OF INTERPOLATION

34. The various interpolatory methods of estimation of the LD 50 are illustrated here for Woodard's data. Woodard used eight doses with ten animals on each. The log doses and the proportional mortalities are shown in the first two columns of Table 3. The log-dose intervals are unequal.

35. Two possible methods of extending the dose range in Kärber's method, when the log doses are unequally spaced, were discussed in § 14. We illustrate here only method (*a*), in which the interval between the first or last dose, and the extrapolated value, is made equal to the adjacent interval. These extrapolated values are shown in brackets in Table 3, together with the assumed mortalities, 0 and 1.

Table 3. *Kärber's method applied to Woodard's data*

Unmodified				Modified			
x	p	$p_i - p_{i-1}$	x'	x	p	$p_i - p_{i-1}$	x'
(0·4542)	(0)						
		0·2	0·4657				
0·4771	0·2			(0·3979)	(0)		
		−0·1	0·4886			0·1	0·4490
0·5000	0·1			0·5000	0·1		
		0·1	0·5511			0·1	0·5511
0·6021	0·2			0·6021	0·2		
		0·1	0·6511			0·1	0·6511
0·7000	0·3			0·7000	0·3		
		0·1	0·7250			0·1	0·7250
0·7500	0·4			0·7500	0·4		
		0·3	0·7750			0·3	0·7750
0·8000	0·7			0·8000	0·7		
		0·1	0·8500			0·1	0·8500
0·9000	0·8			0·9000	0·8		
		0·1	0·9250			0·1	0·9250
0·9500	0·9			0·9500	0·9		
		0·1	0·9750			0·1	0·9750
(1·0000)	(1·0)			(1·0000)	(1·0)		
$M = Sx'(p_i - p_{i-1}) = 0·7445.$				$M = Sx'(p_i - p_{i-1}) = 0·7451.$			
LD 50 = antilog (0·7445) = 5·55.				LD 50 = antilog (0·7451) = 5·56.			

The left half of Table 3 illustrates the straightforward application of Kärber's method, using equation (6). (The familiar method of equation (5) can only be used when the log doses are equally spaced.) The third column gives the differences between adjacent mortalities, while the fourth column gives the arithmetic means of adjacent log doses. The log LD 50 is estimated as the sum of products of corresponding entries in the third and fourth columns.

The modified Kärber method is shown in the right-hand half of Table 3. The preliminary estimate of M, the log LD 50, was 0·7445, to which the nearest log dose is the fifth, 0·7500. For symmetry, then, the first dose level is omitted, and the calculations proceed as before.

36. Dr Irwin's extension of the Reed-Muench method is illustrated in Table 4.

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Table 4. *Dr Irwin's extension of the Reed-Muench method, applied to Woodard's data*

Unmodified

x	l	D	S	ΣDl	ΣSl	p'
0·4771	0·0229	2	8	0·0458	2·7887	0·0162
0·5000	0·0625	1	9	0·1083	2·6055	0·0399
0·6021	0·1000	2	8	0·3083	2·0430	0·1311
0·7000	0·0740	3	7	0·5303	1·2430	0·2990
0·7500	0·0500	4	6	0·7303	0·7250	0·5018
0·8000	0·0750	7	3	1·2553	0·4250	0·7471
0·9000	0·0750	8	2	1·8553	0·2000	0·9027
0·9500	0·0500	9	1	2·3053	0·0500	0·9788

$$M = 0{\cdot}7000 + \frac{(0{\cdot}2010)\,(0{\cdot}0500)}{(0{\cdot}2028)} = 0{\cdot}7496.$$

$$\text{LD } 50 = \text{antilog } (0{\cdot}7496) = 5{\cdot}62.$$

Modified

x	l	D	S	ΣDl	ΣSl	p'
0·5000	0·1021	1	9	0·1021	2·9619	0·0333
0·6021	0·1000	2	8	0·3021	2·0430	0·1288
0·7000	0·0740	3	7	0·5241	1·2430	0·2966
0·7500	0·0500	4	6	0·7241	0·7250	0·4997
0·8000	0·0750	7	3	1·2491	0·4250	0·7461
0·9000	0·0750	8	2	1·8491	0·2000	0·9024
0·9500	0·0500	9	1	2·2991	0·0500	0·9787

$$M = 0{\cdot}7500 + \frac{(0{\cdot}0003)\,(0{\cdot}0500)}{(0{\cdot}2464)} = 0{\cdot}7501.$$

$$\text{LD } 50 = \text{antilog } (0{\cdot}7501) = 5{\cdot}62.$$

The upper half of Table 4 illustrates the straightforward application of the generalized Reed-Muench method. The second column shows, at each dose, half the sum of the two adjacent log-dose intervals. The numbers of deaths and survivals at each dose are given in the third and fourth columns. The cumulated sums of products are shown in the fifth and sixth columns, and the last column shows the index p', which is calculated by dividing the entry in the fifth column by the sum of the entries in the fifth and sixth columns. The log LD 50 is estimated by linear interpolation in the first and last columns.

As in Kärber's method, the modification requires that the first dose be omitted. The calculations for the modified Reed-Muench method are shown in the lower half of Table 4. It is not necessary in practice to calculate p' over the whole dose range, since not more than two values are required for interpolation.

37. Thompson's method, based on moving averages of three adjacent observations, is illustrated in Table 5.

The third and fourth columns of Table 5 show the moving averages x'' and p'' of three successive values of the log dose x and the mortality p, respectively. M, the log LD 50, is obtained by linear interpolation in the x'' and p'' columns. As we remarked above in connexion with the Reed-Muench method, it is unnecessary in practice to calculate x'' and p'' over the whole dose range.

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Table 5. *Thompson's 3-span method applied to Woodard's data.*

x	p	x''	p''
0·4771	0·2	—	—
0·5000	0·1	0·5264	0·1667
0·6021	0·2	0·6007	0·2000
0·7000	0·3	0·6840	0·3000
0·7500	0·4	0·7500	0·4667
0·8000	0·7	0·8167	0·6333
0·9000	0·8	0·8830	0·8000
0·9500	0·9	—	—

$$M = 0{\cdot}7500 + \frac{(0{\cdot}0333)\,(0{\cdot}0667)}{0{\cdot}1667} = 0{\cdot}7633.$$

$$\text{LD}\,50 = \text{antilog}\,(0{\cdot}7633) = 5{\cdot}80.$$

APPENDIX III. ESTIMATION OF THE STANDARD DEVIATION OF THE TOLERANCE DISTRIBUTION (cf. § 27)

38. In each of the curve-fitting methods, we use some transformation of the observed proportional responses, say $y = f(p)$, chosen in such a way that the population values $Y = f(P)$ are linearly related to the log dose x by an equation of the form

$$Y = \alpha + \beta x. \tag{11}$$

The indices α and β determine jointly the position and spread of the tolerance distribution, and are estimated in some way by statistics a and b.

Now, the variance of the tolerance distribution is given by

$$\sigma_x^2 = \sigma_Y^2/\beta^2,$$

where $\sigma_Y^2 = C^2$ is a constant for any particular type of tolerance distribution. The standard deviation of x may consequently be estimated as

$$C/b. \tag{12}$$

This estimate will be consistent, but biased.

39. For the normal curve, the well-known probit transformation is used, and $C = 1$.

For the logistic distribution defined by the equation

$$P = \{1 + e^{-2(\alpha' + \beta x)}\}^{-1},$$

we have used Finney's logit transformation

$$Y = 5 + \tfrac{1}{2}\log_e\{P/(1-P)\},$$

whence

$$P = \{1 + e^{-2(Y-5)}\}^{-1}, \tag{13}$$

giving the linear relation (equation 11) with $\alpha = 5 + \alpha'$.

We have not been able to find in the literature any derivation of the variance of Y in the logistic distribution (equation 13). The following proof is due to Dr J. O. Irwin.

The moment generating function $M(t)$ for the distribution (equation 13) is

$$M(t) = \int_{-\infty}^{\infty} e^{tY}\, dP(Y).$$

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Substituting $u = e^{-2(Y-5)}$, we have

$$M(t) = e^{5t}\int_0^\infty \frac{u^{-\frac{1}{2}t}du}{(1+u)^2} = e^{5t}\Gamma(1-\tfrac{1}{2}t)\,\Gamma(1+\tfrac{1}{2}t), \quad \text{for } |t|<1.$$

The cumulant generating function $\psi(t) = \log M(t)$ is therefore

$$\psi(t) = 5t + \log\Gamma(1-\tfrac{1}{2}t) + \log\Gamma(1+\tfrac{1}{2}t). \tag{14}$$

On expansion of equation (14) we find, for the first two cumulants of Y,

$$\kappa_1 = 5$$
$$C^2 = \kappa_2 = \tfrac{1}{2}[\Gamma''(1) - \{\Gamma'(1)\}^2] = \tfrac{1}{12}\pi^2. \tag{15}$$

(Cf. equation (12·5·7) of Cramér, 1946.) From equation (15),

$$C = \pi/\sqrt{12} = 0{\cdot}9069.$$

40. For the angular transformation, the tolerance distribution is assumed to be of the form

$$P = \sin^2(\alpha+\beta x) \quad (0 \leqslant \alpha+\beta x \leqslant 90),$$

and equation (11) gives the usual transformation

$$Y = \sin^{-1}\sqrt{P}.$$

The mean value of Y is $45° = \frac{1}{4}\pi$ radians, and the variance of Y is

$$C^2 = (180/\pi)^2\left\{\int_0^{\frac{1}{2}\pi} Y^2 \sin 2Y\,dY - \tfrac{1}{16}\pi^2\right\}$$
$$= \left(\frac{180}{\pi}\right)^2\left(\frac{\pi^2}{8} - \frac{1}{2} - \frac{\pi^2}{16}\right),$$

and

$$C = \left(\frac{180}{\pi}\right)\left(\frac{\pi^2}{16} - \frac{1}{2}\right)^{\frac{1}{2}} = 19{\cdot}586.$$

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