

Estimation of relative potency in a 4-point, parallel line assay

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Agenda

- **Relative potency** in the regulatory context
- The **estimation** of relative potency
- The **two-step analysis** of relative potency
- The **%REL POT** macro
- Questions

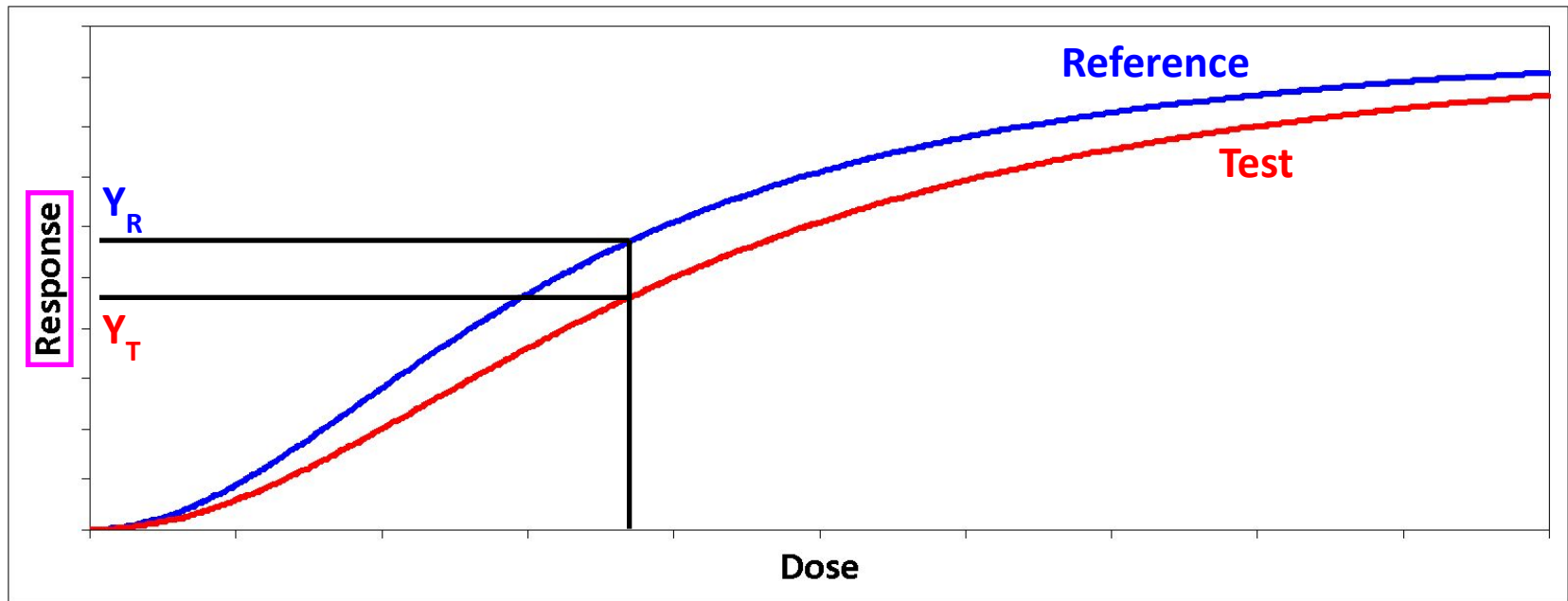
Regulatory context

- **EMA** Guideline on the Requirements for Clinical Documentation for **Orally Inhaled Products** (CPMP/EWP/4151/00 Rev. 1, 2009)
- Objective: **demonstration of therapeutic equivalence** in respect of efficacy of inhaled asthma drugs
- The comparison between products should be performed in both the following ways:
 - **demonstration of equivalence for at least two dose levels** on the pharmacodynamic endpoint (response-axis analysis)
 - analysis of **relative potency** (dose-axis analysis)

Regulatory context

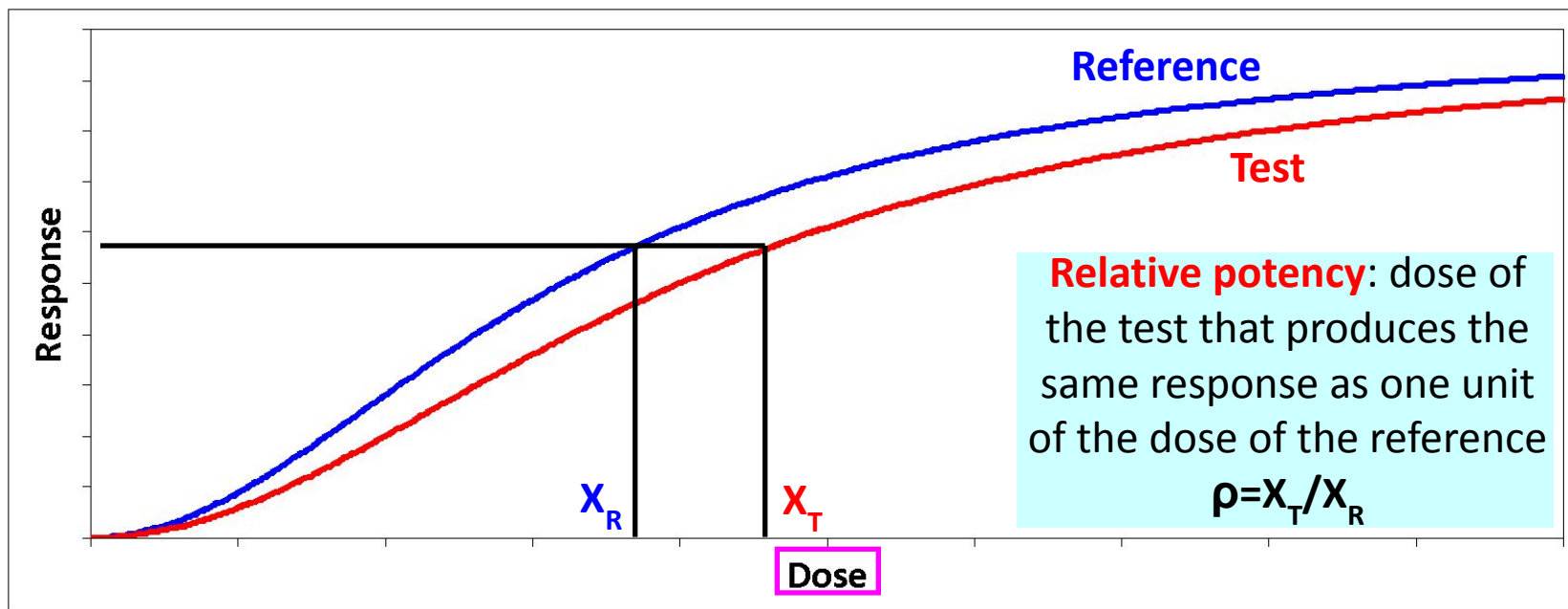
- For either approach to be acceptable a minimum requirement is that the study has **assay sensitivity**, therefore:
 - at least two non-zero dose levels of both the test and reference products need to be studied
 - one dose level needs to be shown to be superior to the other
- We will focus the **analysis of relative potency in a 4-point assay**
 - The same two dose levels of each product being compared are administered (e.g., test high dose, test low dose, reference high dose, reference low dose)
 - Typical design for the comparison of different formulations of the same medication, for example a dry powder inhaler and a pressurized metered-dose inhaler

Response-axis analysis



- The usual approach for the evaluation of equivalence is based on the comparison between products in terms of response
- We focus on the **responses to the two products at the same dose**

Dose-axis analysis

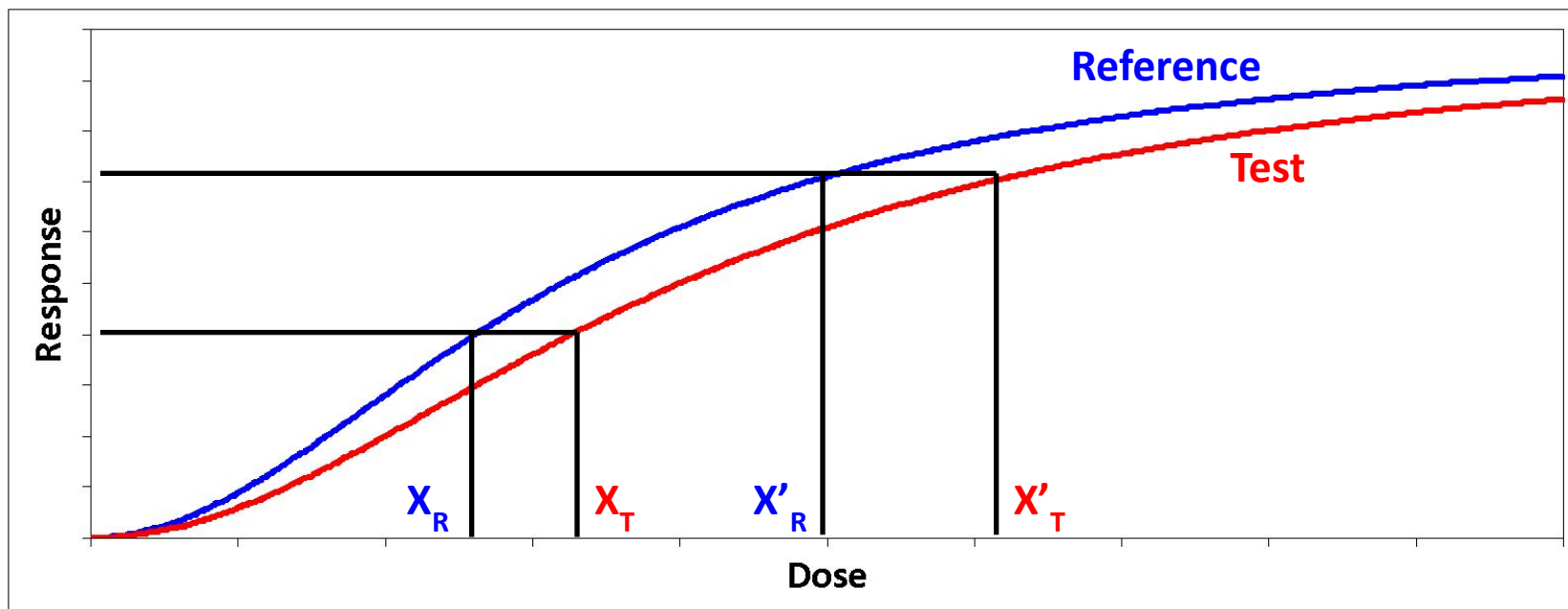


- The evaluation of the relative potency corresponds to a comparison between products on the dose axis
- We focus on the **doses of the two products producing the same response**

Dose-axis analysis

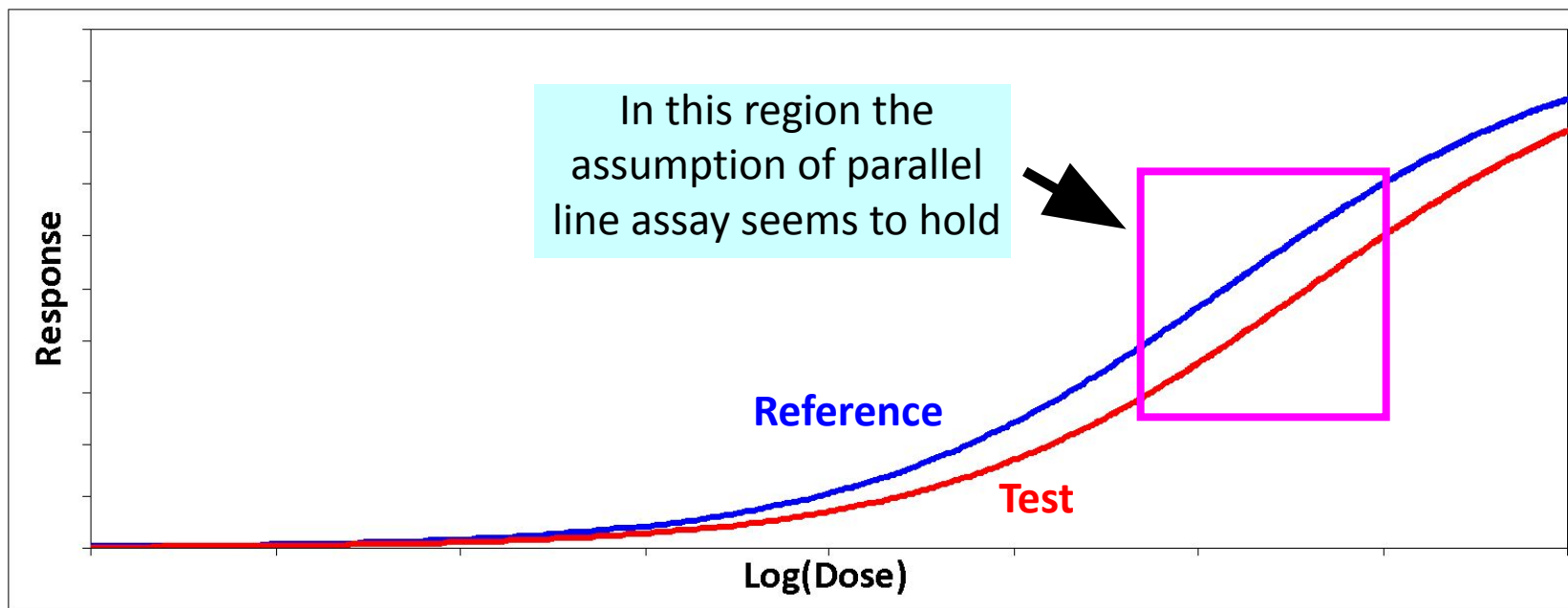
- Based on this definition, if **relative potency** is:
 - **< 1**, then a dose of the test produces the same result as does a higher dose of the reference (**test is more potent**)
 - **= 1**, then the two products produce the same result at the same dose (**test and reference are equipotent**)
 - **> 1**, then a dose of the test produces the same result as does a lower dose of the reference (**test is less potent**)
- The **EMA guideline** requires the CI of relative potency to lie within the limits **0.67-1.5**
- The confidence level to be used is not explicitly specified in the guideline.
- A clarification on this point has been recently provided by García-Arieta (AEMPS assessor): **90% confidence level** (*Br J Clin Pharmacol*, in press)

Estimation of relative potency



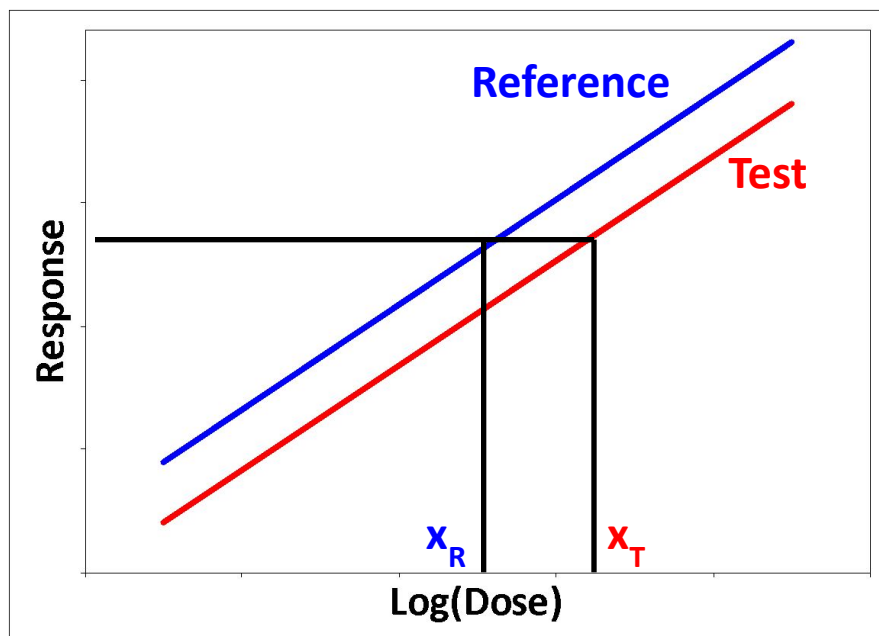
- **Problem:** based on these dose-response curves, relative potency depends on the response level considered (in general $X_T/X_R \neq X'_T/X'_R$)
- In order to postulate a unique value for relative potency, an **assumption on the shapes of dose-response curves** should be introduced

Estimation of relative potency



- We assume a **parallel line assay**, i.e. linear and parallel dose-response curves on log-dose scale
- Since the dose-response curves are linear, two points for each product (**4-point assay**) allow for their estimation

Estimation of relative potency



- x_T, x_R : doses of test and reference producing the same response
- $x_T = \log(X_T), x_R = \log(X_R)$
- **Constant horizontal distance** between the curves on the $\log(\text{dose})$ scale

- Constant difference $x_T - x_R \rightarrow$ **constant ratio X_T/X_R**
- **Unique value for relative potency**, irrespective of the response level considered

Estimation of relative potency

- The log relative potency $\log(\rho)$ corresponds to the difference $x_T - x_R$, since

$$\log(\rho) = \log(X_T/X_R) = \log(X_T) - \log(X_R) = x_T - x_R$$

- The regression lines for the dose-response curves can be expressed as

$$Y_T = \alpha_T + \beta \cdot x_T \quad \text{and} \quad Y_R = \alpha_R + \beta \cdot x_R$$

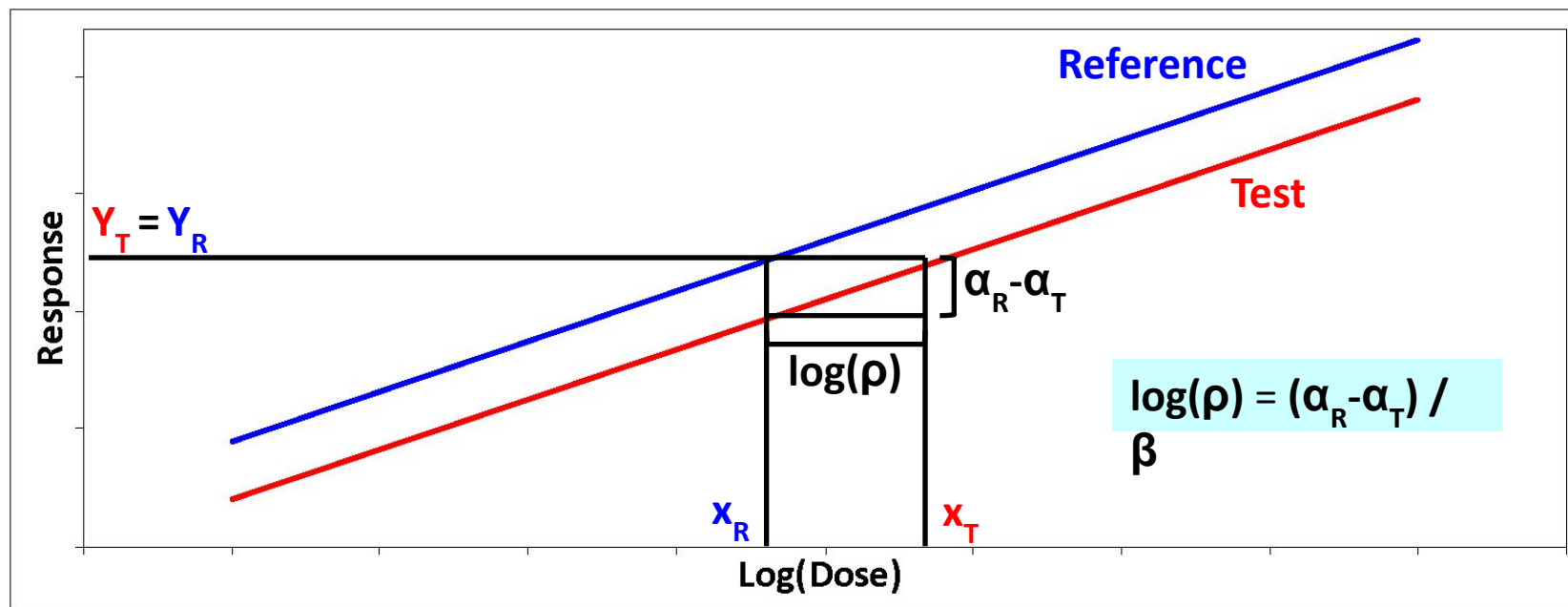
- The log relative potency is the difference $x_T - x_R$ when test and reference produce the same response. By equating the responses of the two products we obtain

$$Y_T = Y_R$$

$$\alpha_T + \beta \cdot x_T = \alpha_R + \beta \cdot x_R$$

$$\log(\rho) = x_T - x_R = (\alpha_R - \alpha_T) / \beta$$

Estimation of relative potency



- The **log relative potency** can be estimated as the ratio between
 - the estimate of the **overall product effect** $a_R - a_T$ and
 - the estimate of the **common slope of log(dose)** b
$$R = (a_R - a_T) / b$$

Estimation of relative potency

- The calculation of the confidence interval is based on **Fieller's theorem**

$$\frac{R - \frac{gv_{12}}{v_{22}} \pm \frac{t}{b} \left[v_{11} - 2Rv_{12} + R^2v_{22} - g \left(v_{11} - \frac{v_{12}^2}{v_{22}} \right) \right]^{1/2}}{(1-g)}$$

- Where
 - R** is the estimate $(a_R - a_T) / b$ of log relative potency
 - $g = t^2 v_{22} / b^2$
 - v₁₁** is the variance of $a_R - a_T$
 - v₁₂** is the covariance between $a_R - a_T$ and b
 - v₂₂** is the variance of b
 - t is the appropriate percentile of the t-distribution with f DF
 - f** are the DF on which the residual variance is based

Assumptions for the estimation

- Estimation of relative potency in a parallel line assay requires the following main **assumptions** to be satisfied
 - A. Absence of significant deviations from parallelism** of dose-response curves on the log-dose scale
 - B. Significant dose-response relationship.** If the slope b is not significantly different from zero, the data are consistent with a zero value for β and hence an infinite value for the relative potency. Assay sensitivity is also explicitly required by the EMA guideline
 - C. Absence of a significant difference between products.** The difference $a_R - a_T$ should be non-significant. If this assumption does not hold, but relative potency is estimated anyway, the estimate obtained is expected to be far from unity
- Regarding the choice of the **significance level** to be used when testing the assumptions, see Finney (*Statistical Method in Biological Assay*, 1978)
- In our limited experience, we considered the usual significance level of 0.05 for assumptions B and C and a more stringent level of 0.1 for the fundamental assumption A

The two-step analysis: step 1

- The proposed method for the analysis of relative potency is based on a **two-step approach**
- In **step 1** a model is estimated to **verify the required assumptions**
- The **treatment effect is decomposed** into the effects of product, dose on the log-scale and their interaction. With this aim, the 4-level categorical variable commonly used for treatment is replaced by two variables: product and log-dose, to be included in the model with their interaction

Former variable	New variables	
Treatment	Product	log(dose)
Product A - 50 µg	A	3.912
Product A - 200 µg	A	5.298
Product B - 50 µg	B	3.912
Product B - 200 µg	B	5.298

The two-step analysis: step 1

- All the **other appropriate factors** (e.g., baseline, centre, country, subject, period, etc.) can be kept in the model as usual. Therefore the proposed method can be applied to a wide range of models in both parallel group and cross-over designs
- The inclusion of interaction terms involving the treatment (e.g., treatment * centre, treatment * visit) is not allowed
- Based on the estimated model, the assumptions are checked (the significance levels used should not be considered as mandatory)
 - A. Absence of significant deviations from parallelism:** the p-value of the interaction product * log(dose) should be ≥ 0.1
 - B. Significant dose-response relationship:** the p-value of the slope for log-dose should be < 0.05
 - C. Absence of a significant difference between products:** the p-value of the product effect should be ≥ 0.05

The two-step analysis: step 1

- Attention must be paid when considering **assumption C** (absence of a significant difference between products)
 - Since the model includes the interaction term (i.e., non-parallelism is allowed), the estimate of the product effect and its significance **depend on the dose level** considered
 - We suggest to test the product effect
 - **at the two log(dose) levels** corresponding to the doses actually administered and
 - **at the mean log(dose) level**
 - In absence of significant deviations from parallelism, these three tests are expected to lead to consistent results
- In absence of evidence against the assumptions, we can proceed to **step 2**

The two-step analysis: step 2

- The same model used in step 1 is estimated **excluding the non-significant term for interaction**
- The following **assumptions** may be checked for confirmation
 - B. Significant dose-response relationship
 - C. Absence of a significant difference between products
 - This assumption can now be verified without taking into account particular dose levels, since the interaction term is excluded from the model and parallelism is assumed
- Then **log relative potency** can be estimated as the ratio between
 - the estimated product effect $a_R - a_T$ and
 - the estimated slope for $\log(\text{dose})$ b
$$R = (a_R - a_T) / b$$
- The confidence limits can be obtained based on the other results from the model

The %REL POT macro

```
%REL POT (data=, response=,  
          fixed_cov=, random_cov=, categorical_cov=,  
          alpha=) ;
```

- **data**: dataset to be analysed. The variables “product” (“T” or “R”) and “dose” (untransformed dose) are required

	subject	country	treatment	product	dose	baseline	y
1	1	3	T 50	T	50	3.71	6.47
2	149	2	T 200	T	200	6.96	10.01
3	229	2	R 50	R	50	5.8	6.95
4	315	3	R 200	R	200	7.37	9.24

- **fixed_cov**: variables to be included as fixed effects in the model
- **random_cov**: variables to be included as random effects in the model
- **categorical_cov**: categorical variables (fixed or random) included in the model
- **alpha**: $1-\alpha$ is the confidence level of the interval



The %RELPOD macro

- How the parameters involved in the point and interval estimation of the relative potency are derived in step 2?
- ODS table SolutionF** from the MIXED procedure

VIEWTABLE: Solution for Fixed Effects								
	Effect	product	country	Estimate	Standard Error	DF	t Value	Pr > t
1	Intercept			0.2086	0.3493	394	0.60	0.5508
2	product	R	$a_R - a_T$	-0.04660	0.09656	394	$f = 0.48$	0.6296
3	product	T		0	.	.	.	.
4	logdose		b	0.4232	0.06985	394	6.06	<.0001
5	baseline			1.0291	0.02402	394	42.84	<.0001
6	country		1	-0.1929	0.1174	394	-1.64	0.1012
7	country		2	0.08306	0.1193	394	0.70	0.4867
8	country		3	0	.	.	.	.

The %RELPOUT macro

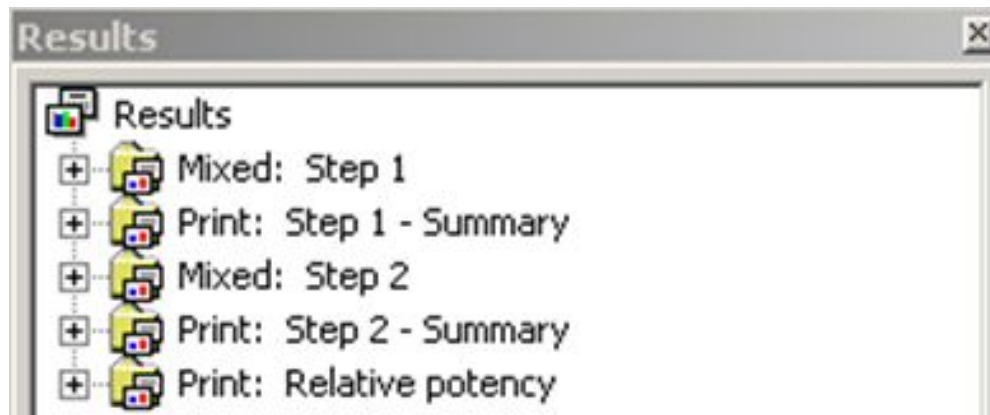
- **ODS table CovB** from the MIXED procedure

VIEWTABLE: Covariance Matrix for Fixed Effects									
	Row	Effect	product	country	Col1	Col2	Col3	Col4	Col5
1	1	Intercept			0.1220	-0.00493	0	-0.02178	-0.000002
2	2	product	R		v_{11}	0.009324	v_{12}	-0.000002	0.000000
3	3	product	T		0	0	0	0	0.000000
4	4	logdose			-0.02178	-0.000002	v_{22}	0.004879	-0.000002
5	5	baseline			-0.00227	0.000065	0	-0.00016	0.000000
6	6	country		1	-0.00788	-0.00027	0	0.000256	-0.000002
7	7	country		2	-0.00867	0.000419	0	0.000063	0.000000
8	8	country		3	0	0	0	0	0.000000

The %REL POT macro

```
%REL POT(data=example,response=y,  
          fixed_cov=baseline country,random_cov=,  
          categorical_cov=country,  
          alpha=.1);
```

- The **output of the MIXED procedure** and a **summary of the relevant results** are presented for both steps and the **estimate of relative potency** is finally printed with its confidence interval



The %RELPOt macro

Step 1 - Summary

Assumption	Effect	logdose	p	Note
Parallelism	logdose*product	.	0.3348	Non-significance required
Dose-response	logdose	.	<.0001	Significance required
Difference between products	product	3.91	0.3064	Non-significance required
Difference between products	product	4.61	0.6291	Non-significance required
Difference between products	product	5.30	0.7332	Non-significance required

Step 2 - Summary

Assumption	Effect	p	Note
Dose-response	logdose	<.0001	Significance required
Difference between products	product	0.6296	Non-significance required

Relative potency

Relative potency	Lower confidence limit	Upper confidence limit
0.89573	0.60006	1.31471



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Any questions?