Bivariate Dose-Response for Quantitative Endpoints

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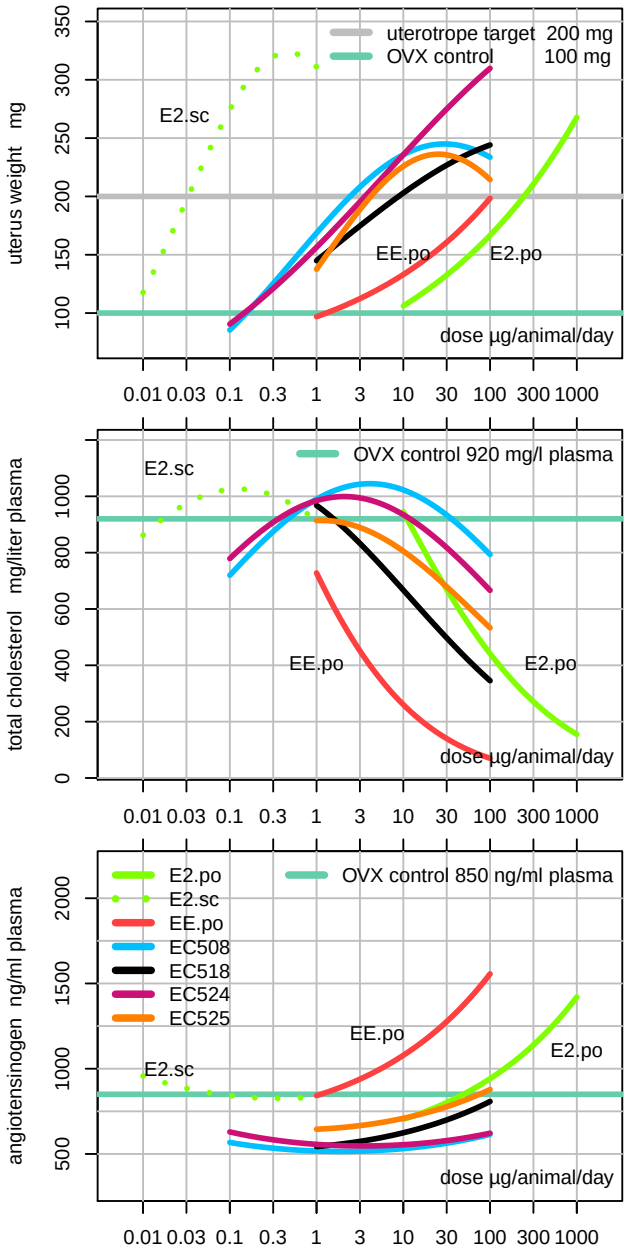
Agenda

Classical and new concepts for bivariate dose–response

- Screening for uterotrope and hepatic effects of estrogen based substances
- Basics
- Models
- Relative efficiency
- New locally fitting model
- Analysis without model function
- Selected results of screening experiment

Screening of new estrogen based prodrugs



Dose-Response for Uterus, Total Cholesterol and Angiotensinogen

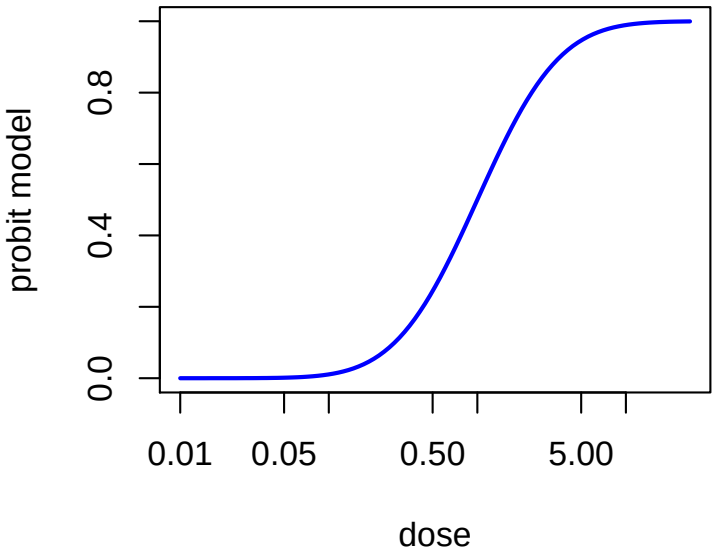
- uterotrope effect intended *benefit*
- hepatic effects adverse *risk*
- wide dose range: five orders of magnitude
- 3-4 doses, n=5-6 rats per dose
- heterogeneous response patterns
- apparent hormesis for some candidates

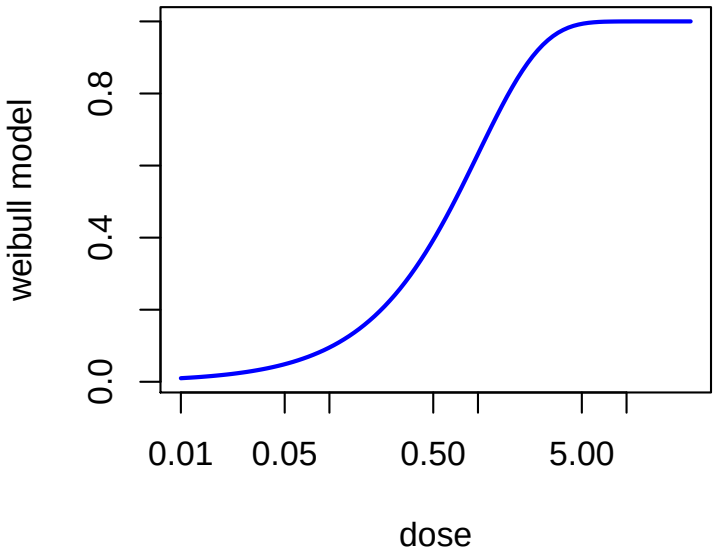
Classical Approach (univariate) Quantal Response

- data: triples d, n, y for a number of increasing doses d
- response: binary or binomial $Y(d) \sim B(n, p(d))$, where p denotes probability of a reaction
- model: $EY/n = P(reaction|d) = F(d)$ with a distribution function F.
- common choice: F belongs to a location scale family, sometimes extra modeling of skewness
- $\log(d)$ instead of dose is most often used: $F(d) = F(\alpha + \beta \log(d), \gamma)$, extra parameter for skewness
- most prominent: probit, logit, weibit
- 2-3 parameters, symmetric, monotonous

Quantal Response Models

Probit as 2 parameter, Weibull as 3 parameter model.

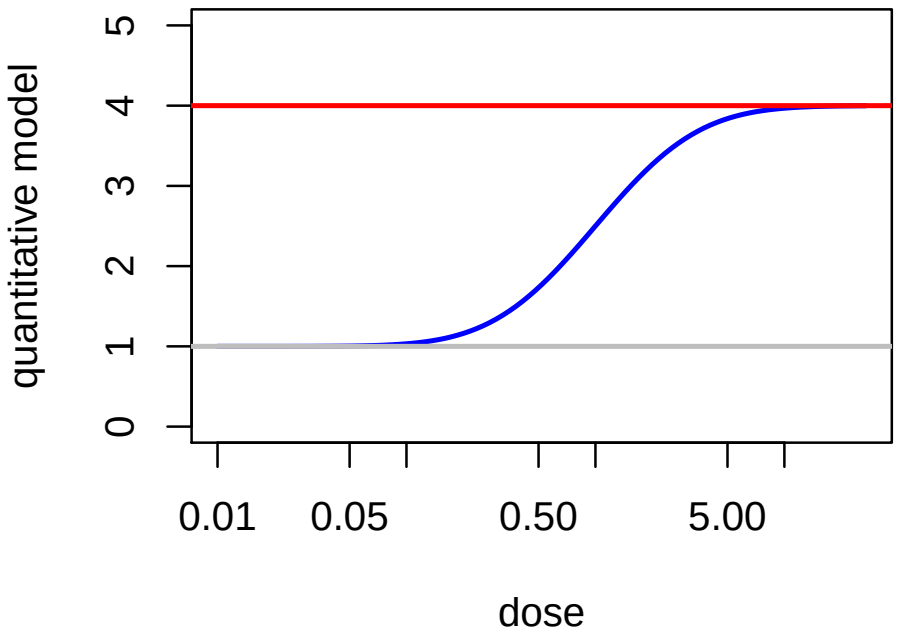




Classical Approach Quantitative Response

Monotonicity and limiting behaviour assumed

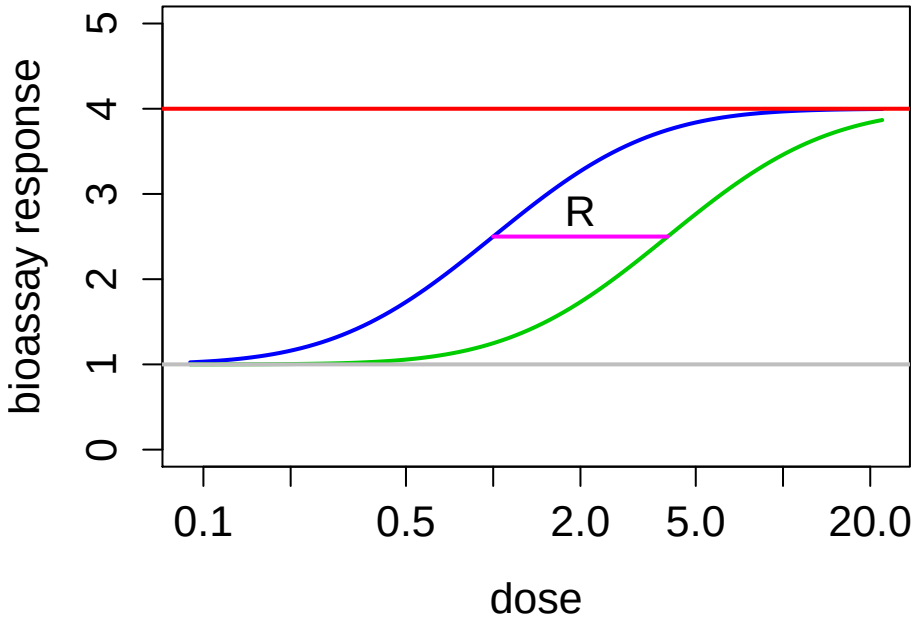
- data: measurement y for each of n subjects per d
- model: $EY = \theta_{low} + \Delta F(\alpha + \beta \log d, \gamma)$
- parms: $\Delta = \theta_{high} - \theta_{low}$



often negative and positive controls are included

The Bioassay – Concentration of a Dilution

Analyse two preparations of a test substance – e.g. digitoxin, where one substance can be regarded as dilution of the other.
$d^{(2)} = R \times d^{(1)}$ assay is used to determine the dilution factor R



Plot of dose response curves for two substances. Curves parallel on log scale.
Estimate horizontal shift in $\log d$. Use as estimate for $\log R$.

Relative Efficiency – Bioassay like

Often substances are compared as if they would behave like dilutions in a bioassay. Factor R is called relative efficiency.
Steps of analysis necessary:

- fit models
- check for identical slope β, (shape γ)
- check for identical asymptotes $\theta_{low}, \theta_{high}$
- estimate $\log R$
- calculate approximate variance using delta-method or use Fieller's method for constructing confidence intervals.

Dose-Response – Two Endpoints

For pharmaceutical products we consider an efficacy endpoint (benefit) and one for unintended toxic response (risk). Quantitative comparison of two substances can be made by combining results.

- determine $R_{benefit}, R_{risk}$ if possible
- the product determines the therapeutic window $R_{ben/risk} = R_{benefit} \,/\, R_{risk}$
- compute variance on log-scale.

Inference is straightforward if two independent dose-response experiments are available, otherwise use the correlation of residuals in order to assess variance.

Analysis in a Non-Perfect World

For dose-response studies there is no law, no guaranty, that

- monotonicity holds
- curves are parallel
- asymptotic behavior is identical
- enough dose levels are available in order to fit five parameter models

Therefore, simple and flexible, locally fitting models are proposed. In case of non-parallel curves, comparison is restricted to given fixed target values.

Addressing Violation of Assumptions – a New Very Flexible Approach

Instead of using a linear approximation of a suitable transform of the dependent variable in log *dose*, we consider a quadratic approx.:

$$E \log y(d) = \alpha + \beta \log d + \gamma (\log d)^2$$

This is a very flexible function. Could be generalized using simple splines, sparse in parameters. Here the back-transformed form

$$\exp\{E \log y(d)\} = A \times d^{\beta} \times d^{\gamma \log d}$$

$\log y$ as Response – Left Hand Side Transformation

For bivariate dose response, log transformation of response appears reasonable. Ratios might be of interest.

- if $\log y(d) \simeq$ normal, this is perfect. Concentrations often behave like this.
- if $y(d) \simeq$ normal and $\sigma_{y(d)} << \mu_{y(d)}$ then also $\log y \simeq$ normal holds true.
- log is a rather useful transformation when looking for inference on ratios as all computations on log scale are linear then.

Fisher (1947) used the log transformation investigating the relation of heart- and body weight of male and female cats.

Screening Study – Biological Background

Oral application of estrogen

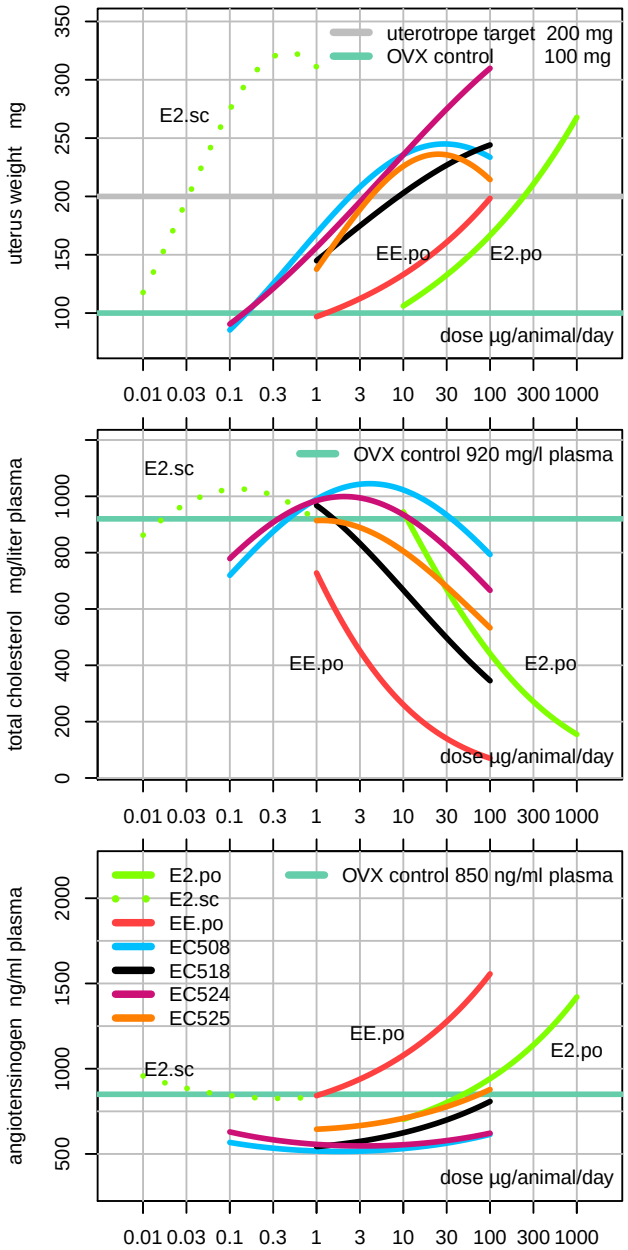
problems

- free estrogen (E2) in plasma is almost totally metabolized ($> 99\%$) at every passage through the liver
- metabolism leads to a decrease of total cholesterol and an increase of angiotensinogen – both indicators of severe side effects

the prodrug approach to solution (simple mind biometricians view)

- E2 molecule is modified by a new ligand
- ligand enables a strong and specific binding to a receptor within erythrocytes
- liver passage therefore in a *safe shelter*
- release of E2 scalable by strength of binding of ligand to E2 molecule.

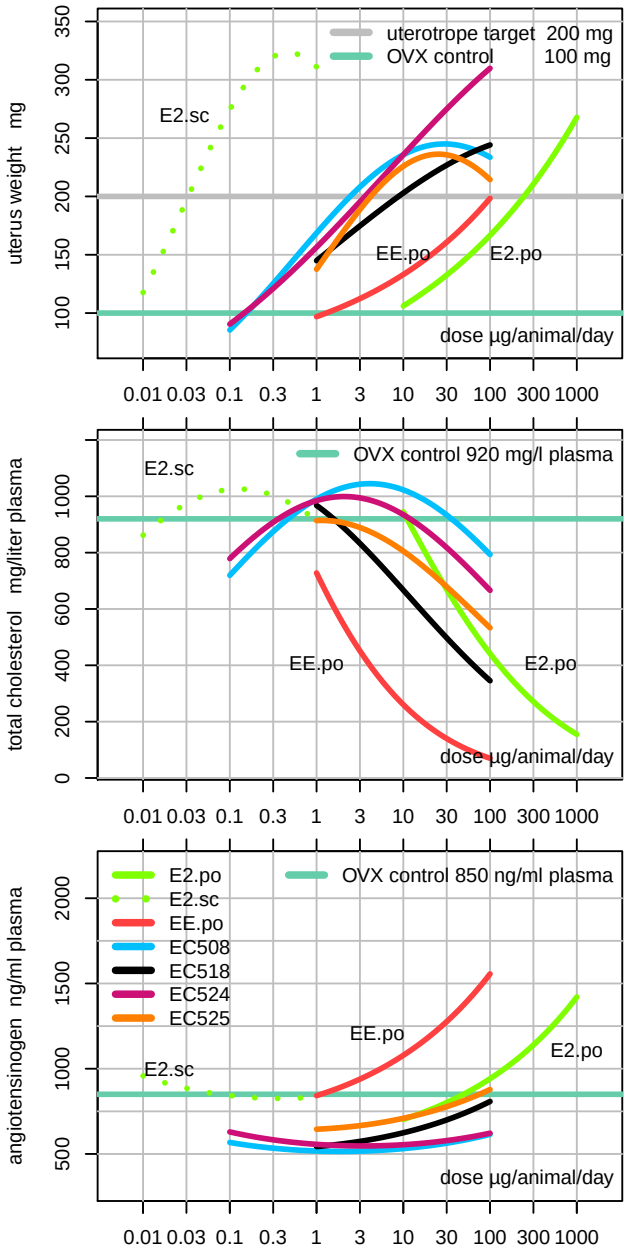
Screening Study – Statistical Analysis



Dose-Response for Uterus, Total Cholesterol and Angiotensinogen

- Fit of second degree polynomials in log d per substance.
- Simplifications result in highly significant loss of fit.
- Delta method used for estimating standard deviations(ut.w). For hepatic effects sds directly from fit.
- Three parameter fit corresponds to saturated model. With four doses sufficient fit was reached.

Screening Study – Selected Results



Dose-Response for Uterus, Total Cholesterol and Angiotensinogen

hepatic activity: uterus weight 200 mg

- total cholesterol
  prodrugs behave like E2.sc (no significant differences)
  EC518 even better
- angiotensinogen
  all prodrugs showed significantly lower values than E2.sc and were all below the OVX reference
  → hormesis
- no relevant violations of assumptions needed for valid statistical inference

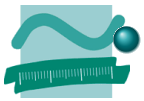
An Approach Without a Dose-Response Model – The Bland Altman Way

Bland and Altman (1986) made a simple but extremely efficient contribution to appropriate comparisons of different methods of measurement.

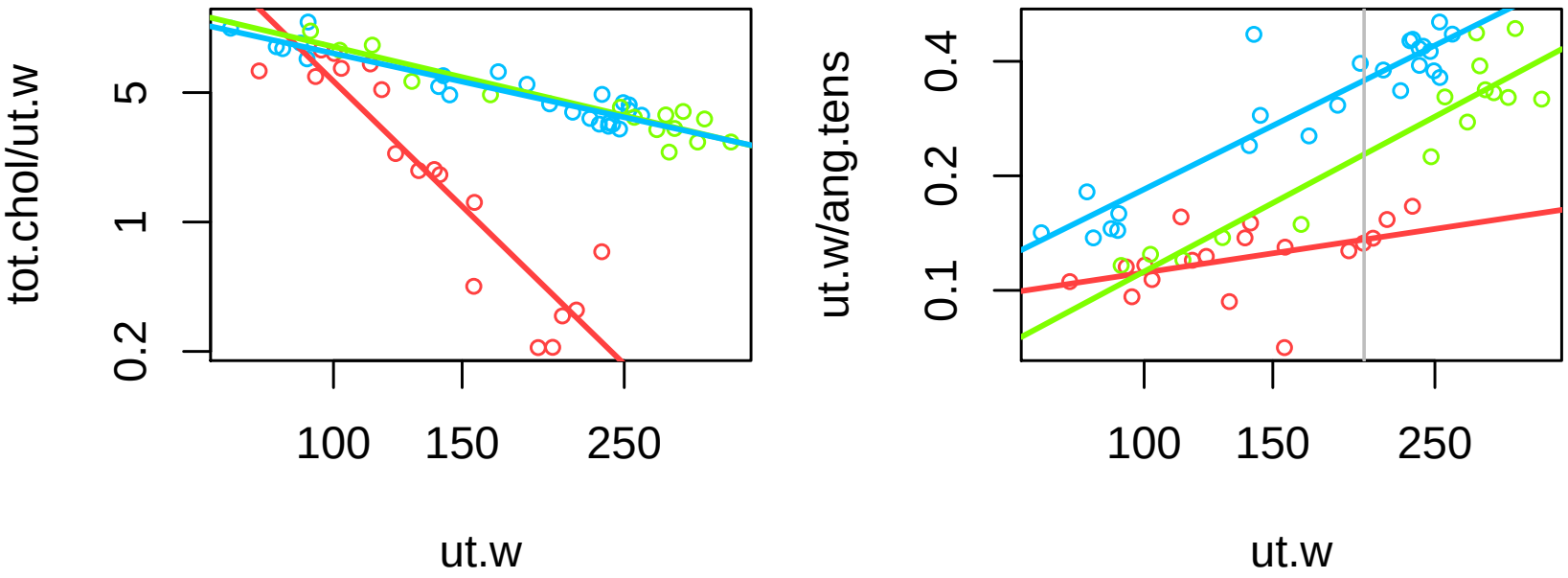
- the idea: display differences $M_2 - M_1$ versus mean $(M_1 + M_2)/2$ or a gold standard
- statistical background: if M_1 and M_2 are bivariate observations and $Var(M_1) = Var(M_2)$ holds true, then difference and mean are uncorrelated.
- violations, and more can be detected in the Bland Altman plot.

Application of principle to bivariate dose response

- select a *beneficial* and a toxic *risk* endpoint.
- display benefit-risk ratio versus benefit, both on log-scale
- compare benefit-risk ratio relations of different substances



Screening study – selected comparisons



Benefit-risk ratios in log-log display. Accidently, the relation appears linear. For total cholesterol decreasing behavior, for angiotensinogen increasing behavior.

Remark: Comparison of substances possible over the whole range of bebeficial target values possible

References

Bland JM, Altman DG. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. Lancet, i, 307-310.
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R. A. Fisher (1947) The analysis of covariance method for the relation between a part and the whole, Biometrics 3, 65–68.