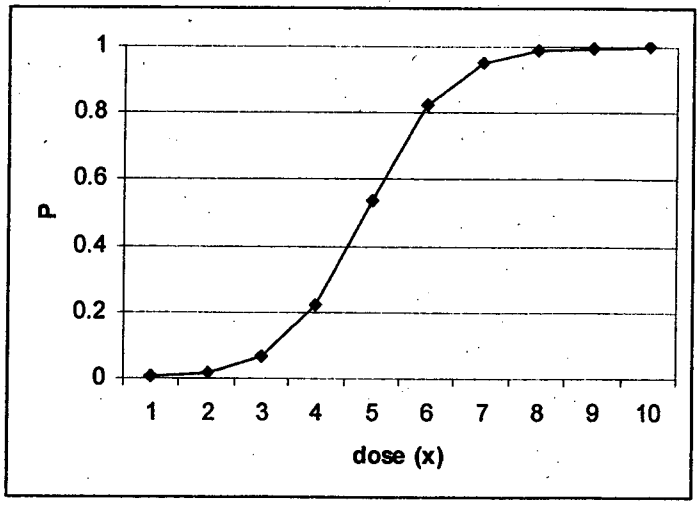
4.

Statistical Analysis of Bioassays

Biological assays (bioassay, for short) are methods for estimating the potency of a drug or material by utilizing the reaction caused by its application to experimental subjects that are living. In a typical assay, the measurement is whether each unit responds or not for the dose applied. The response of this nature is regarded as binary (responded or not) and thus binary data analysis techniques can be used to analyze bioassay data.

In an insecticidal trial, if the dose applied to a given batch is low, none of the insects in that batch may die whereas, if a high dose is applied, they may all die. Whether or not an insect dies when exposed to a given dose depends on the tolerance of the insect to the compound. Those insects with a low tolerance will be more likely to die from exposure to a given dose than those with a high tolerance. Thus the tolerance varies between insects and a distribution of tolerance levels can be considered. Accordingly, those insects with a tolerance level less than x_i will die when expose to dose x_i. When the dose x_i increases, the proportion of death, p_i increases. A typical dose response relationship is given by the figure below.



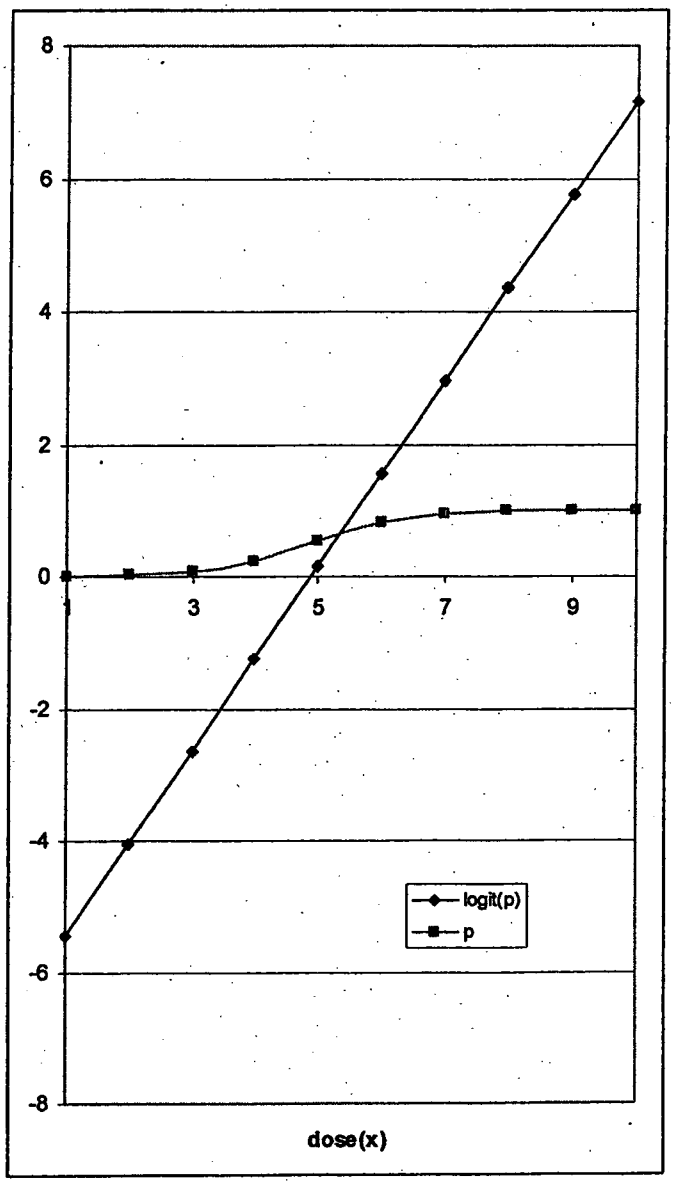
With appropriate link functions, above non linear relationship can be transformed into a linear relationship. Common transformations used for P in these situations are,

i) $\text{logit}(p) = \log\left(\frac{p}{1-p}\right)$

ii) $\text{probit}(p) = \phi^{-1}(p)$

iii) $\text{Complimentory log}-\log(p) = \log[-\log(1-p)]$

For instance, with logit transformation, the above relationship will be in the following form.



Similar linearity can be achieved with probit and complementary log-log transformations.

Goodness of fit of the model

Once a model is fitted, fitted model can be evaluated using goodness of fit test statistics. Most common test statistic used for models of above nature is called *Deviance* (G^2) which has asymptotic χ^2 with $n-q$ degree of freedom where q is the number of parameter in the fitted model. When the best fitting model is obtained, that model can be used for prediction. For instance, the dose that kills 50% of the population, which is called LD_{50}, can be predicted from the model.

Example.
Suppose following data have been obtained from a dose response trial.

Dose	No of deaths out of 50
0.0030	02
0.0060	08
0.0120	12
0.0240	27
0.0480	45

The output from SAS after fitting the linear logistic model is as follows.

Maximum Likelihood Analysis

Maximum likelihood computations

converged.

Maximum Likelihood Analysis of

Variance

Source	DF	Chi-Square	Pr > ChiSq
Intercept	1	75.13	<.0001
dose	1	62.56	<.0001
Likelihood Ratio	3	3.68	0.2987

Analysis of Parameter Estimates

Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	-2.5011	0.2885	-3.0666	-1.9355	75.13	<.0001
dose	1	104.1712	13.1703	78.3579	129.9845	62.56	<.0001

Model in Terms of Tolerance Distribution

MU	SIGMA
0.02400913	0.00959958

The G^2, which is indicated in the output as likelihood ratio, is 3.68 with 3 df and the probability (P) associated with the given test statistic, which is also known as level of significance, is 0.2987. Since P is large (value larger than 0.05 is usually considered as large), H_0 is not rejected and thus the fitted model is good. Since the fitted model is good, other statistics and estimates can be used to make inference. The statistic corresponding to dose in the maximum likelihood ANOVA is 62.56 with 1 df and $P < 0.0001$. Very small P value indicates highly significant dose effect on the response. The estimate for the dose effect given under analysis of parameter estimates is 104.17 with $P < 0.0001$ and this also indicates higly significant dose effect on the response. From the analysis, the fitted model obtained is

$$\log\left(\frac{\hat{p}_i}{1-\hat{p}_i}\right) = -2.50 + 104.17 dose_i$$

From the above fitted model predictions can be made. In fact, *mu* and *sigma* given in the output under *model in terms of tolerance distribution* can be interpreted as estimated mean and the standard deviation of the tolerance distribution. The two values given in the output are 0.02400913 and 0.00959958 respectively. It can be shown that *mu* is in fact estimated LD_{50} value.

In the above model fitting, if log(dose) is used instead of dose itself, G^2 is 4.38 with 3 df and $P = 0.2229$. Thus it is seen that G^2 with log dose is larger than G^2 with dose itself and it indicates that there is no improvement in the model by using log(dose) instead of dose itself. Hence dose should be used as the predictor variable and not log(dose) in this particular example.

Confidence Interval for LD_{50}

In order to have more information about the true value, confidence interval can be constructed. For the true LD_{50}, confidence interval (CI) is given by

$$\hat{LD}_{50} \pm Z_{\alpha/2} \bullet SE\left(\hat{LD}_{50}\right).$$

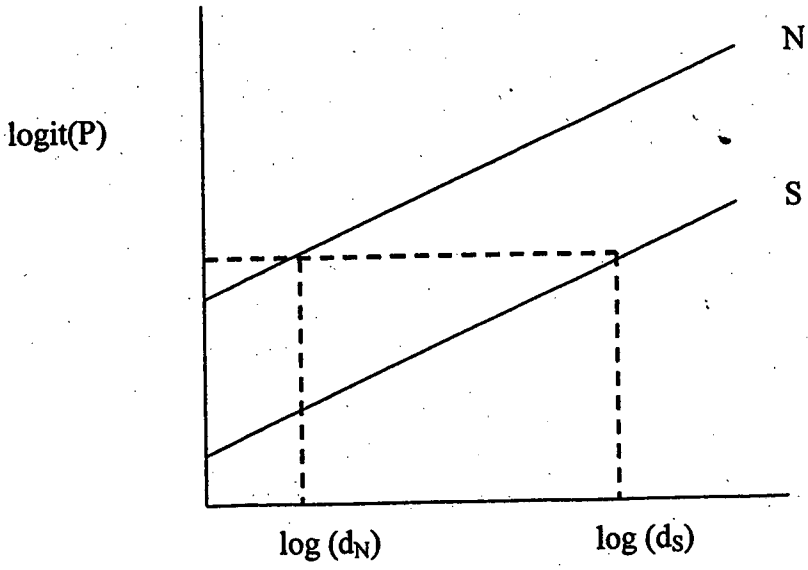
According to the Fieller's theorem, the SE of $\hat{LD}_{50}$ can be computed using two parameter estimates $\hat{\beta}_0$ and $\hat{\beta}_1$, and variances and covariance of the parameters estimates. For the above example the 95% CI for LD50 is 0.0207 to 0.0280.

Relative potency

Often we need to compare two compounds, such as newly developed one and a standard. For each compound, the proportion of individuals responding at a series of increasing doses is recorded. For the comparison, three types of linear logistic models are fitted and evaluated. The three types of models fitted are

i) two different lines
ii) two parallel lines
iii) a common line

If the lines are different, the difference between the effects of two compounds is not a constant. If there is a common line, the two chemicals are equally effective and if the lines are parallel, relative potency can be assessed (parallel line assay) (see the figure given below).



With parallel lines, let log (d_N) and log (d_S) have produced the same response. Then the r_{NS}, the potency of new compound relative to the standard, is

$$r_{NS} = \frac{d_S}{d_N} = \exp\left\{\frac{\alpha_N - \alpha_S}{\beta}\right\}$$

where α_N and α_S are the intercepts for the new and standard curves and β is the common slope. The confidence intervals for the r_{NS} can also be constructed using Fieller's theorem.

Example

Suppose following data (proportion of insects died) have been recorded from a study.

Insecticide	Deposit of insecticide (mg/10 cm^2)					
	2.00	2.64	3.48	4.59	6.06	8.00
A	3/50	5/49	19/47	19/38	24/49	35/50
B	2/50	14/49	20/50	27/50	41/50	40/50

For the separate lines, an extract of the output is as follows.

Maximum Likelihood Analysis of Variance (chem1)

Source	DF	Chi-Square	Pr > ChiSq
Intercept	1	58.66	<.0001
d	1	51.20	<.0001
Likelihood Ratio	4	8.78	0.0668

Maximum Likelihood Analysis of Variance (chem2)

Source	DF	Chi-Square	Pr > ChiSq
Intercept	1	66.12	<.0001
d	1	70.01	<.0001
Likelihood Ratio	4	8.16	0.0860

Therefore, for separate lines, the G^2 is 16.94 with 8 df.

For the parallel lines, an extract of the output is as follows.

Maximum Likelihood Analysis of Variance

Source	DF	Chi-Square	Pr > ChiSq
Intercept	1	124.79	<.0001
d	1	121.35	<.0001
chem	1	9.20	0.0024
Likelihood Ratio	9	18.37	0.0311

Therefore, for parallel lines the G^2 is 18.37 with 9 df.

For a common line, an extract of the output is as follows.

Maximum Likelihood Analysis of Variance

Source	DF	Chi-Square	Pr > ChiSq
Intercept	1	124.30	<.0001
d	1	120.94	<.0001
Likelihood Ratio	10	27.74	0.0020

Therefore, for a common line the G^2 is 27.74 with 10 df

By evaluating the change in the G^2 it can be said that the parallel lines case is the best. The LD_{50} values for chem1 and chem2 are 5.417 and 4.158. Therefore the relative potency of chem2 to chem1 is 5.417/4.158.