## Statistical analysis of bioassay and the PLA model

## What is a bioassay?

- Substance
- Subject
- Response

Standard preparation vs. Tested preparation

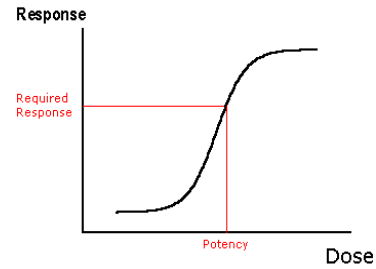
## Bioassay objective

To measure the potency of some new compound relative to some standard compound,

Where

potency is the dose of a compound required to cause a particular response.

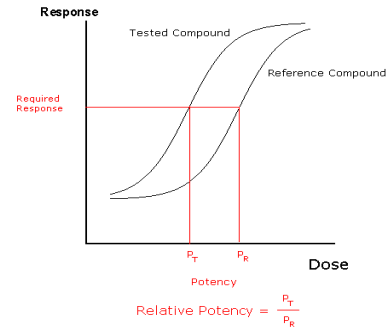
## Potency




## Relative potency

Relative potency is the ratio of the tested compound potency and the reference compound potency.

## Relative Potency (cont.)



## Statistical Models

- Parallel Line model (PLA)
- Slope Ratio model

Both models are linear.

At TEVA we routinely utilize the PLA model.

## Basic requirements

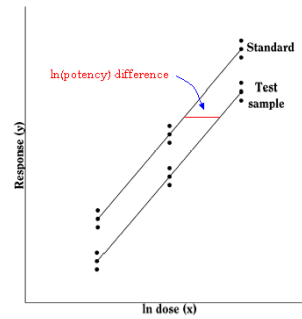
- Randomization
- Responses are Normally distributed
- Homogenous variances

EP recommends a logarithmic transformation to improve compliance with second and third requirements when necessary

## Requirements for PLA model

- The relationship between the logarithm of the dose and the response can be represented by a straight line
- For any unknown (tested) preparation the straight line is parallel to that of the standard

## 1 picture = 1000 words



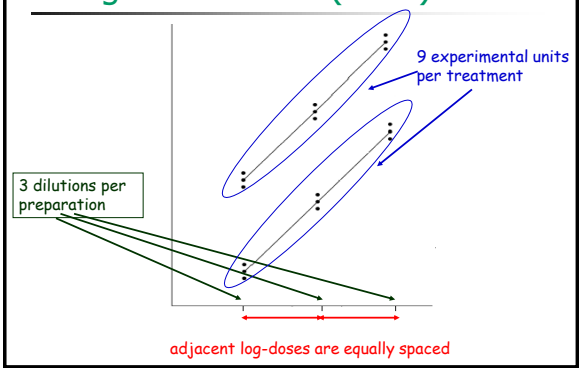

## PLA in practice

- Design restrictions imposed by the EP
- Experimental design
- Analysis of variance
- Tests of validity
- Potency estimation and confidence limits
- Handling missing values
- Troubleshooting
- Software

## Design restrictions

- Each preparation must be tested with the same number of dilutions
- The ratio of adjacent doses must be constant for all treatments
- There must be an equal number of experimental units to each treatment

## Design restrictions (cont.)




## Experimental design

Completely randomized design – if experimental units are reasonably homogeneous.

EP also discusses :

- Randomized block design.
- Latin-square designs
- Cross-over designs

## PLA model is a linear model

- Response is continuous
- Two explanatory variables:
  - Log(dose) - continuous
  - Preparation - classifying variable
  - Log(dose) and preparation interaction - optional

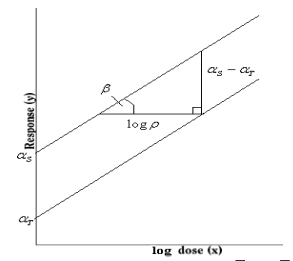
## Tests of validity

- Dose response: linear regression term must be significant
- Parallelism: interaction between log(dose) and preparation must not be significant
- Linearity must be verified

## Methods for assessing linearity

- Just look at $R^2$
- Add a quadratic term to the model and verify that it is non-significant
- Model dose/dilution as a class variable, and compare the results to the "correct" model

## Relative Potency estimation




$$\log \hat{\rho} = \bar{x}_S - \bar{x}_T - \frac{\bar{y}_S - \bar{y}_T}{b}$$

## Fiducial limits

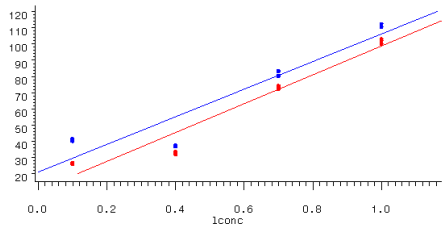
Filler's theorem

$$\bar{x}_S - \bar{x}_T + \frac{1}{1-g}\cdot\left[\log\hat{\rho} + \bar{x}_S - \bar{x}_T \pm \frac{t_{\alpha/2}\cdot s}{b}\cdot\sqrt{(1-g)\cdot\left(\frac{1}{N_S}+\frac{1}{N_T}\right)+\frac{(\log\hat{\rho}+\bar{x}_S-\bar{x}_T)^2}{SS_x}}\right]$$

## Example

```
data in;
  input substance $ conc od1 od2 od3;
  lconc=log(conc);
datalines;
RS  10      112.5   110.8   110.6
RS   5       80.7    80.3    83.4
RS   2.5     36.9    37.6    37.6
RS   1.25    41.5    40.6    40.3
DP  10      102.1   100     103.2
DP   5       74.3    72.4    74.2
DP   2.5     32      32.2    33.6
DP   1.25    26.8    26      26.6
;
run;
```

## Take a look




## Run 1 - validate assumptions

- Run full model with:
  - Log(dose)
  - Preparation
  - Log(dose) and preparation interaction

## GLM RUN 1 – output

| R-Square | Coeff Var | Root MSE | y Mean |
|---|---|---|---|
| 0.907974 | 16.32399 | 10.31268 | 63.17500 |

Regression

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| lconc | 1 | 20478.08133 | 20478.08133 | 192.55 | <.0001 |
| substance | 1 | 205.21144 | 205.21144 | 1.93 | 0.1801 |
| lconc*substance | 1 | 9.63333 | 9.63333 | 0.09 | 0.7665 |

Linearity

parallelism

## Run 2 - actual PLA run

- Run ~~full~~ model with:
  - Log(dose)
  - Preparation
  - ~~Log(dose) and preparation interaction~~

## GLM run 2 - estimates

| Parameter | Estimate | | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|
| Intercept | 20.13242531 | B | 4.44260359 | 4.53 | 0.0002 |
| lconc | 86.79090803 | | 6.11769563 | 14.19 | <.0001 |
| substance DP | -9.1166666 7 | B | 4.11796490 | -2.21 | 0.0380 |
| substance RS | 0.00000000 | B | . | . | . |

$\alpha_S - \alpha_T$ $\beta$

$\log\rho = 9.11/86.79 = 0.1049$

$\rho = 10^{0.1049} = 1.274$

## GLM Run 2 – Stuff for Fiducial limits calculation

| R-Square | Coeff Var | Root MSE | y Mean |
|---|---|---|---|
| 0.907974 | 16.32399 | 10.31268 | 63.17500 |

s

X'X Generalized Inverse (g2)

| | Intercept | lconc | substance DP | substance RS | y |
|---|---|---|---|---|---|
| Intercept | 0.1939807328 | -0.201743804 | -0.083333333 | 0 | 20.132425308 |
| lconc | -0.201743804 | 0.3678402089 | 0 | 0 | 86.790908026 |
| substance DP | -0.083333333 | 0 | 0.166666667 | 0 | -9.116666667 |
| substance RS | 0 | 0 | 0 | 0 | 0 |
| y | 20.132425308 | 86.790908026 | -9.116666667 | 0 | 2136.662 |

$v_{22}$ $v_{11}$

Fiducial Limits by Fieller's theorem: 1.015-1.616

## Troubleshooting 1.01

- Problem: Non-linearity.
- Solution: removal of a dose – to select the "best range" of linearity.

## Troubleshooting 1.02

Problem: Exceptionally high residual error (Root MSE)

Solution: this is an indication of technical problem – check the bioassay process

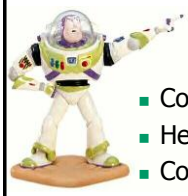
## Troubleshooting 1.03

Problem: Exceptionally low residual error (Root MSE) may cause F values to exceed critical values

Solution: replace residual error by estimate from historical data

## Software

- SAS®
- Any other decent statistical software
- PLA® - tailored software for bioassay analysis



## Beyond this talk

- Combination of assay results
- Heterogeneity of variances
- Correlated errors
- Nonlinear dose-response models

## Guidelines and references

- EP Chapter 5.3
- USPC General Chapter <111>
- Statistics in the Pharmaceutical Industry: Ch. 3