Ecotoxicology 2.0
Modelling to better evaluate

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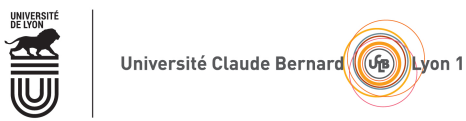


Table of content

Table of content

Ecotoxicology

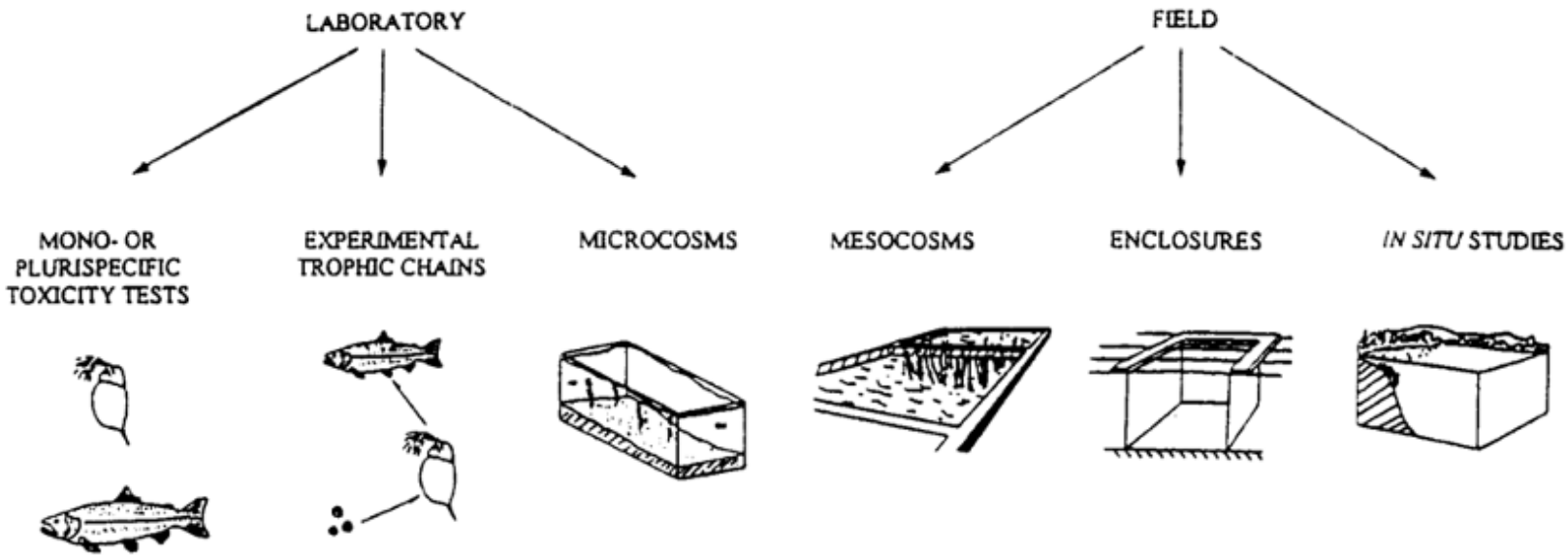
- A scientific field at the bridge of chemistry, toxicology and ecology

  The branch of toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animals (including humans), vegetables and microorganisms, in an integrated context [Truhaut, 1977]

  Ecology in the presence of toxicants [Chapman, 2002]

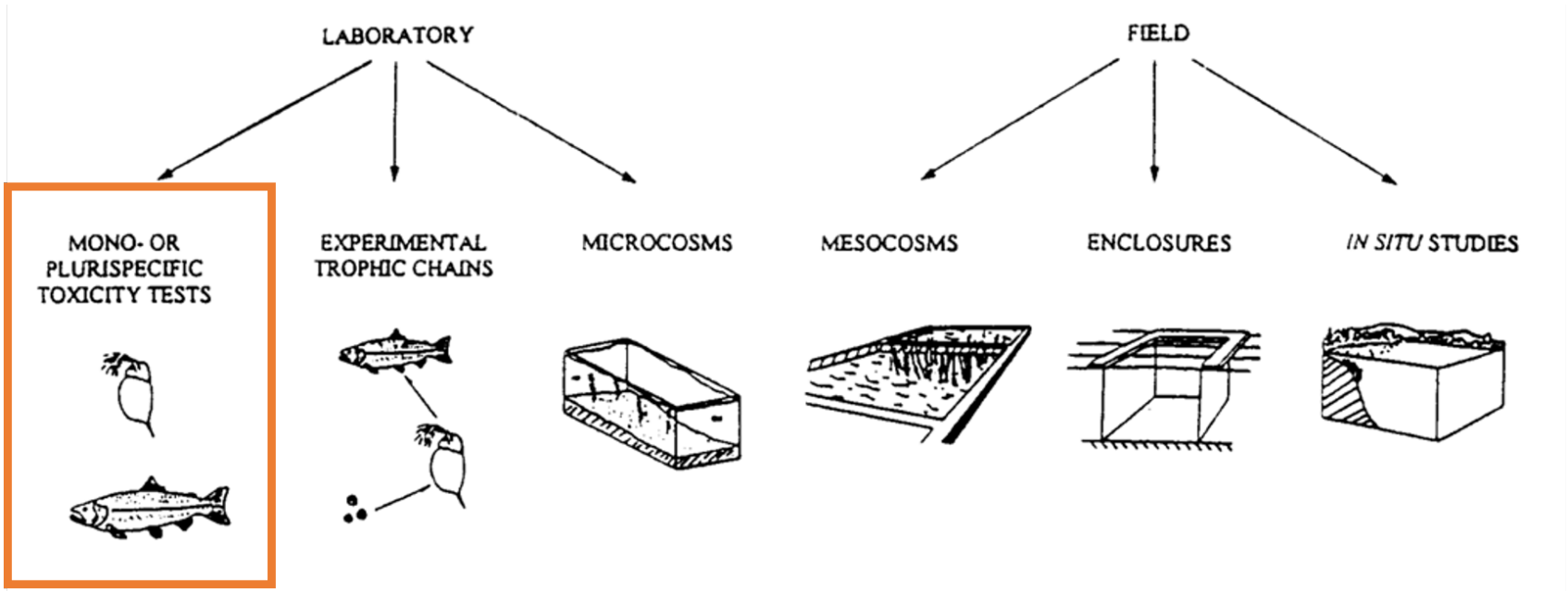
- In ecotoxicology, the answer of the ecosystem to environmental perturbations (physical, chemical and/or biological) is studied in all compartments of the biosphere (air, soil and water) and at all levels of biological organization [Walker et al., 2006]

A variety of experimental devices



[Caquet et al., 1996]

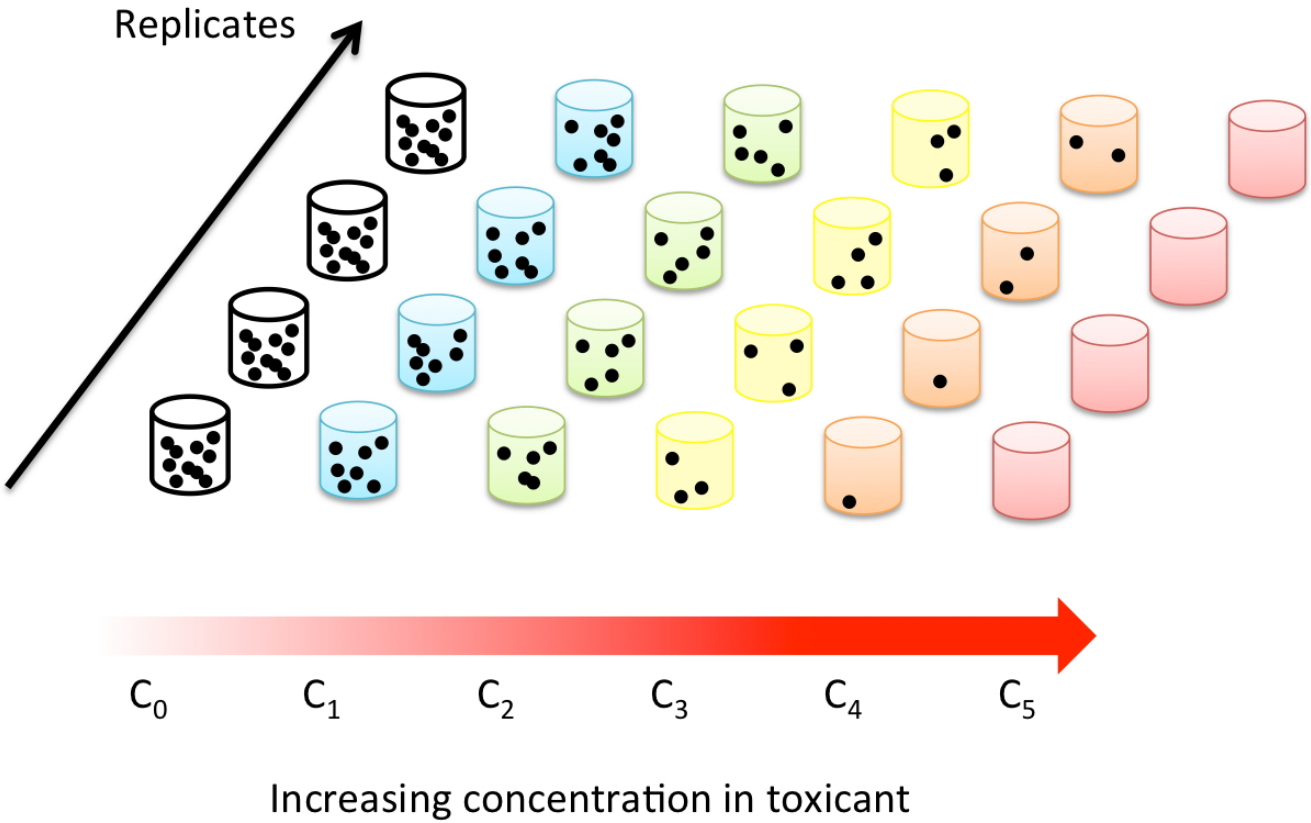
A variety of experimental devices



[Caquet et al., 1996]

Standard experimental design

Under standardized protocols, individuals are counted **over time**, that is at regular time points.
Endpoints can be survival, growth and/or reproduction for example.



Example of a toxicity test

Daphnia magna, acute immobilisation test (OECD 202, 1984) and chronic reproduction test (OECD 211, 2012)

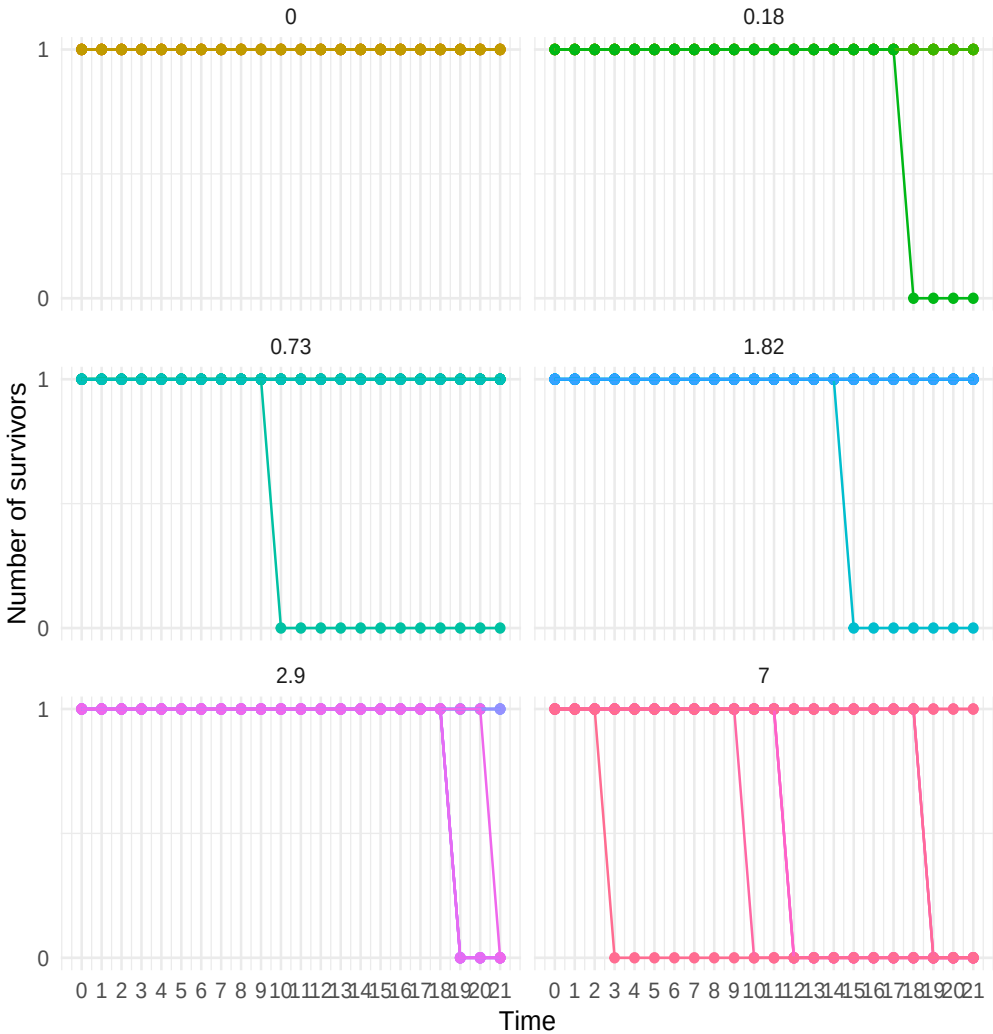
Daphnia magna

Acute test: the number of immobile daphnids is determined for each concentration at 24 and 48 hours.

Chronic test: offsprings are daily counted during 21 days.

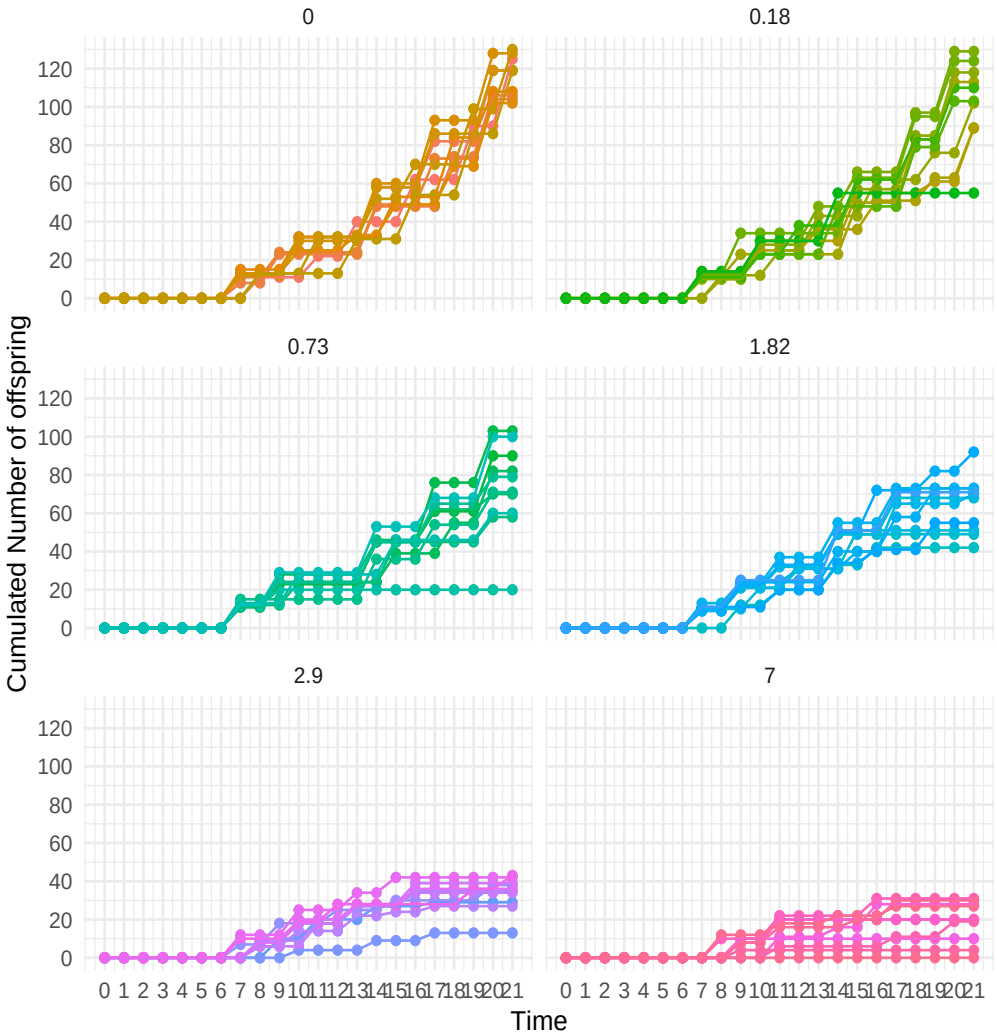
Example of survival data

Effect of chlordane on *D. magna* survival during 21 days (10 replicates of 1 individual):



Example of reproduction data

Effect of chlordane on *D. magna* reproduction during 21 days (10 replicates of 1 individual):



NOEC/LOEC: severely criticized for multiple reasons

Shortcomings

- necessarily one of the tested concentrations (hence strongly dependent on the experimental design);
- based on a wrong interpretation of the p-value (*absence of evidence is not evidence of absence*);
- strongly dependent on the sample size
  $\rightarrow$ unprotectrive with small sample sizes: the lower the sample size, the higher the NOEC;
- cannot always be determined (e.g., if the first concentration leads to a significant difference);
- no uncertainty limits are associated.

x% effective or lethal concentrations (EC_x/LC_x)

Alternative to the NOEC, EC_x/LC_x are now **strongly recommended**.
$\rightarrow$ obtained by fitting a dose-response model to toxicity test data at a chosen target time point, then deriving the dose which corresponds to a given effect level (usually x =10, 20 or 50%).

Advantages

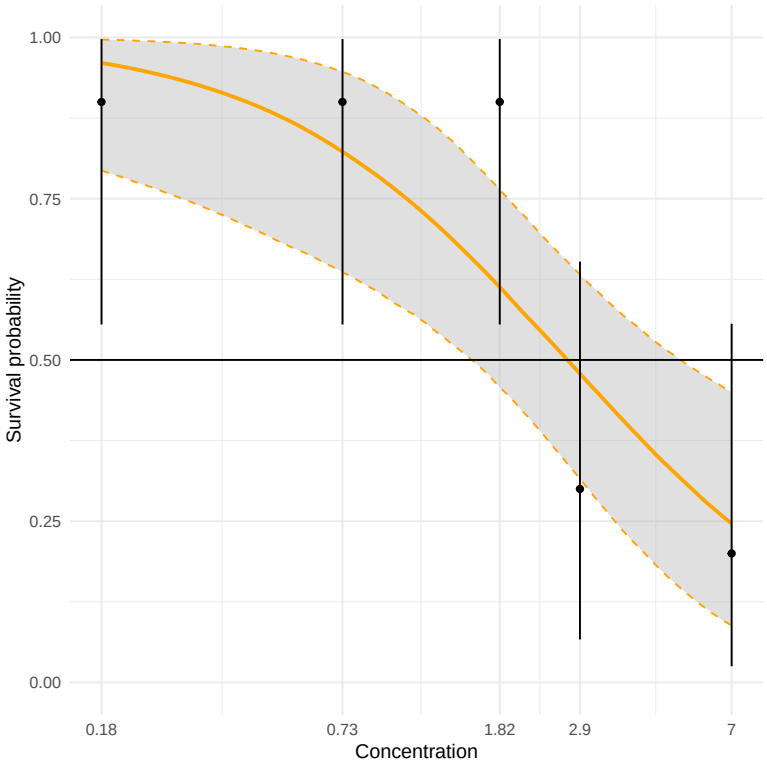
- capture and account for the whole dose-response curve;
- slightly dependent on the experimental design;
- may be associated to uncertainty limits.

Shortcomings

- sometimes technical difficulties when fitting;
- choice of a model;
- choice of an effect level x;
- choice of the exposure duration.

Example of LC_x estimation

Use of survival data at the end of the experiment (day 21)



	Median	2.5%	97.5%
LC_5	0.22	0.0074	0.71
LC_{10}	0.41	0.033	1.04
LC_{20}	0.82	0.16	1.6
LC_{50}	**2.67**	**1.50**	**5.3**

Table of content

(Dose or Concentration)-(response or effect) relationships?

A few vocabulary:

- **Dose** refers to the internal concentration, *i.e.*, the amount of toxicant **within** the body of organisms. But in ecotoxicology, only the **exposure concentration** is usually known.
  $\rightarrow$ We rather speak about **concentration**-response or effect relationships.
- Concentration-**response** relationships refer to the link between the exposure concentration and the proportion of individuals responding with an all-or-none effect.
- Concentration-**effect** relationships refer to the link between the exposure concentration and the magnitude of the induced biological change, measured in appropriate units.

What is a concentration-response/effect relationship?

A **concentration-response/effect relationship** is a simple X-Y graph relating increasing levels of exposure (X) to the response/effect (Y) **at a certain exposure time**.

Examples of **responses**:

- **Quantal** (or binary) data, expressed as proportion or probability (*e.g.*, mortality or immobilization).

Examples of **effects**:

- Ordered descriptive categories (*e.g.*, severity of a lesion);
- **Counts** (or discrete) (*e.g.*, reproduction products like eggs or clutches);
- **Continuous** measurements (*e.g.*, body size).

What is a regression model to be fitted on data?

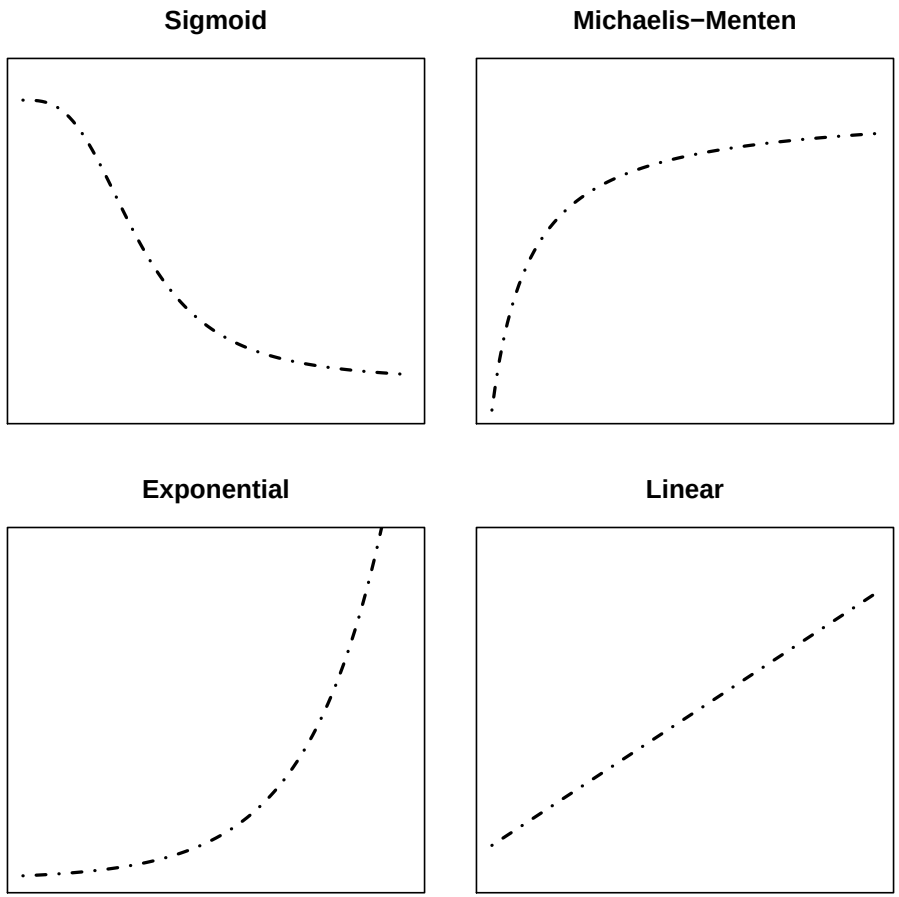
From concentration-response/effect experiments, if there is a reasonable number of concentrations (usually ≥ 5) of the toxicant and a reasonably well-behaved response/effect, it is straightforward to **fit a regression model**.

A regression model relating a dependent variable Y (the response or the effect) to an explanatory variable X (the concentration) is composed of two parts:

1. a **deterministic part**, which describes the mean value (or curve) (*e.g.*, a log-logistic model);
2. a **stochastic part**, which represents the distribution around the mean curve (*e.g.*, a normal distribution).

$\rightarrow$ Each part depends on the nature of data to analyze.

Four shapes to describe dynamic in life science

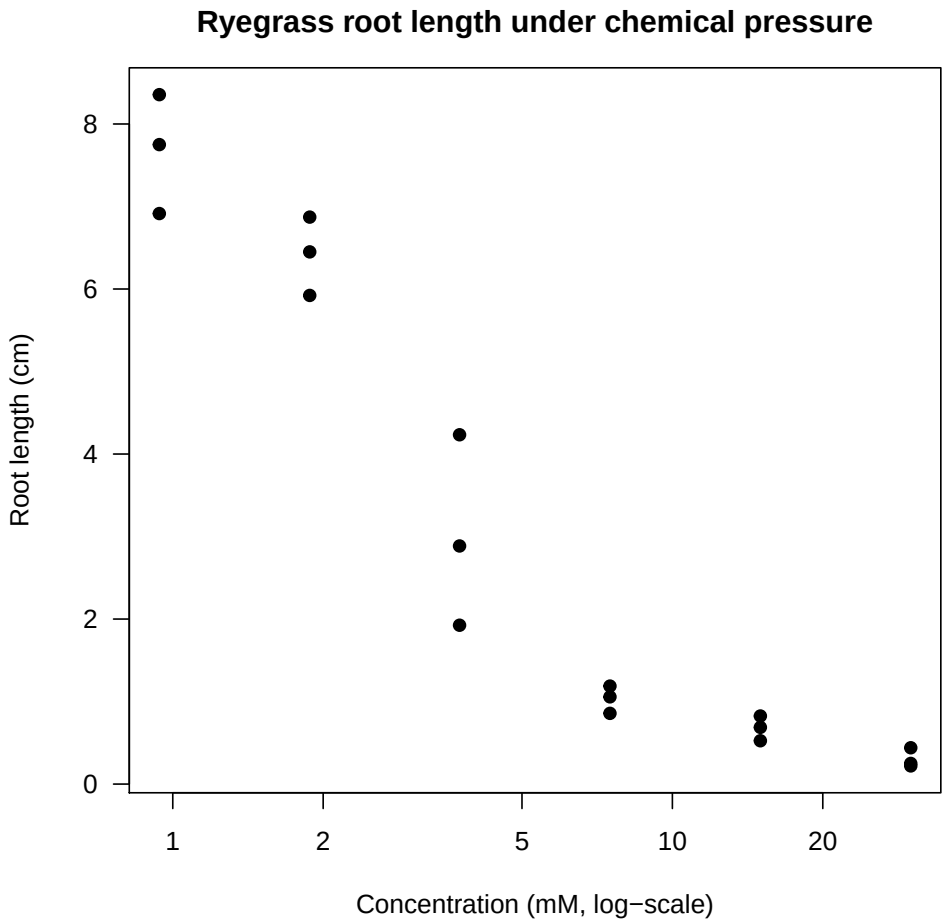


From http://bioassay.dk/bioassay/.

Example of **sigmoidal** relationship

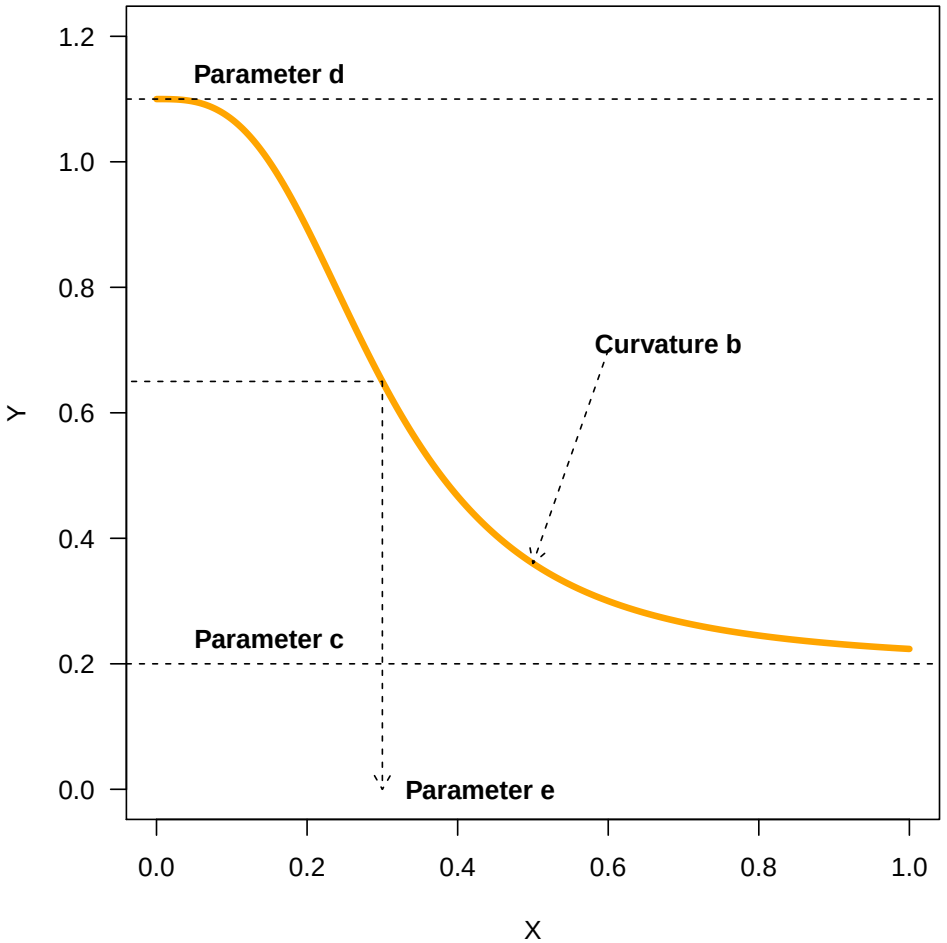
Data from a single dose-effect relationship between root lengths of perennial ryegrass (*Lolium perenne* L.) and concentration of ferulic acid.

$(n = 24)$



The log-logistic model - Graph

$b = 3, c = 0.2, d = 1.1$ and $e = 0.3$



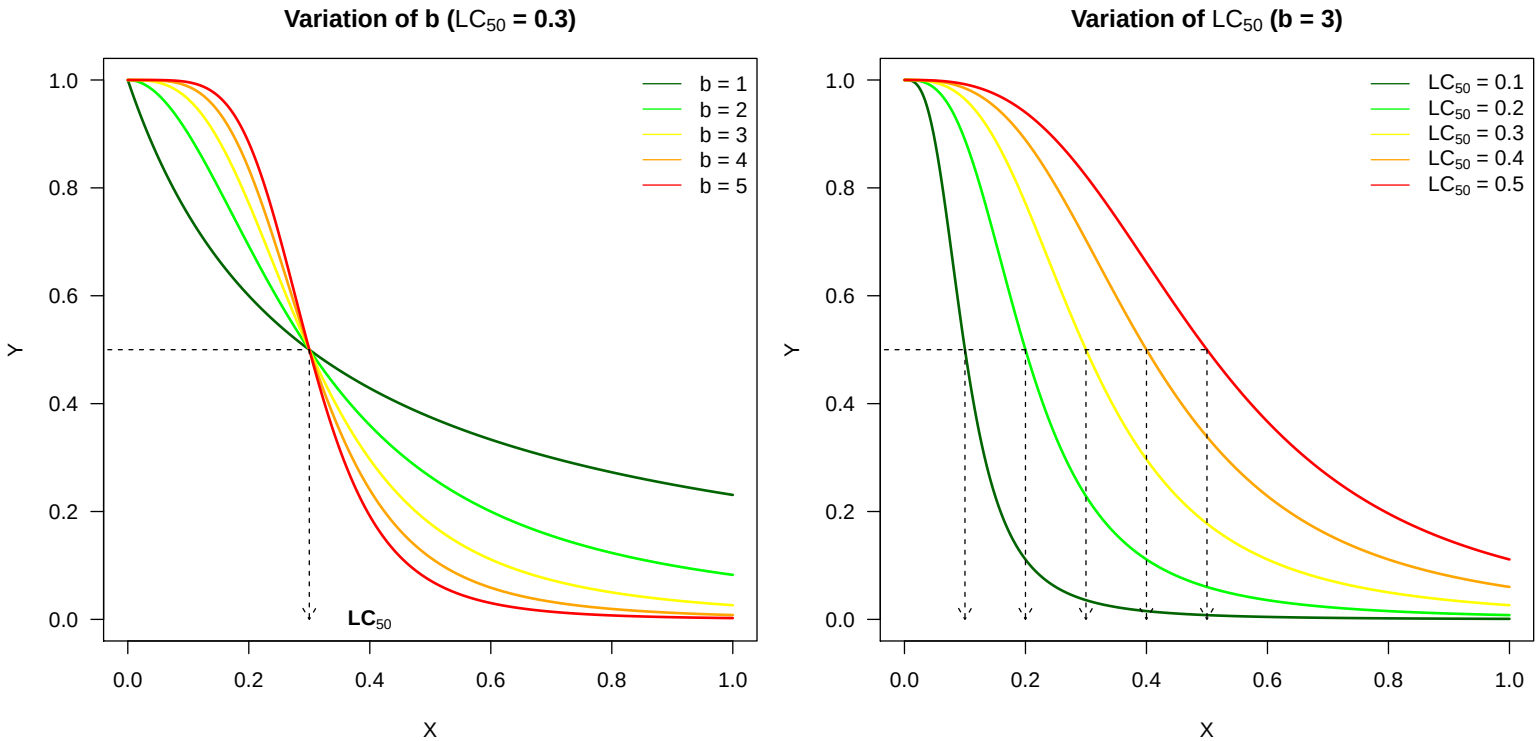
$$Y = c + \frac{d - c}{1 + (\frac{X}{e})^b}$$

$e = EC_{50}$ or LC_{50}
(here, in arbitrary unit)

For **survival**, d is the natural mortality (may be fixed to 1) and c is fixed to 0.

The log-logistic model - Morphology

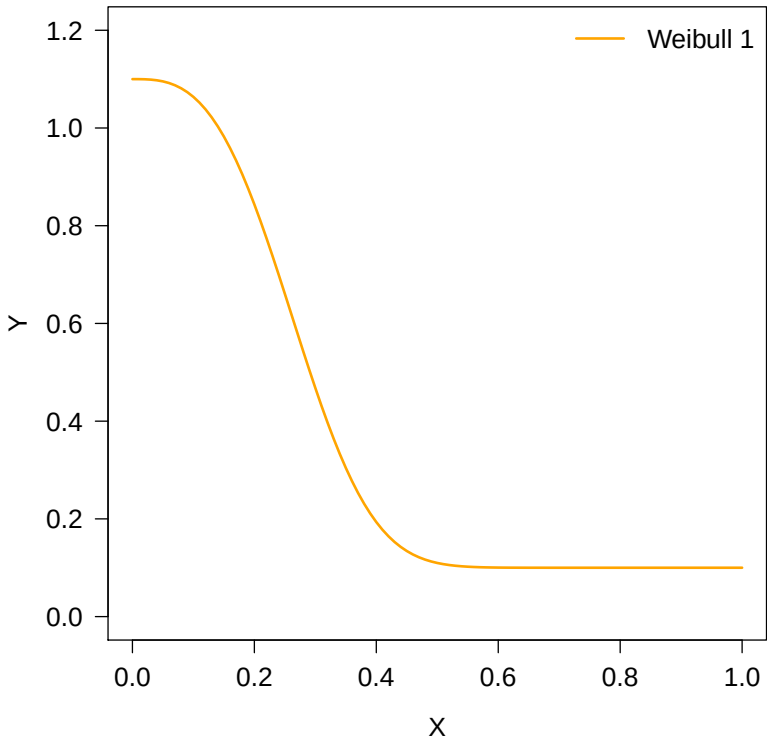
Case of survival, with $c = 0$ and $d = 1$: $e = LC_{50}$.

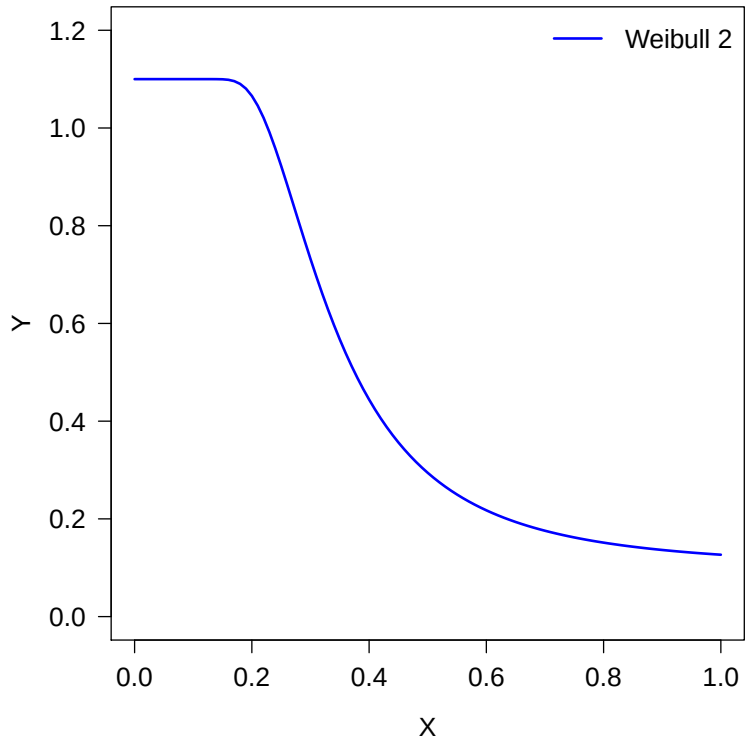


Other sigmoidal models

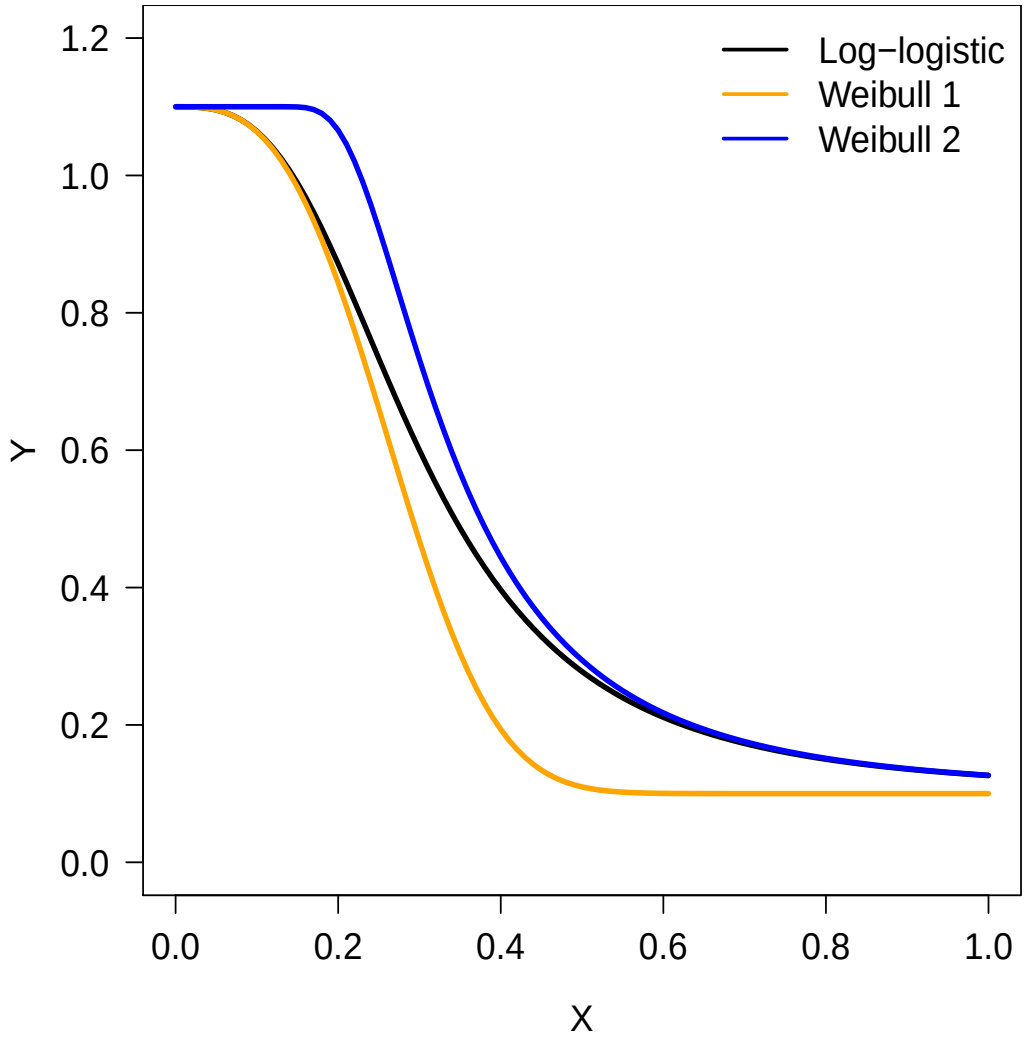
Many other models exist to describe sigmoidal shapes of dose-response curves as for example the Weibull's models:

$$Y = c + (d - c)e^{-(\frac{x}{e})^b} \quad \text{or} \quad Y = c + (d - c)(1 - e^{-(\frac{x}{e})^b})$$





Comparison of sigmoidal curves



About the stochastic part

Its role

Modelling of the variability **around** the mean tendency of the data
$\rightarrow$ requires the choice of the appropriate probability distribution

- **Quantal (or binary) data**: use of a binomial distribution.

$$Y \sim \mathcal{B}\left(p(X,\theta), n\right)$$

- **Count (or discrete) data**: use of a Poisson distribution

$$Y \sim \mathcal{P}(\lambda) \quad \text{with} \quad \lambda = f(X,\theta)$$

- **Continuous data**: use of a normal distribution

$$Y \sim \mathcal{N}(f(X,\theta), \sigma)$$

In brief: a wide variety of models

1. A **deterministic part**: linear or non-linear,
   and its associated parameters:
   for example, (α, β) or (b, c, d, e);

2. A **stochastic part**: Gaussian or not,
   and its associated parameters:
   for example, p (binomial), λ (Poisson) or σ (normal).

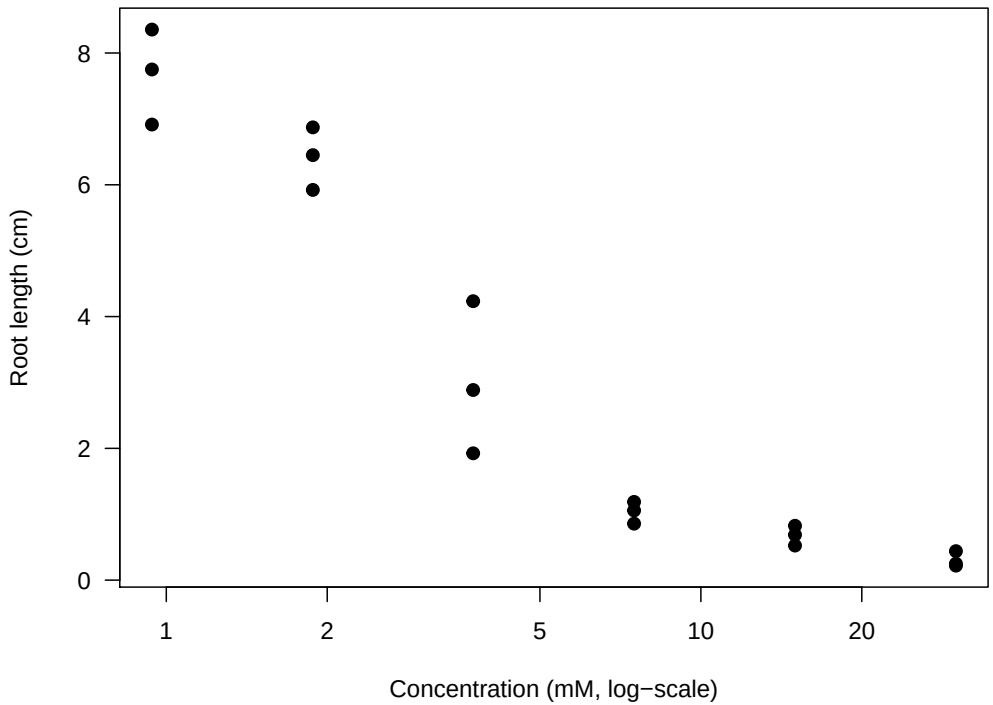
A battery of regression types

- **Gaussian linear regression**: simple linear regression, polynomial regression, multiple regression,...;
- **Generalized linear regression**: logistic regression, Poisson regression, multinomial logit regression, probit regression,...;
- **Gaussian non-linear regression**: least-square regression, simple or multiple;
- **Generalized non-linear regression**.

Table of content

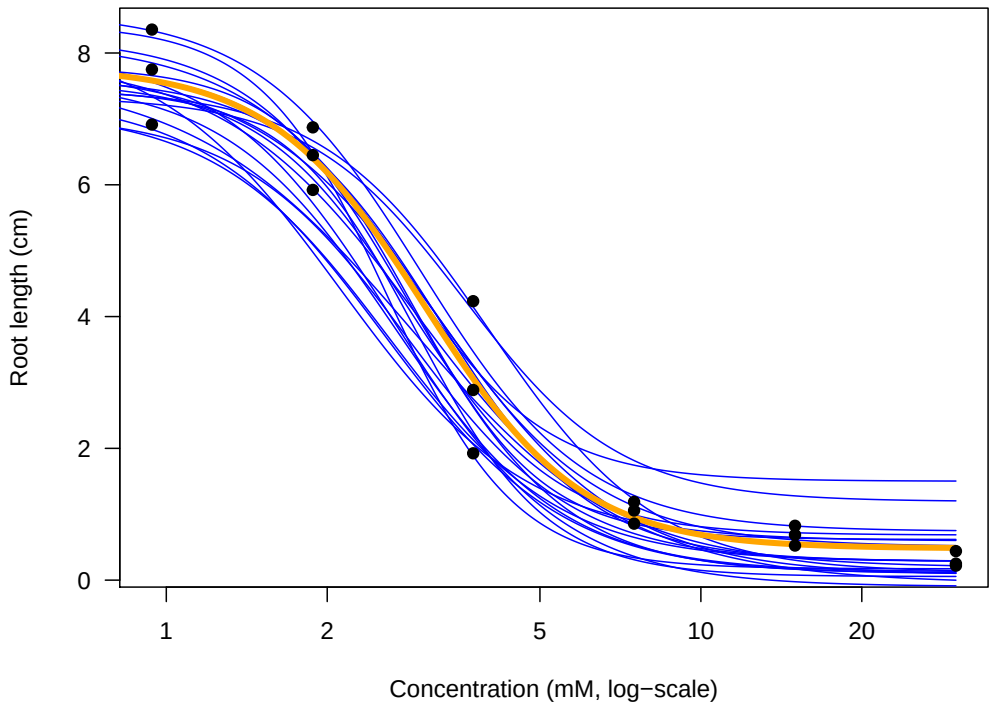
What is inference ?

Inference implies the use of both observed data and a model.



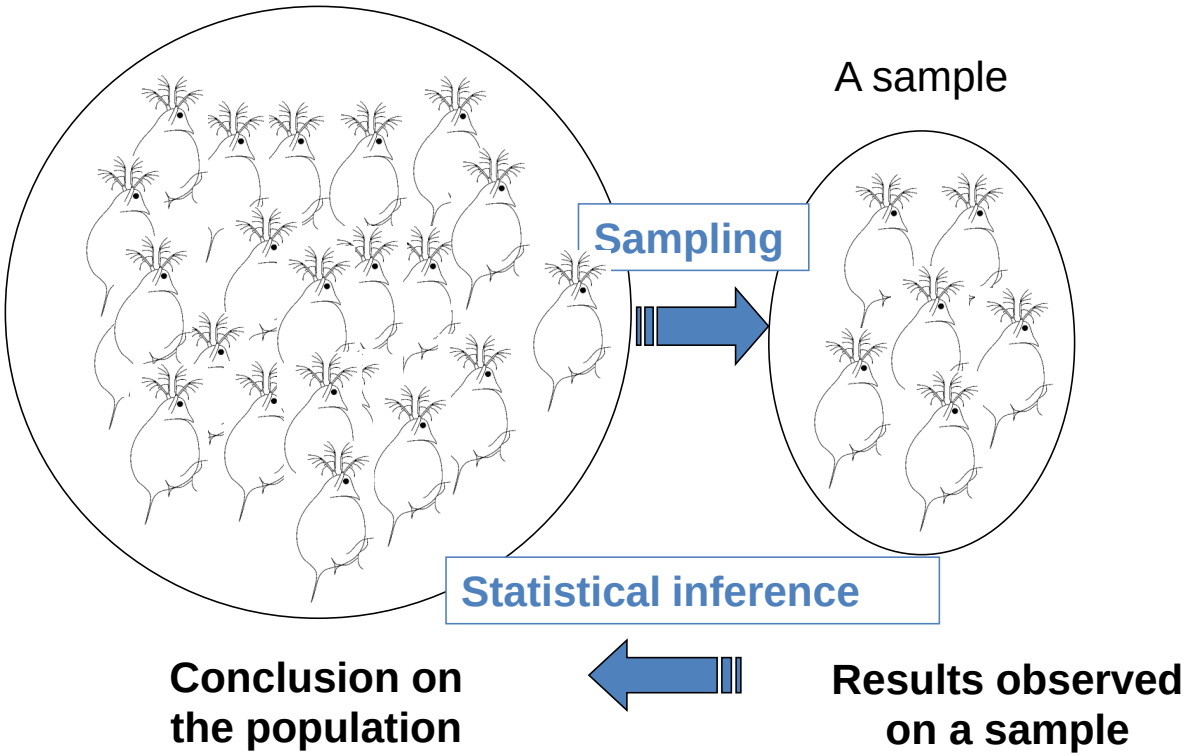
What is inference ≡ Get parameter estimates

Several criteria may provide the best fit parameter values.



Inference: generalization to population

Inference necessarily implies **generalization** from a sample to population, and the calculation of **uncertainty** in the estimated parameters, especially uncertainty due to the sampling error.



Inference: how to perform?

Two main ways of practicing:

The **frequentist** framework

Based on the principle of maximizing the probability of the data given the model, namely the **likelihood** $P(Y|\theta)$.

The **Bayesian** framework

Based on the principle of maximizing the probability of the model given the data, namely the **joint posterior distribution** $P(\theta|Y)$, combining both the likelihood and **prior information** available on parameters **in advance**.

Frequentist framework

- **Parameter θ is supposed fixed but unknown**;
- Parameter inference only uses observed data;
- Confidence intervals are based on repeated sampling from the model, the probability being associated to the relative occurrence frequency of an outcome.

Frequentist framework

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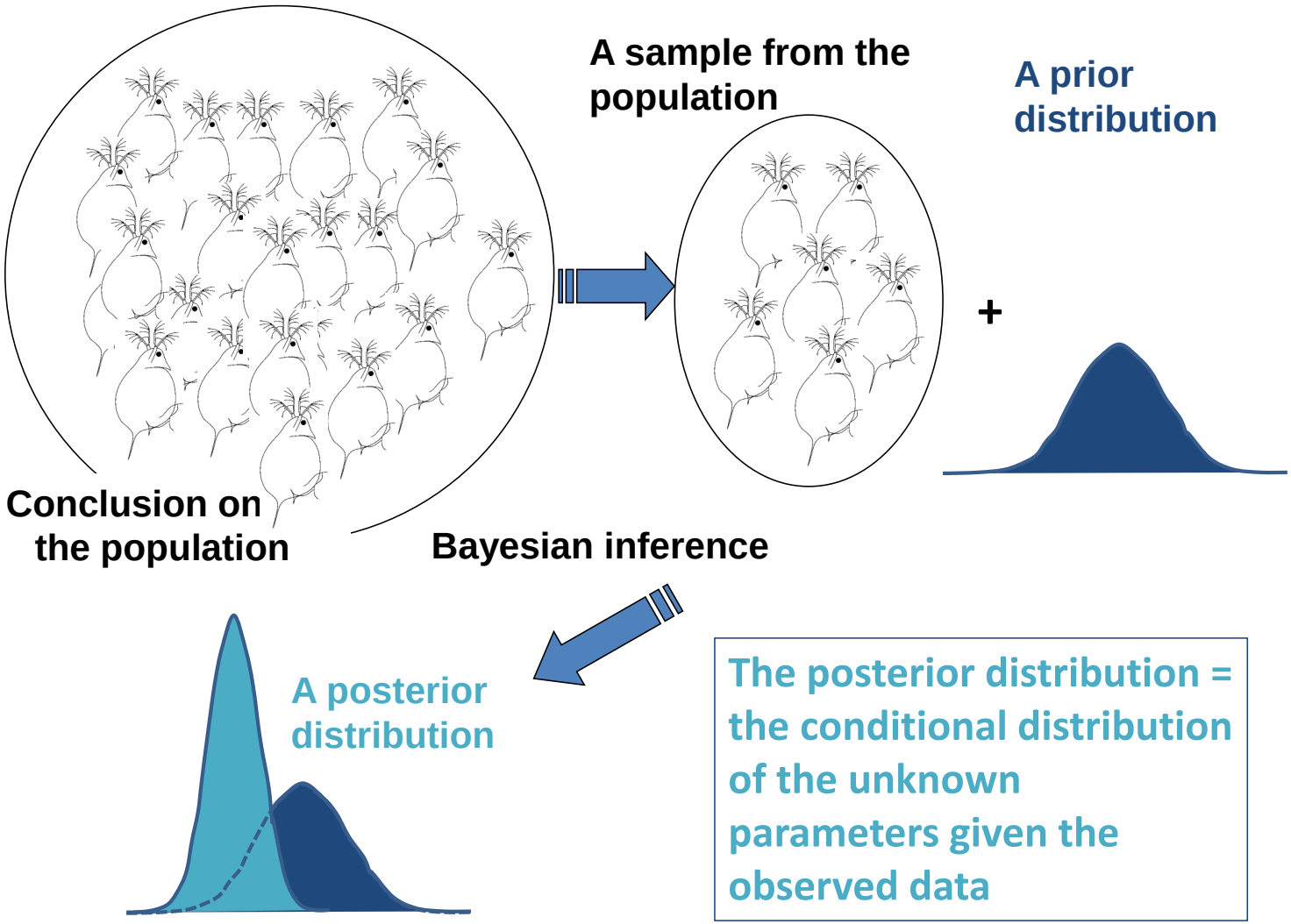
Bayesian framework

- **Parameter θ is considered as a random variable, associated to a probability distribution**;
- Parameter inference uses both observed data and prior information (prior distribution);
- Credible intervals are defined from the posterior distribution and can be easily interpreted: **95% is the probability that the true parameter value lies within its 95% credible interval**.

The Bayesian framework in pictures



Advantages of the Bayesian framework

Use of the posterior distribution for parameter estimation

- **Point estimate:**
  Mean, median or mode of the posterior distribution
- **Interval estimate:**
  Definition of a **credible interval** (or Bayesian confidence interval) from posterior distribution quantiles:
  $\rightarrow 2.5\%$ and 97.5% quantiles for a 95% credible interval.
  Easy interpretation: the probability that the parameter lies in a 95% credible interval is 95%.
- There is **no more need** of hypothesis tests, nor of p-value calculation: one can make decisions directly from the posterior distribution.

Inference: tools for practice

Under the **frequentist** framework

TOX Rat®

Excel macro : REGTOX

Under the **Bayesian** framework

https://mosaic.univ-lyon1.fr/

Table of content