1. Introduction
One type of assay which has been found valuable in many different fields, but especially in toxicological studies, is that dependent upon the quantal, or all-or-nothing, response. Though quantitative measurement of a response is always to be preferred when available, there are certain responses which permit of no graduation and which can only be expressed as ‘occurring’ or ‘not-occurring’. The most obvious example of this kind of response is death; although workers with insects have often found difficulty in deciding precisely when an insect is dead, in many investigations the only practical interest lies in whether or not it has a test insect is dead, or perhaps in whether or not it has reached a degree of inactivity such as is thought certain to be followed by early or early death. In fungicidal investigations, failure of a spore to germinate is a quantal response of similar importance. In studies of drug potency, the response may be the cure of some particular morbid condition, no possibility of partial cure being under consideration. This lecture is concerned with the statistical techniques needed in the analysis of quantal response data.

2. Frequency distribution of quantal response
In quantal assays, occurrence or non-occurrence will depend upon the intensity of the stimulus. For any one subject, under controlled conditions, there will be a certain level of intensity below which the response does not occur and above which the response occurs. Such a value has often been called a threshold or limen, but the term tolerance is now widely accepted. This tolerance value will vary from one member to another of the population used, frequently between quite wide limits. When the characteristic response is quantitative, the stimulus intensity needed to produce a response of any given magnitude will show similar variation between individuals. In either case, the value for an individual also is likely to vary from one occasion to another as a result of uncontrolled internal or external condition.

For quantal response data it is therefore necessary to consider the distribution of tolerances over the population studied. If the dose, or intensity of the stimulus, is measured by $\lambda$, the distribution of tolerance may be expressed by

$$dP = f(\lambda) \, d\lambda;$$

This equation states that a proportion $dP$, of the whole population consists of individuals whose tolerance lie between $\lambda$ and $\lambda + d\lambda$, where $d\lambda$ represents a small interval on the dose scale, and that $dP$ is the length of this interval multiplied by the appropriate value of the distribution function $f(\lambda)$.

If a dose $\lambda_0$ is given to the whole population, all individuals will respond whose tolerances are less than $\lambda_0$, and the proportion of these is $P$, where
\[ P = \int_0^{\lambda_0} f(\lambda) d\lambda ; \]

The measure of dose is here assumed to be a quantity that can conceivably range from zero to \(+\infty\).

Distribution of tolerances, as measured on the natural scale, may be markedly skew, but it is often possible, by a simple transformation of the scale of measurement, to obtain a distribution which is approximately normal. The transformation

\[ x = \log_{10} \lambda \]

generally brings normality in the response variable, however, for some fungicides a better transformation may be

\[ x = \lambda^i, \]

where usually \( i \leq 1 \).

3. Effective dose

In these assays, the earlier attempts were made to characterize the effectiveness of a stimulus in relation to a quantal response referred to the minimal effective dose, or, for a more restricted class of stimuli, the minimal lethal dose, terms, which failed to take account of the variation in tolerance within a population. The logical weakness of such concepts is the assumption that there is a dose for any given chemical, which is only just sufficient to kill all or most of the animals of a given species, and that doses a bit lesser would not kill any animal of that species. Any worker, however, accustomed to the estimation of toxicity knows that these assumptions do not represent the truth.

It might be thought that the minimal lethal dose of a poison could instead be defined as the dose just sufficient to kill a member of the species with the least possible tolerance, and also a maximal non-lethal dose as the dose, which will just fail to kill the most resistant member. Undoubtedly some doses are so low that no test subject will succumb to them and others so high as to prove fatal at all, but considerable difficulties attend determination of the end-points of these ranges. Even when the tolerance of an individual can be measured directly, to say, from measurements on a sample of ten or a hundred that the lowest tolerance found indicated the minimal lethal dose would be unwise; a larger sample might contain a more extreme member. When only quantal responses for selected doses can be recorded the difficulty is increased, and the occurrence of exceptional individuals in the batches at different dose levels may seriously bias the final estimates. The problem is, in fact, that of determining the dose at which the dose response curve for the whole population needs the 0% or 100% levels of kill and even a very large experiment could scarcely estimate these points with any accuracy.

An escape from the dilemma can be made by giving attention to a different and more satisfactorily defined characteristics, the median lethal dose, or, as a more general term to include response other then death, the median effective dose. This is the dose that will produce a response in half the population. The median effective dose is commonly referred to as the ED 50, the more restricted concept of median lethal dose as the LD 50. Analogous symbols were used for doses effective for other proportions of the population, ED 90 being the dose that causes 90% to respond. With a fixed total number of subject effective doses in the neighborhood of ED 50 can usually be estimated more precisely then those for more extreme percentage levels and
this is, therefore, particularly favoured in expressing the effectiveness of the stimulus. The ED 50 alternatively be regarded as the median of the tolerance distribution that is to say the level of tolerance such that exactly half the subject lie on either side of it.

For any distribution of tolerance, the ED 50, $\Lambda$, satisfies the equation

$$\int_0^{\Lambda} f(\lambda)d\lambda = 0.5.$$ 

When a simple normalizing transformation for the doses is available, so that $x$, the normalizing measure of dosage, has a normally distributed tolerance, equation (1) is transformable to

$$dP = \frac{1}{\sigma \sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2} dx.$$ 

Where $\mu$ is the center of the distribution and $\sigma$ its variance. The $\mu$ is the population value of the mean dosage tolerance, or median effective dosage, and efforts must be directed at estimating it from the observational data. For the present case the transformation is logarithmic, so that $\mu$ is the log ED 50; the results obtained are in the main true for any other transformation, at least as far as they relate to the measure of dosage, $x$, but modifications are required in transforming back from $x$ to the $\lambda$ scale.

The ED50 alone does not fully describe the effectiveness of the stimulus. Two insecticides/fungicides may require the same rate of application in order to be lethal to half of the population, but, if the distribution of tolerances has a lesser 'spread' for one than for the other, any increase or decrease from this rate will produce a greater change in mortality for the first than for the second. This spread is measured by the variance, $\sigma^2$, the smaller the value of $\sigma^2$, the greater is the effect on mortality of any change in dose. Stimuli which produce their effects by similar means, often have approximately equal variances of their log tolerances for any given population of test subjects, even though they differ substantially in their median lethal doses. An assessment of the relative potencies can then be made from median doses alone.

4. Probit Analysis

The ED 50 or LD 50 can easily be calculated using the Probit Analysis. The form of analysis now used to estimate the parameters $\mu$ and $\sigma^2$ of the distribution of tolerances, is generally based upon the probit transformation of the experimental results. For doing this, we conduct an experiment on different doses of an insecticide applied under standardized conditions to samples of an insect species and record the number of insects killed and the number of insects exposed. Now the ratio of the number insects died to that of the number of insects exposed gives the probability of the insects killed at a particular dose. Now this probability data is subjected to probit transformation that is nothing but the 5 more than the normal equivalent deviate(this is done to simplify the arithmetical procedure by avoiding negative values). The probit of the proportion $P$ is defined as the abscissa which corresponds to a probability $P$ in a normal distribution with mean 5 and variance 1; in symbols, the probit of $P$ is $Y$, where
The expected proportion of insects killed by a dosage $x_0$ is

$$P = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} e^{-\frac{1}{2}u^2} du. \quad (3)$$

Comparison of two formulae for $P$ then shows that the probit of the expected proportion killed is related to the dosage by the linear equation

$$Y = 5 + \frac{1}{\sigma} (x - \mu). \quad (5)$$

By means of probit transformation, experimental results may be used to give an estimate of this equation, and the parameters of the distribution may then be estimated; in particular, the median effective dosage is estimated as that value of $x$ which gives $Y = 5$.

**Note:** The choice of an efficient experimental design is based on the nature of the variability in the experimental material, environmental conditions and objectives for conducting a bioassay. The design may be a randomized complete block design, an incomplete block design, design for factorial experiments etc.

### 5. Estimation of Parameters

When experimental data on the relationship between dose and mortality have been obtained, either a graphical or an arithmetical process can be used to estimate the parameters. Both depend on the probit transformation. The graphical approach is much more rapid and is sufficiently good for many purposes, but for some, more complex problems, or when an accurate assessment of the precision of estimates is wanted, the more detailed arithmetical analysis is necessary.

In order to make either type of estimate, the percentage kill observed for each dose must first be calculated and converted to probits. The probits are then plotted against $x$, the logarithm of the dose and straight line is drawn by eye to fit the points as satisfactorily as possible. In drawing the line and judging its agreement with the data, only the vertical deviations of the points must be considered. The line in statistical terminology, the weighted regression line of the mortality probit on $x$.

Now LD50 is estimated from the line as $m$, the dosage at which $Y = 5$. The slope of the line, $b$, which is an estimate of $1/\sigma$, is obtained as the increase in $Y$ for a unit increase in $x$. These two parameters are then substituted in equation (5) to give the estimated relationship between dosages and kill. To test whether the line is an adequate representation of the data, a $\chi^2$ test may be used. A value of $\chi^2$ within the limits of random variation indicates satisfactory agreement theory (the line) and observation (the data).
**Example:** Table 1 contain the data on effect of a series of concentrations of rotenone when spraying on *Macrosiphoniella sanborni*, the chrysanthemum aphis, in batches of about fifty.

### Table 1: Toxicity of Retenone to *Macrosiphoniella sanborni*

<table>
<thead>
<tr>
<th>Concentration (mg./1.)</th>
<th>No. of insects (n)</th>
<th>No. of affected</th>
<th>%kill (p)</th>
<th>Log concentration (x)</th>
<th>Empirical probit</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.2</td>
<td>50</td>
<td>44</td>
<td>88</td>
<td>1.01</td>
<td>6.18</td>
</tr>
<tr>
<td>7.7</td>
<td>49</td>
<td>42</td>
<td>86</td>
<td>0.89</td>
<td>6.08</td>
</tr>
<tr>
<td>5.1</td>
<td>46</td>
<td>24</td>
<td>52</td>
<td>0.71</td>
<td>5.05</td>
</tr>
<tr>
<td>3.8</td>
<td>48</td>
<td>16</td>
<td>33</td>
<td>0.58</td>
<td>4.56</td>
</tr>
<tr>
<td>2.6</td>
<td>50</td>
<td>6</td>
<td>12</td>
<td>0.41</td>
<td>3.82</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Now the probits are plotted against dosage. They almost lie on a straight line. All predictions can be made from the probit diagram. In the present example a probit value of 5 is given by a dosage of \( m = 0.687 \); this therefore is the estimate of log LD50, and the LD50 is estimated as a concentration of 4.86 mg/l. Similarly the logLD90 corresponds to a probit of 6.28 and is therefore and is therefore 1.003; the LD90 is thus estimated as 10.1 mg/l. Again an increase of 0.8 in dosage is associated with an increase of 3.21 in the probit. Hence the estimated regression coefficient of probit on dosage, or the rate of increase of probit value per unit increase in \( x \), is

\[
b = \frac{1}{s} = 4.01,
\]

Where \( s = 0.25 \) is an estimate of \( \sigma \), the standard deviation of the distribution of log tolerances.

The relationship between probit and dosage may be written

\[
Y = 5 + 4.01(x - 0.687), \quad \text{or} \quad Y = 2.25 + 4.01x \tag{6}
\]

Equation (6) may be used to calculate expected numbers of insects killed at each concentration. By substitution of the values of \( x \) used in the experiment, the equation gives the values of \( Y \). In Table 2 expected probits are calculated. Thus a probit of 6.30 corresponds to a percentage of between 90 and 91, or, more accurately, 90+2/6%. If the expected proportion for any concentration is multiplied by \( n \), the number of insects tested at that concentration, the result is the expected number of affected insects, or the average number which would be affected in a batch of size \( n \) if equation () represented the true relationship between dosage and kill. These numbers, \( np \), may then be compared with the actual numbers affected, \( r \), in order to judge the adequacy of the equation.

### Table 2: Comparison of Observed and Expected Mortality.

<table>
<thead>
<tr>
<th>Log concentration (x)</th>
<th>Y</th>
<th>P</th>
<th>No. of insects (n)</th>
<th>No. affected</th>
<th>Discrepancy (r-np)</th>
<th>( (r-nP)^2/nP(1-P) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.01</td>
<td>6.30</td>
<td>90.3</td>
<td>50</td>
<td>44</td>
<td>-1.2</td>
<td>0.33</td>
</tr>
<tr>
<td>0.89</td>
<td>5.83</td>
<td>79.7</td>
<td>40</td>
<td>42</td>
<td>2.9</td>
<td>1.06</td>
</tr>
<tr>
<td>0.71</td>
<td>5.10</td>
<td>54.0</td>
<td>46</td>
<td>24</td>
<td>-0.8</td>
<td>0.06</td>
</tr>
<tr>
<td>0.58</td>
<td>4.58</td>
<td>33.7</td>
<td>48</td>
<td>16</td>
<td>-0.2</td>
<td>0.00</td>
</tr>
<tr>
<td>0.41</td>
<td>3.90</td>
<td>13.6</td>
<td>50</td>
<td>6</td>
<td>-0.8</td>
<td>0.11</td>
</tr>
</tbody>
</table>

\( \chi^2_{[3]} = 1.56 \)
A test of significance of the discrepancies may be obtained by squaring each, diving the square by \((1-P)\), and again dividing by the tabulated value \(nP\). The sum of these quantities is, to a sufficiently close approximation if the line has been well drawn. A \(\chi^2\); the degree of freedom are two less than the number of concentrations tested. The value of \(\chi^2\) is 1.56, which, being less than the table value, is clearly sufficiently small to be attributed to random fluctuations about the relationship specified in (5). Thus the regression line obtained appears to be a very satisfactory representation of the results of the experiment.

When a parameter such as the median lethal dose has been estimated from experimental data it is natural to wish to infer within what limits its true value may reasonably be expected to lie. A statement about the probability, by the use of which probabilities can be assigned only to statements about the occurrence of observations or of statistics calculated from the observations. In order to overcome this difficulty, the concept of fiducial probability has been introduced. These limits can easily be calculated after obtaining the standard errors of the Ld values. In the present example the fiducial limits of LD 50 are \(m = 0.687 \pm 0.023\).

Besides knowing the ED 50 of a particular chemical preparation, the experimenter may be interested in comparing the relative potencies of the several chemical preparations. One is required to fit the probit regression lines for each of the chemical preparations separately. These regression lines are required to be tested for parallelism. If the probit regression lines are parallel for the different chemical preparations, then the relative potency is constant at all levels of the response.

**Note:** Empirical probits can be obtained using Table. This Table is available in *Statistical Tables for Biological, Agricultural and Medical Research* by Fisher and Yates (as Table IX). There are also other methods available to obtain the probit regression line.

*This note is prepared from the book ‘Probit Analysis’ by D. J. Finney*