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State of the art in dose-response modelling

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HERBA INUTILIS NON FACILE PERIT

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Before Paracelsus

- Noah sent forth the dove which returned with an olive leaf and Noah knew the water had abated, the endpoint being the olive leaf (quantal response)
- Later numerous kings used cupbearers to avoid poisoning, the endpoint being the survival/health of the cupbearer (quantal / quantitative response)

Paracelsus

What is not poisonous:
everything is a poison
and nothing is inert.
The dose alone decides whether a thing
is a poison or not

Paracelsus (1494-1541)

After Paracelsus

- Modern society produces countless biological compounds
- We need sophisticated bioassay protocols to ensure compounds work as intended without unintentionally harming the environment, man and beast
- Erlich's standardization of *diphtheria* antitoxin in late nineteenth century
- Standardization in pharmacology, toxicology and pesticides becomes crucial
- Bioassay and dose-response curves are like two peas in a pod
- Some of the pioneers
  - Bliss C.
  - Finney, J
  - Jerne, H.K
  - Plackett R.L & Hewlett P.S.

An opinion

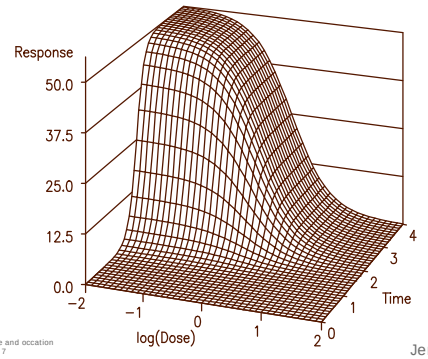
"... biological assay appears to have somewhat specialised appeal. Although few statisticians have worked on it intensively, to the majority is appears as a topic that can be neglected, either because of its difficulties or because it is trivial... Despite this ... many features of bioassay are outstanding good for concentration the mind on important part of biometric practice and statistical inference."

Finney 1979

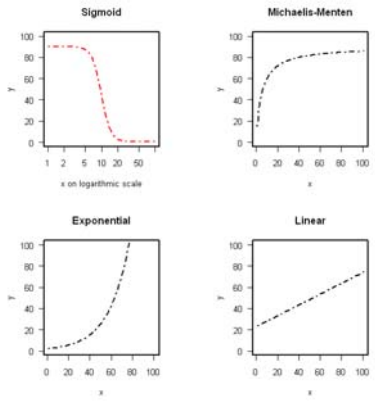
Dose-response data:

- **Dose**: A concentration of a chemical that might evoke a reaction in living organism
- **Response**: biological reaction to a given dose (also called: endpoint or outcome or y)
- **Types of response:**
  - Continuous
    - biomass, length, enzyme activity
  - Discrete/quantal
    - Binary (dead/alive, immobile/mobile)
    - Counts (number of offspring, colonies)
    - Multinomial (severity of damage)
    - Survival (time to failure)

Dose-response curves give only a snapshot, and in living organism developmental stage is crucial for a result



Jensen, 1995



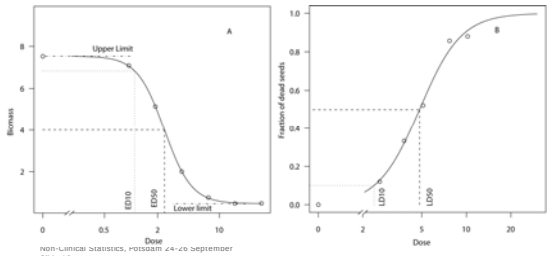
Parametric models

1. Logistic and log-logistic
   - Log-logit (logistic regression)
   - Michaelis–Mentens
2. Log-normal
   - Log-probit
3. Weibull-1
   - Exponential log-log link
4. Weibull-2
   - Complementary log-log link, first order multistage

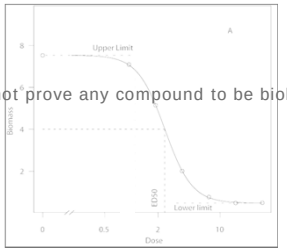
The natural hierarchical structure among models above is very convenient as models that are special cases inherit most characteristics from the parent model. **They all have parameters that are easily biologically interpretable.**

Others
- Inverse j-shaped/u-shaped hormesis models (models for initial adverse response stimulation)

Sigmoid curve (log-logistic)



Dilemma with dose-response curves



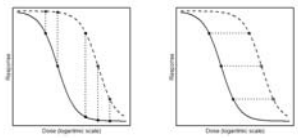
We cannot prove any compound to be biologically inert

Dose-response vs ANOVA

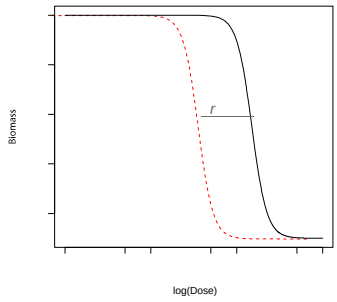
The advantage of using dose-response regression instead of the unfortunately popular of analysis of variance is that you can compare potency with different choice of dose range as long as dose-ranges give a reasonable presentation of the response range. Used to assess:

No Observable Effect Levels (NOEC) and Lowest Observabel Effect Levels (NOEC) ...

Dose-response curves can be compared, e.g., at EDx levels, say ED50, even though the dose ranges are different and the assays have been repeated in time and space with different doses.



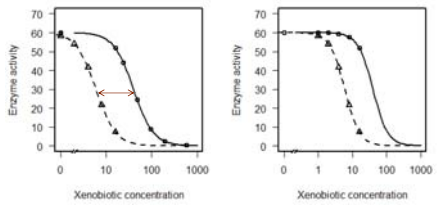
Similar curves (analytical assays) are a special case



Analytical vs comparative assay
(Jerne & Wood 1949 and Finney 1978)

1. Analytical assays, similar curves for standard and test preparation
   - Substitute for chemical analyses
   - Site of action studies: It is a necessary, but not a sufficient conditions that dose-response curves are similar in all parameters except this that moves it horizontally on the dose-axis
2. Comparative assays, no need for similar curves, even though it would be nice and makes interpretation easy
   - Enzyme inhibition
   - physiological effect
   - effect on the whole organisms

Resistance to xenobiotics by comparing EDx



Philosophy of the R add-on package *drc*

- Self starters
- After fitting
- Choice of distribution

Towards a unified approach to dose-response analysis 1

- Use Self starter
- Engine function drm(y~x, fct=....)
  - Various functions (fct=)
  - Log-logistic (LL.)
  - Logistic (L.)
  - Weibulls (W1. or W2.)
  - Michaelis-Mentens (MM.)
  - Exponential (EXD.)
  - Non monotomeously decreasing/increasing curves (BC.)
  - **Interface much like lm() and glm() in R**
- One or more curves fitted separately or simultaneously
- Parameter constraints possible

Towards a unified approach to dose-response analysis 2

- Responses
  - Continuous
  - Counts
  - Quantal
- After fitting functions
  - ED() calculate effective dose (EDx, LDx)
  - SI() relative potency at any one EDx
  - ..more
- Graphics: plot.drc()

One Curve

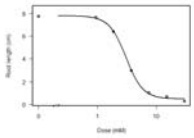
```
library(drc)
ryegrass.m1 <- drm(rootl ~ conc, data = ryegrass, fct = LL.4())
```

$$y = c + \frac{d-c}{1+\exp[b\cdot(\log(x)-\log(e))]}$$

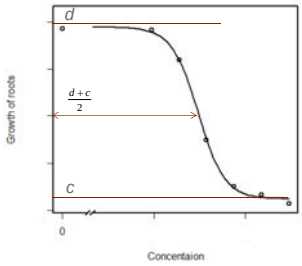
```
summary(ryegrass.m1)
```

```
Parameter
estimates:    Estimate Std. Error t-value      p-value
b:(Intercept) 2.98222 0.46506     6.41251      0.0000
c:(Intercept) 0.48141 0.21219     2.26876      0.0345
d:(Intercept) 7.79296 0.18857     41.32722     0.0000
e:(Intercept) 3.05795 0.18573     16.46440     0.0000
```

```
plot(ryegrass.m0, broken=TRUE, xlab="Dose (mM)", ylab="Root length (cm)")
```



The dose-response curve



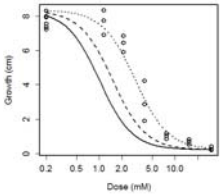
$$y = c + \frac{d-c}{1+\exp[b\cdot(\log(x)-\log(e))]}$$

Self- starter

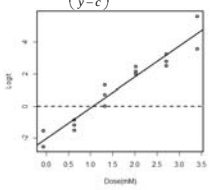
Self-starter functions render estimation in nonlinear regression almost as easy as for estimation of linear regressions.

Guestimate vs Self-starter

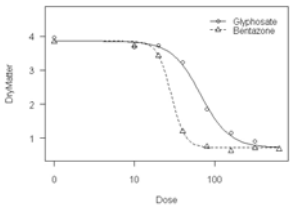
$$y = c + \frac{d-c}{1+\exp[b\cdot(\log(x)-\log(e))]}$$

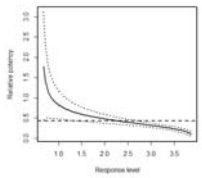


$$\log\left(\frac{d-y}{y-c}\right) = a + b\cdot\log(x)$$



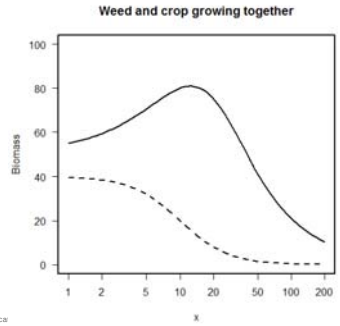
More curves and relative potency



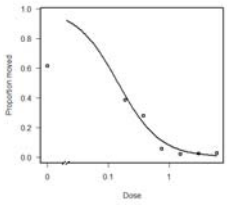


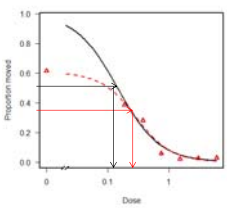
If not similar no constant relative potency

Monotome and non-monotome curves

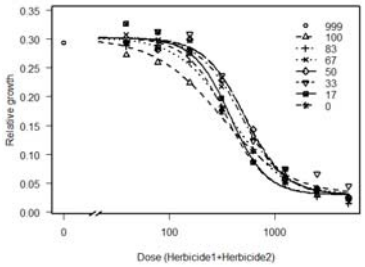


Earthworm avoidance test (Binomial data)





Do acifluorfen and diquat enhance the effect on *Lemna minor*?



A ray design

Mixtures

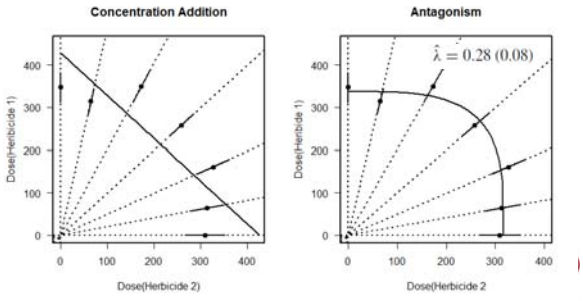
- Concentration Addition (CA)
  - At any response level:

$$\left(\frac{z_1}{Z_1}\right)^{1/\lambda_1} + \left(\frac{z_1}{Z_1}\right)^{1/\lambda_2} = 1$$

- Model functions for the universal response surface approach (URSA) for the quantitative assessment of drug interaction
  - provide a parametric approach for modeling the joint action of several agents. The model allows quantification of synergistic effects through a single parameter.

Isoboles tested against each other

$$\left(\frac{z_1}{Z_1}\right) + \left(\frac{z_1}{Z_1}\right) = 1 \qquad \left(\frac{z_1}{Z_1}\right)^{1/\lambda} + \left(\frac{z_1}{Z_1}\right)^{1/\lambda} = 1$$



Toxicology and Ecotoxicology

Model diagnostics

- Mean structure
  - Plots of fitted regressions
  - Residual plots
  - Lack-of-fit
- Variance homogeneity
- Build in transform-both-sides
- Normal distribution
- Independence
- Preparation of doses in dilution series
  - Additive and multiplicative effects of variations in the dose

Other important aspect?

- Protocol?
- Distribution of doses?
- Equidistant dose distribution or decided upon a part of the curve
- Number of replication vs number of doses?

Applications

- **Screening of Drugs**
  - Analysis of high-throughput dose-response data
- **Toxicology and Ecotoxicology of Xenobiotic**
  - Estimation of effect concentrations (e.g. EC/ED/LC/LD50)
  - Selectivity, joint action of mixtures *in vivo* and *in vitro*
- **Weed Science and plant Ecology**
  - Modeling herbicide selectivity, seed germination, yield loss and competition among weeds and crops

A "random" sample of use of *drc*

Ritz C & Streibig JC. Bioassay analysis using R. *J. Stat. Soft. 2005;12:1-22.*

Ritz C. Toward a unified approach to dose–response modeling in ecotoxicology. Environmental Toxicology and Chemistry 2010; 29: 220–229.

Bozic, D. Resistance of sunflower hybrids to imazamox and tribenuron-methyl. 2012; 39: 1-10

Kniss AR. Nonlinear Regression Analysis of Herbicide Absorption Studies. Weed Science. 2011; 59: 601-610.

Wang Y et al. A Grid Algorithm for High Throughput Fitting of Dose-Response Curve Data. Curr Chem Genomics. 2010; 4: 57–66.

Defawe OD. Optimization and qualification of a multiplex bead array to assess cytokine and chemokine production by vaccine-specific cells. Journal of Immunological Methods 2012; 382: 117–128

Freyberger A. Assessment of a recombinant androgen receptor binding assay: Initial steps towards validation. Reproductive Toxicology 2010; 30: 2–8

Bjergager, M et al. Synergy in microcosms with environmentally realistic concentrations of prochloraz and esfenvalerate. Aquatic Toxicology 2011; 101 : 412-422

List of wishes

- Bootstrap and other types of confidence intervals
- Extending mixed model capabilities
- Handling other types of response
- Robust starting value procedures
- Automation in R & D of pesticides in laboratory and field
- Graphic interface in teaching and research
- **Demystify nonlinear regression for the end user**

Thank you for your attention