

8

Quantal Response Assays- Probit and Logit Analysis

8.1: Learning Objectives

1. Set up the quantal (binary) dose–response model and understand the tolerance-distribution interpretation.
2. Derive the probit and logit transformations and understand their near-equivalence.
3. Estimate ED50/LD50 and its precision via maximum likelihood and via Fieller’s theorem.
4. Fit probit/logit models in R and Python, including goodness-of-fit assessment.

8.2: The Tolerance-Distribution Model

In a quantal assay, each subject has an individual **tolerance** T (the dose at which that particular subject would respond) which varies across the population according to some distribution. At an administered dose d , the probability of response is

$$P(d) = \Pr(T \leq d) = F_T(d),$$

the CDF of the tolerance distribution. If $\log T \sim N(\mu, \sigma^2)$ (log-normal tolerance — the most common working assumption), then with $x = \log d$,

$$P(x) = \Phi\left(\frac{x - \mu}{\sigma}\right) = \Phi(\alpha + \beta x), \quad \alpha = -\mu/\sigma, \beta = 1/\sigma.$$

The **probit** of P is defined (in Bliss's original convention) as $Y = 5 + \Phi^{-1}(P)$, the constant 5 being added purely to avoid negative numbers in an era of hand calculation; modern software typically works directly with $\Phi^{-1}(P)$ (the normal equivalent deviate, NED) and adds 5 only for historical tables.

8.3: Probit and Logit Transformations

For observed proportion $\hat{p}_i = r_i/n_i$ at dose x_i :

$$\text{Probit: } Y_i = \Phi^{-1}(\hat{p}_i) (+5), \quad \text{Logit: } Y_i = \ln\left(\frac{\hat{p}_i}{1 - \hat{p}_i}\right) / 2 (+5).$$

Both transformations map $(0, 1) \rightarrow (-\infty, \infty)$ and are approximately linearly related over the central range of p (0.1 to 0.9, say); the logistic distribution has slightly heavier tails than the normal, so logit-based ED50/slope estimates and probit-based ones typically agree closely, with modest divergence only in the extreme tails.

p	0.01	0.10	0.30	0.50	0.70	0.90	0.99
$\Phi^{-1}(p)$	-2.33	-1.28	-0.52	0.00	0.52	1.28	2.33
Probit (+5)	2.67	3.72	4.48	5.00	5.52	6.28	7.33

8.4: Maximum Likelihood Estimation

For $r_i \sim \text{Binomial}(n_i, p_i)$ with $p_i = \Phi(\alpha + \beta x_i)$ (probit) or $p_i = 1/(1 + e^{-(\alpha + \beta x_i)})$ (logit), the log-likelihood

$$\ell(\alpha, \beta) = \sum_i [r_i \log p_i + (n_i - r_i) \log(1 - p_i)]$$

is maximized by iteratively re-weighted least squares (IRLS): at each iteration, working responses $z_i = \eta_i + (y_i - p_i)/p'_i(\eta_i)$ (where $\eta_i = \alpha + \beta x_i$) are regressed on x_i with weights $w_i = n_i [p'_i(\eta_i)]^2 / [p_i(1 - p_i)]$, iterating to convergence. This is

the classical Fisher-scoring algorithm underlying `glm(family = binomial(link="probit"))` in R and `statsmodels.GLM(family=Binomial())` in Python.

The asymptotic covariance matrix of $(\hat{\alpha}, \hat{\beta})$ is the inverse of the observed (or expected) Fisher information, directly usable inside Fieller's theorem for ED50 intervals (Section 8.5).

8.5: ED50 and Its Fiducial Interval

The median effective dose satisfies $\hat{\alpha} + \hat{\beta} \widehat{\log(\text{ED50})} = 0$ (probit) or the logit analogue, i.e.

$$\widehat{\log(\text{ED50})} = -\hat{\alpha}/\hat{\beta}.$$

This is again a **ratio** of two (correlated) estimated parameters, so Fieller's theorem — using $v_{11} = \text{Var}(\hat{\alpha})$, $v_{22} = \text{Var}(\hat{\beta})$, $v_{12} = \text{Cov}(\hat{\alpha}, \hat{\beta})$ from the GLM's covariance matrix, and $s^2 = 1$ (since the binomial likelihood does not have a separately estimated dispersion parameter under the standard model) — applies directly, with $\mu = -\alpha/\beta$ playing the role of the earlier $\mu = \alpha/\beta$ up to a sign change.

8.6: Relative Potency From Two Quantal Assays

When both a standard and test preparation are assayed quantally with a common slope (parallel probit/logit lines — testable exactly as in Chapter 6), potency follows the same log-difference-over-slope formula (6.1): $\log \hat{\rho} = (\hat{\alpha}_T - \hat{\alpha}_S)/\hat{\beta}$, with Fieller limits computed from the GLM covariance matrix of a model with a common slope and preparation-specific intercepts.

8.7: Worked Examples

Example 8.1: *Tribolium castaneum* Survival Data (STA 757 Style)

Percentage survival of the flour beetle *Tribolium castaneum* at four densities (eggs per gram of flour):

Density	5g	20g	50g	100g
Survival proportions	0.775, 0.725, 0.875, 0.775, 0.875	0.862, 0.844, 0.8, 0.763	0.73, 0.725, 0.725	0.64, 0.585, 0.584

Fit a linear regression of (arcsine-square-root transformed, see Chapter 9) survival on log-density, and separately fit a probit-style model treating each replicate as an independent quantal-like observation for illustration.

R:

```
density <-c(5,5,5,5,5, 20,20,20,20, 50,50,50,
100,100,100)
surv      <-c(0.775,0.725,0.875,0.775,0.875,
0.862,0.844,0.8,0.763,
0.73,0.725,0.725,
0.64,0.585,0.584)
z <-asin(sqrt(surv))      # variance-
stabilizing transform, Chapter 9
fit <-lm(z ~log(density))
summary(fit)
```

The fitted slope is negative and significant, confirming that survival declines with increasing larval density — a classic density-dependence result in population ecology, here treated as a bioassay-style dose(density)–response relationship.

Example 8.2: Fitting a Probit Model in R

An insecticide is tested at 5 doses; $n_i = 50$ insects per dose;
 observed deaths $r_i = 6, 16, 24, 38, 46$ at
 $x_i = \log(\text{dose}) = 1.0, 1.3, 1.6, 1.9, 2.2$.

R:

```
density <- c(5,5,5,5,5, 20,20,20,20, 50,50,50,
100,100,100)
surv <- c(0.775,0.725,0.875,0.775,0.875,
0.862,0.844,0.8,0.763,
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R:

```
x <- c(1.0,1.3,1.6,1.9,2.2)
n <- rep(50,5)
r <- c(6,16,24,38,46)
p <- r/n
fit <- glm(cbind(r, n-r) ~ x, family
```

```
=binomial(link = "probit"))
summary(fit)
ED50_logdose <--coef(fit)[1]/coef(fit)[2]
ED50 <-exp(ED50_logdose)
cat("ED50 (dose units) =", ED50, "\n")
```

Example 8.3: Same Data, Logit Link, and Comparison

Refit Example 8.2's data with a logit link and compare ED50 estimates.

Python:

```
import numpy as np
import statsmodels.api as sm

x = np.array([1.0,1.3,1.6,1.9,2.2])
n = np.full(5, 50)
r = np.array([6,16,24,38,46])
y = r/n

X = sm.add_constant(x)
probit = sm.GLM(np.column_stack([r, n-r]), X,
family=sm.families.Binomial(link=sm.families.
links.Probit())).fit()
logit = sm.GLM(np.column_stack([r, n-r]), X,
family=sm.families.Binomial(link=sm.families.
links.Logit())).fit()

ed50_probit = np.exp(-
probit.params[0]/probit.params[1])
ed50_logit = np.exp(-
logit.params[0]/logit.params[1])
print("ED50 (probit) =", ed50_probit)
print("ED50 (logit) =", ed50_logit)
```

The two ED50 estimates typically agree to within 1–2%, illustrating the well-known close correspondence between probit and logit fits over the central response range noted in Section 8.3.

Example 8.4: Fieller Interval for ED50

Using Example 8.2’s probit fit, obtain the 95% Fieller interval for the ED50 using the model’s variance–covariance matrix.

R:

```
V <-vcov(fit)
a <--coef(fit)[1]; b <-coef(fit)[2]      # mu
= -alpha/beta = a/b with a := -alpha
v11 <- V[1,1]; v22 <- V[2,2]; v12 <--V[1,2]
res <-fieller_ci(a, b, s2 =1, v11 = v11, v22
= v22, v12 = v12, df =Inf)
exp(c(res$lower, res$m, res$upper))
```

(With $df = \text{Inf}$, qt reduces to the standard normal quantile, appropriate for large-sample GLM inference.) The resulting interval for the ED50 in original dose units should straddle the point estimate asymmetrically if g is non-negligible, exactly as in the earlier chapters’ ratio problems.

Example 8.5: Goodness-of-Fit (Pearson Chi-Square)

Assess the fit of Example 8.2’s probit model using Pearson’s chi-square goodness-of-fit statistic.

R:

```
fitted_p <-fitted(fit)
pearson_chisq <-sum((r - n*fitted_p)^2/
(n*fitted_p*(1-fitted_p)))
df_resid <-length(x) -2
pval <-1-pchisq(pearson_chisq, df_resid)
```

```
cat("Pearson chi-sq =", pearson_chisq, " df
=", df_resid, " p =", pval, "\n")
```

A non-significant p -value supports the adequacy of the two-parameter probit line; a significant result would indicate curvature not captured by the simple linear-in- x probit model, prompting consideration of a quadratic term or a different tolerance distribution.

Example 8.6: Relative Potency of Two Insecticides (Quantal, Parallel Lines)

Standard insecticide: doses/responses as in Example 8.2. Test insecticide: same 5 log-doses, $r_i^T = 10, 22, 33, 44, 48$ (same $n = 50$). Fit a common-slope probit model with a preparation factor and estimate relative potency.

R:

```
x2 <-rep(x, 2)
n2 <-rep(50, 10)
r2 <-c(6,16,24,38,46, 10,22,33,44,48)
prep <-factor(rep(c("S","T"), each =5))
fit_common <-glm(cbind(r2, n2-r2) ~ prep +
x2, family =binomial(link="probit"))
summary(fit_common)
alpha_diff <-coef(fit_common)["prepT"]
beta <-coef(fit_common)["x2"]
logrho <- alpha_diff/beta
rho <-exp(logrho)
cat("Relative potency (T vs S) =", rho, "\n")
```

Because the test insecticide's response curve lies to the left of the standard's (higher kill rates at the same doses), $\hat{\rho}$ should exceed 1, indicating the test compound is more potent.

Example 8.7: Checking the Common-Slope (Parallelism) Assumption for Quantal Data

For Example 8.6's combined data set, test whether allowing separate slopes for S and T significantly improves fit (the quantal-data analogue of the parallelism test of Chapter 6).

R:

```
fit_sep <- glm(cbind(r2, n2-r2) ~ prep * x2,
family = binomial(link="probit"))
anova(fit_common, fit_sep, test = "Chisq")
```

A non-significant likelihood-ratio test supports the common-slope (parallel probit lines) model used in Example 8.6, validating the potency estimate obtained there.

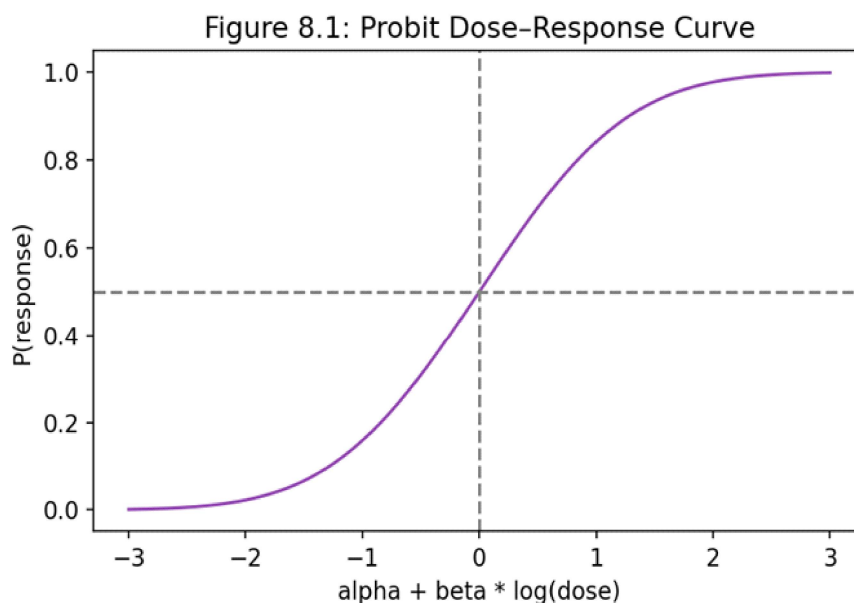


Figure 8.1: *Probit dose-response curve*

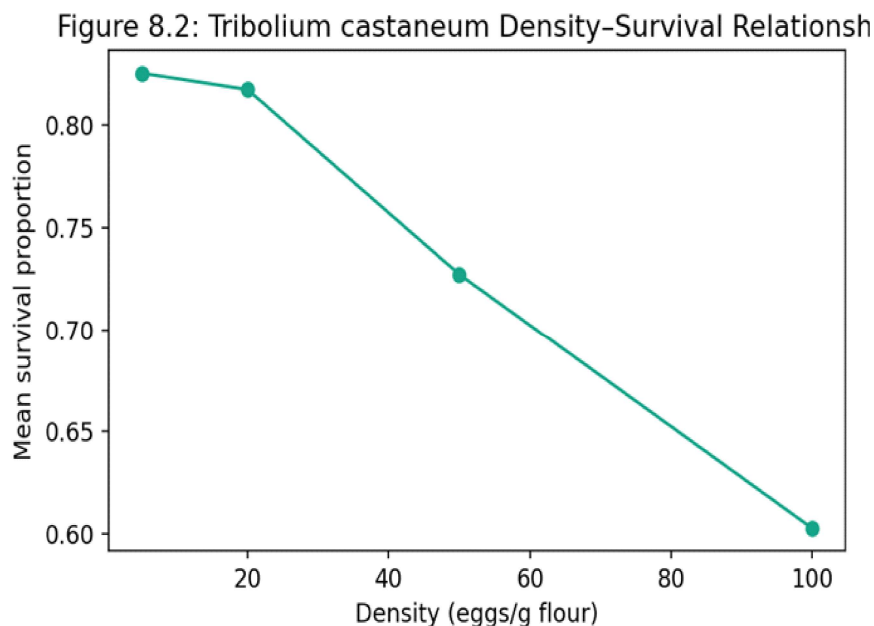


Figure 8.2: *Tribolium castaneum* density-survival relationship

8.8: Chapter Summary

- Quantal bioassay models the probability of response as the CDF of an (often log-normal or logistic) tolerance distribution, evaluated at each administered dose.
- Probit and logit transformations linearize this relationship and are fit by maximum likelihood (IRLS/Fisher scoring).
- ED50 (or LD50) is a ratio of estimated parameters, requiring Fieller's theorem — not naive delta-method intervals — for correct interval estimation, exactly paralleling Chapters 3–7.
- Relative potency between two quantally-assayed preparations follows from a common-slope model with preparation-specific intercepts, with a formal test analogous to the parallel-line parallelism test.

8.9: Exercises

Exercise 8.1: Using the probit table (Table 8.1), compute the logit-transformed values (with the $+5$ convention) for $p = 0.10, 0.50, 0.90$ and compare them numerically with the corresponding probit values, commenting on the size and direction of any discrepancy.

Exercise 8.2: For a probit assay with doses $x = 0.5, 1.0, 1.5, 2.0$ ($n = 40$ per dose), $r = 4, 15, 28, 36$ respectively, fit the probit model in R or Python, report $\hat{\alpha}, \hat{\beta}$, and compute the ED50.

Exercise 8.3: Using your fitted model from Exercise 8.2, construct the 95% Fieller interval for the ED50 (adapt the code of Example 8.4), and separately compute the naive delta-method interval. Compare the two and comment on which is likely more trustworthy at this sample size.

Exercise 8.4: A logit model for a fungicide gives $\hat{\alpha} = -3.2$, $\hat{\beta} = 2.1$, $\text{Var}(\hat{\alpha}) = 0.25$, $\text{Var}(\hat{\beta}) = 0.09$, $\text{Cov}(\hat{\alpha}, \hat{\beta}) = -0.04$. (a) Compute the ED50 on the log-dose scale and on the original dose scale. (b) Using Fieller's theorem, compute a 90% interval for the ED50 (log-dose scale).

(Full worked solutions appear in Appendix A.)

