

# Analysis of bioassay data using the Wadley's Problem technique in probit analysis — a neglected option

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## Abstract

Data from bioassays of the type encountered in testing insect growth regulators in grain or disinfestation treatments for fruit flies often involve only the levels of treatment and the number of survivors. The number of insects tested is not known directly. Some authors have adapted probit analysis appropriate to data from conventional bioassays to these data by procedures which assume the number of insects in untreated controls correspond exactly to the number tested. The resulting statistics may be biased and the limits too narrow. Better estimates may be obtained by probit analysis using the Wadley's problem technique, an option available in standard statistical packages.

Simulation studies suggesting that analysis based on the Poisson or Poisson-lognormal distributions are superior to probit analysis have relied on the modified probit technique and are not accepted.

Results of analysis of data from grain bioassays with the methoprene insect growth regulator are presented using both procedures.

## Introduction

Probit analysis (Finney 1971) is widely used for the analysis of bioassay data in which treatments are applied at several levels ( $x$ ) to known number of test organisms ( $n$ ) and the number of organisms responding ( $r$ ) is observed. The calculations are tedious and computer programs are available to undertake the necessary computations.

However, in bioassays of treatments such as insect growth regulators in grain or disinfestation treatments for fruit flies, the number of test organisms in each treatment is not usually known directly. Data typically comprise the treatment levels ( $x$ ) and the number of organisms surviving ( $s$ ). The number of organisms surviving in the untreated control is designated ( $s_0$ ).

Clearly, in assays of this type there are only two data arrays instead of the usual three and a modification of the usual probit analysis is therefore required.

## Modified Probit Analysis

One approach has been to modify the data to the form required for the standard analysis by computing the number of organisms responding as  $r = (s_0 - s)$  and by using the number of organisms in untreated controls as the number of insects tested ( $n$ ) (Hallman and Sharp 1990; Sharp and Picho-Mar-

tiny 1990). The data set then comprises the assays  $x$ ,  $n$  and  $r$  and analysis proceeds in the conventional manner.

## Wadley's Problem Technique

A direct method of analysing data of this type was given by Wadley (1949) who used the case where the initial infestation conformed to the Poisson distribution. Amscombe (1949) discussed the more general negative binomial distribution. The variance of the initial infestation adds to the binomial variance due to the treatment. Details of the procedure have been simplified and given by Finney (1971) under the heading 'Wadley's Problem'.

## An Example

The alternative analyses may be illustrated by data on the bioassay of methoprene on wheat against *Rhyzopertha dominica* (Fabricius). Parent insects (50) were added to the grain (67 g) and allowed to oviposit for 26 days at 25°C and 55% relative humidity and were then removed. The grain was then held at 30°C to allow development of F<sub>1</sub> progeny which were counted after 10 weeks. There were 3 replicates of 6 doses plus an untreated control.

The data and a summary of the resulting analyses by both methods are given in Table 1. The regression equations are somewhat different and the LD<sub>50</sub> values differ by over 5% and have different limits.

**Table 1.** Comparison of probit analysis by the Wadley's problem technique and by modified probit analysis, assuming the number of insects tested equals the mean number in untreated controls. Data on the response of *Rhyzopertha dominica* to methoprene in wheat with F<sub>1</sub> progeny counted after 10 weeks at 25/30°C and 55% relative humidity.

Dose (mg/kg)	Number of progeny (3 replicates)
0	946, 853, 809
0.0025	971 <sup>a</sup> , 903 <sup>a</sup> , 940 <sup>a</sup>
0.005	948 <sup>a</sup> , 911 <sup>a</sup> , -
0.01	736, 883 <sup>a</sup> , 827
0.025	372, 490, 387
0.05	132, 71, 102
0.1	20, 15, 12
Wadley's Problem	Mean number of progeny in controls 869.3. Maximum likelihood estimate $v = 928.2$ $Y = 3.400x + 10.602$ LD <sub>50</sub> = 0.023 (0.020 – 0.025)
Modified probit	$Y = 3.475x + 10.650$ LD <sub>50</sub> = 0.024 (0.021 – 0.026)

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<sup>a</sup>Number of progeny greater than the mean number of progeny in controls and must be deleted from the modified analyses.

The Wadley's problem technique permitted the use of all data and, in addition to the regression equation, gives a maximum likelihood estimate of the number of progeny in untreated grain, in this case 928 compared with the estimate from controls of 869. The modified probit model required six data to be deleted since in these instances the number of progeny (at treatment levels exercising minimal control) exceeded the mean number in untreated and would lead to negative probits.

Such deletions led to an underestimate of the number of progeny exposed to each treatment so that in the experimental range the LD values were underestimated. The deletions also led to an overestimate of the slope of the regression. Finally, the modified procedure ignored the variability in the number of progeny exposed to each treatment and resulted in limits which were too narrow.

### Criticisms of Probit Analysis

Recently it was claimed that analysis of observed survivors with Poisson or Poisson-lognormal methods is preferable to probit analysis (Preisler and Robertson 1992). The conclusion was based on a simulation study in which the modified probit analysis procedure was used. The study simulated an experiment aimed at determining an  $LT_9$  which was fixed at 65 minutes. For data with a heterogeneity factor of 14.3 it was necessary to delete 11.4% of the simulated samples and the actual value of the  $LT_9$  was within two standard errors of the mean in only 67.5% of 500 runs. The mean estimate of the  $LT_9$  was 66.67 which is an error of 2.6%. With more homogeneous data (heterogeneity factor 0.96 and 0.2% of samples discarded) the mean  $LT_9$  value was 65.04 and 79.2% of runs were within the expected interval. Errors of the magnitude demonstrated here may be of little consequence in many

bioassays but may be of crucial importance in applications such as establishing quarantine treatment for fruit flies.

These poor results appear to be a consequence of the use of the modified probit analysis method instead of Wadley's problem technique and as such cannot be accepted as a fundamental criticism of the probit model. The superiority of the Poisson or Poisson-lognormal models remains to be established.

### Computer Programs

Suitable computational procedures for analyses of the Wadley's problem technique available as options in standard computational programs (Lane et al. 1987).

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