Probit Analysis: A Tool for Analyzing Dose-Response Relationship

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ABSTRACT

Probit analysis is commonly used in concentration dose response bioassays to study mortality or survival. Bioassay data usually show a curved relationship between dose and response, which is difficult to analyze directly. Probit analysis simplifies this by converting response percentages into probit values and relating them to log-transformed doses, resulting in an almost straight-line relationship. This helps in estimating concentrations or doses that produce specific levels of biological effect with good accuracy. The method also provides information on the rate of increase in mortality, variability in responses and how well the data fit the model. These outputs allow reliable comparison of toxicity among different treatments and populations. Because of its simplicity and reliability, probit analysis remains a standard method for analyzing dose–mortality relationships in toxicological and entomological studies.

INTRODUCTION

Probit analysis is a specialized statistical method used in concentration dose response studies (bioassays) to analyze quantal or binomial responses, such as death or survival (Kulkarni *et al.,* 2015). Toxicity data from these experiments are typically expressed as proportions or percentages of responding organisms at defined doses or concentrations, producing a characteristic sigmoidal relationship between dose and response.

Because direct analysis of this non-linear relationship is statistically complex, probit analysis provides a structured approach for quantifying dose–mortality relationships and estimating key toxicological endpoints, including the median lethal concentration or dose (LC_{50} or LD_{50} or any other response), along with their associated confidence limits.

❖ Meaning of the term "Probit"

The term probit comes from the phrase *"probability unit."* It refers to a transformed value obtained by converting the observed proportion of response (such as mortality) into a scale based on the cumulative normal distribution, which allows easier statistical analysis of dose–response data (Nelson, 2018).

❖ Historical background

Probit analysis was first introduced by Chester Ittner Bliss in 1934 to analyze all-or-nothing responses in pesticide bioassays. The method was later formalized by David J. Finney (Finney, 1949), who established its statistical theory and computational procedures. These developments provided a rigorous framework for analyzing binomial response data arising from dose–response experiments.

❖ Why to do probit analysis ?

Probit analysis is used because it changes the usual S-shaped (Fig. 1) dose–response curve into a nearly straight line (Fig. 2). This makes the data easier to analyze using simple regression methods. It works by converting response percentages (such as mortality) into probit values and relating them to doses on a logarithmic scale (Prentice, 1976). The method assumes that individuals in a population differ normally in their tolerance to a toxicant. Probit analysis helps in accurately estimating important values like LC_{50} or LD_{50} and allows clear comparison of toxicity between different treatments, populations, or time periods. For these reasons, it is widely used in bioassay studies.

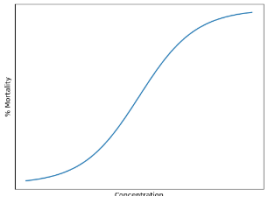

Fig. 1 Mortality sigmoid curve

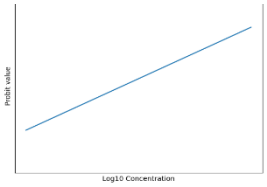

Fig. 2 Probit *v/s* Log_{10} concentration line

❖ How to do probit analysis manually?

1) **Convert mortality to proportions:** Change mortality from 0–100 per cent into a proportion between 0 and 1. This makes the data easier to work with. E.g., 40 per cent mortality becomes 0.4.

2) **Find the Z value:** Use the standard normal Z-table to find the Z value corresponding to the proportion. The Z value shows how far the response is from the average in standard deviation units.

3) **Convert Z to Probit value:** Add 5 to the Z value to get the probit value. Z values can be negative and difficult to use directly. Adding 5, shifts all values to a positive scale without changing their meaning. This makes 50 per cent response equal to probit 5 and simplifies interpretation.

4) **Log-transformed concentrations:** Convert each tested dose or concentration into a logarithmic scale (Log_{10}). This spreads out very low and very high doses, making the data easier to analyze and plot.

5) **Plot the points:** Plot log concentration on the X-axis and probit value on the Y-axis. Each point shows how the population responded to that dose.

6) **Draw the regression line:** Draw a straight line that best fits the points. This line shows the trend of increasing mortality with increasing concentration.

7) **Find concentration showing 50 per cent effect:** Locate probit 5 on the Y-axis (50% mortality), project to the regression line and then find the corresponding concentration on the X-axis and take anti-log value. This is the LC_{50}, the dose expected to kill half the population or any other effect on halt of the population. You can also use the line to estimate LC_1 to LC_{99} for other response levels.

❖ **Meaning of probit analysis outcomes**

1) **LC_{50} or LD_{50}**: The concentration (LC_{50}) or dose (LD_{50}) expected to kill 50 per cent of the test population. It is the most commonly used measure of toxicity because it represents a standard, reliable midpoint of the dose-response curve. LC_{50} is used because the natural dose–response curve is S-shaped, and the steepest part of the curve occurs around 50 per cent mortality. At this point, a small change in concentration causes a large change in mortality, making the measurement more sensitive and reliable. This sharp response does not occur at lower or higher mortality levels, which makes LC_{50} the standard for comparing toxicity.

2) **LC_{90} or LD_{90}**: The concentration or dose expected to kill 90 per cent of the population, useful for understanding higher-level effects, but less commonly used than LC_{50} for routine comparisons.

3) **Slope ± SE**: The slope of the probit regression line shows how quickly mortality increases with dose. A steep slope means small changes in dose cause large changes in mortality. SE (standard error) indicates the reliability of the slope estimate.

4) **Heterogeneity**: Shows how well the observed data match the model. Low heterogeneity means the data points are close to the predicted line, so the model fits well. High heterogeneity means the data vary a lot from the line, suggesting the model may not explain the responses accurately.

5) **Chi-square (χ^2)**: A test to check how well the model fits the observed data. The χ^2 value is compared with a critical value from the chi-square table, which depends on the degrees of freedom (df) and the chosen significance level (usually 0.05). If χ^2 (calculated) < χ^2 (table value), the model fits the data well. If χ^2 (calculated) > χ^2 (table value), the model does not fit properly, and the results may be unreliable.

6) **Degrees of freedom (df)**: The number of independent data points available to estimate parameters, used with χ^2 to evaluate model fit.

7) **Standard error (SE)**: Shows the precision of estimated values (like LC_{50}). Smaller SE means higher precision.

❖ **Precautions and limitations in probit analysis**

- Corrected mortality should be calculated (e.g., using Abbott's formula) to eliminate the effect of natural mortality in controls.
- Use sufficient replicates for each concentration to reduce random errors and increase reliability.
- Test a range of concentrations that produces partial mortality, ideally between 20–80 per cent, so the probit regression can be estimated accurately.
- Avoid concentrations that cause 0 per cent or 100 per cent mortality only, as they do not contribute to the regression line.
- Ensure the test population is uniform in age, size, and health to reduce variability.

- Maintain consistent experimental conditions (temperature, humidity and handling) across all treatments.
- Use enough data points along the curve to allow a reliable regression line; too few points can give inaccurate LC_{50} estimates.
- Check for goodness-of-fit (χ^2 and heterogeneity) to confirm the probit model is appropriate.

CONCLUSION

Probit analysis is a reliable and practical method for analyzing dose–response data in bioassays. By converting non-linear mortality responses into a linear form, it allows accurate estimation and comparison of toxicological endpoints, provided its assumptions and precautions are carefully followed.

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