The Benchmark Dose

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Definitions - p_0 and BMR

Background level - $\mathbf{p_0}$

- Probability of an adverse response in the unexposed population (the control group)
- (Or simply the response level for the unexposed population)
- May be estimated from the model or specified, e.g. 0.05

BMR – Benchmark response

- Largest increase in the probability of an adverse response (above the background level) that is not deemed harmful to the organisms
- Typical values are in the range 0.01 - 0.1

Definitions - BMD and BMDL

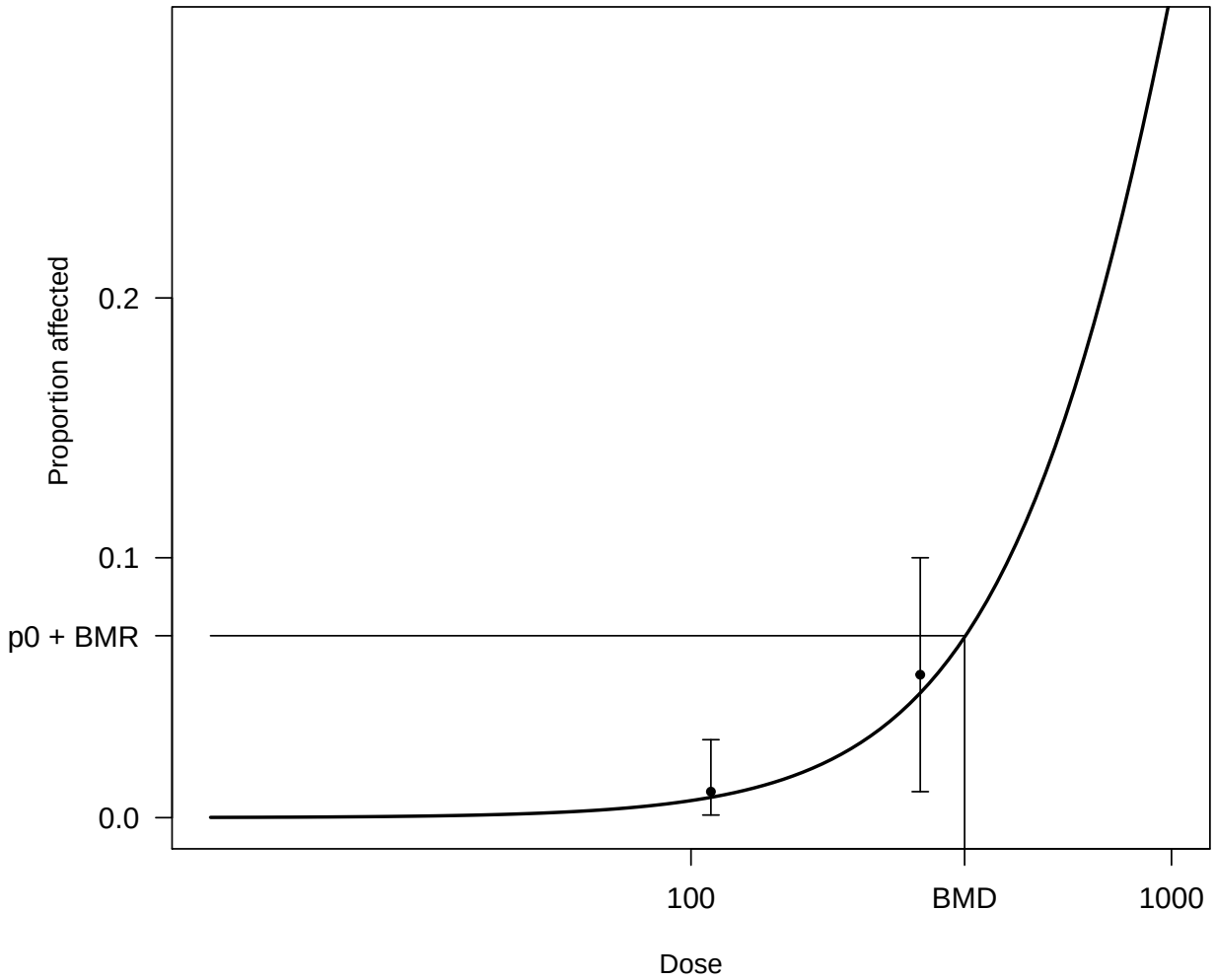
BMD – Benchmark dose

- Dose (or concentration) resulting in a small pre-specified acceptable change, BMR, from the background level

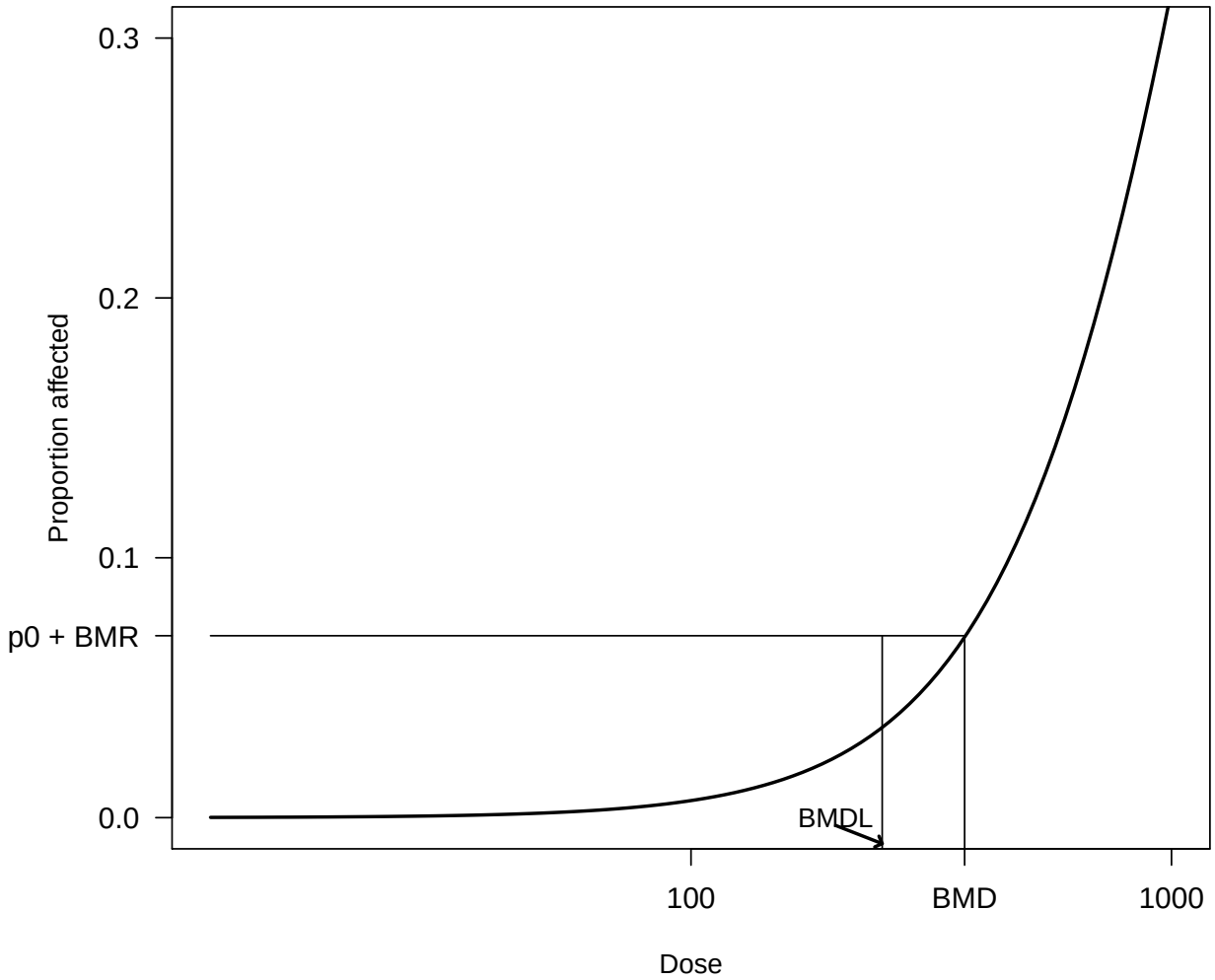
BMDL – Benchmark dose lower limit

- Lower limit of the confidence interval for BMD
- The point of departure used for generating reference values in risk assessment

Benchmark dose, illustration



Benchmark dose, illustration



Advantages of the BMD methodology

- The full dose response curve is utilized in the analysis
- Independent of choice of doses
- Biologically interpretable parameters estimated
- Does not need to be a dose in the experiment
- Good experimental designs are rewarded
- BMD is based on a consistent response level across studies
- More information and better estimates to be obtained from a given number of animals

Benchmark doses for binomial response data

Two definitions

Additional risk :

$$BMR = f(BMD; \beta) - p_0$$

Excess risk:

$$BMR = \frac{f(BMD; \beta) - p_0}{1 - p_0}$$

where f is the assumed model function

The DoseResponse repository at GitHub

The *DoseReponse* repository contains the most updated versions of *drc*, *drcData* and *bmd*.

To install packages from GitHub, first install and load *devtools*

```
library(devtools)
install_github("DoseResponse/drc")
install_github("DoseResponse/drcData")
install_github("DoseResponse/bmd")
```

Now load all three packages

```
library(drc)
library(drcData)
library(bmd)
```

Example: An earthworm toxicity test

- Six concentrations of the herbicide chloroacetamide (five active and a control)
- Forty earthworms used for each concentration
- The resulting number of dead earthworms was counted at end of experiment

Data are found in *drcData* with the name: chlorac

```
chlorac
```

```
##   conc total num.dead
## 1    0    40        3
## 2   10    40        5
## 3   20    40        6
## 4   40    40       38
## 5   80    40       40
## 6  160    40       40
```

Hoekstra (1987)

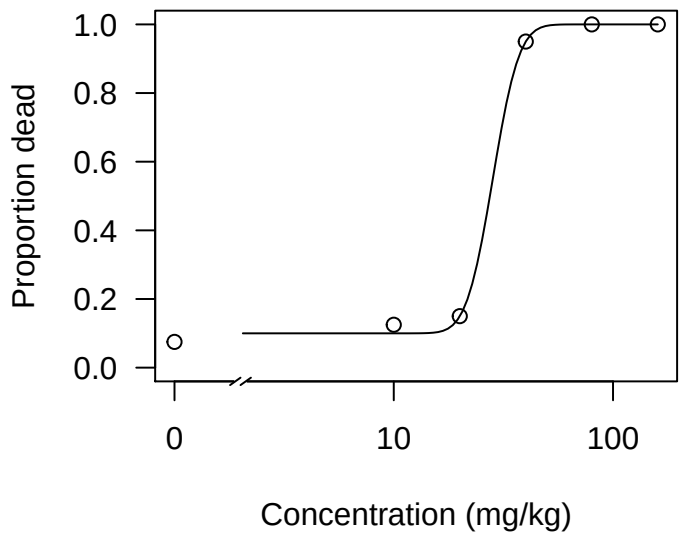
Model fitting

Fitting a three-parameter log-normal model with estimated lower limit to data

```
chlorac.LN.3 <- drm(num.dead/total ~ conc,
                    weights = total,
                    data = chlorac,
                    fct = LN.3u(),
                    type = "binomial")
```

Plotting the fitted model

```
plot(chlorac.LN.3, broken=TRUE, ylim=c(0,1),
     ylab=c("Proportion dead"), xlab = c("Concentration (mg/kg)"))
```



How to find the benchmark dose

Estimated parameters from the fitted model

```
coef(chlorac.LN.3)
```

```
## b:(Intercept) c:(Intercept) e:(Intercept)
##    4.60377303    0.09998766   28.29192242
```

That is, $p_0 = 0.1$

With additional risk and BMR=0.05, BMD is the concentration associated with a proportion of dead earthworms equal to

$$f(BMD) = BMR + p_0 = 0.15$$

With excess risk and BMR=0.05, BMD is the concentration associated with a proportion of dead earthworms equal to

$$f(BMD) = BMR(1 - p_0) + p_0 = 0.145$$

Estimating the BMD

BMD based on the additional risk definition with BMR = 0.05

```
bmd(chlorac.LN.3,
    bmr = 0.05,
    backgType = "modelBased",
    def = "additional")
```

```
##       BMD      BMDL
##  20.0155 15.39552
```

BMD based on the excess risk definition with BMR = 0.05

```
bmd(chlorac.LN.3,
    bmr = 0.05,
    backgType = "modelBased",
    def = "excess")
```

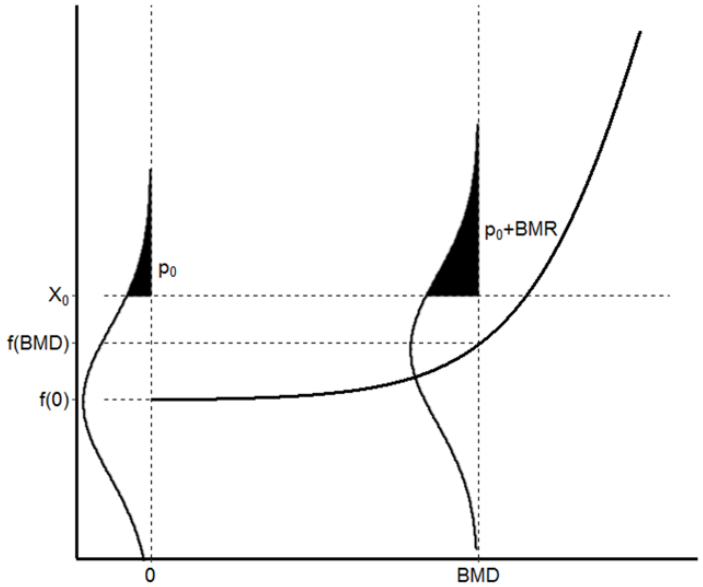
```
##       BMD      BMDL
##  19.79229 15.15075
```

Benchmark doses for continuous response data - The hybrid approach

Defining p_0

Probability of an abnormal response in an unexposed organism, assuming a normal distribution:

$$p_0 = 1 - \Phi\left(\frac{x_0 - f(0, \beta)}{\sigma}\right)$$



Defining BMD

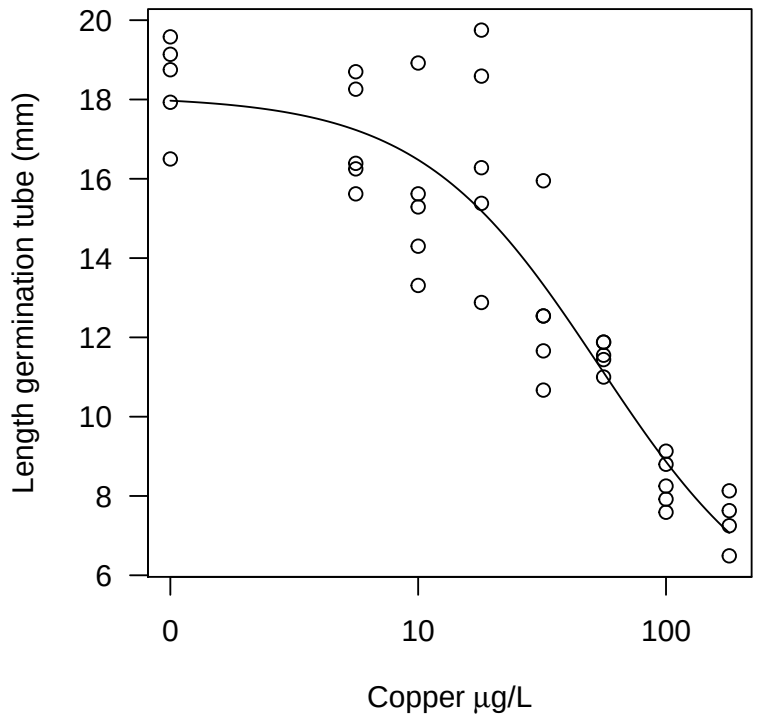
Probability of an abnormal response in an exposed organism (with dose BMD) should equal p_0 + BMR

$$BMR = 1 - \Phi\left(\frac{x_0 - f(BMD, \beta)}{\sigma}\right) - p_0$$

Example: Giant kelp exposed to copper

Giant kelp (*Mactocystis pyrifera*) was exposed to one of eight different concentrations of copper in an aquatic ecosystem with up to five replications for each concentration.

The length of the germination tubes were measured with a shorter length indicating a higher toxic response.



Chapman et al. (1995)

Model fitting

Data are found in *drcData* with the name: GiantKelp

```
head(GiantKelp)
```

```
##   tubeLength dose
## 1      19.58  0.0
## 2      18.75  0.0
## 3      19.14  0.0
## 4      16.50  0.0
## 5      17.93  0.0
## 6      18.26  5.6
```

Fitting a four-parameter log-logistic model to data

```
kelp.m1 <- drm(tubeLength ~ dose,
               data = GiantKelp,
               fct=LL.4())
```

Estimating the BMD - the expert knowledge cut-off

First, what is an adverse event?

Assume a germination tube shorter than 14 mm would imply inability of the Giant kelp to function properly

Estimating BMD based on the hybrid approach, defining an adverse event as a tube length < 14 mm, with BMR $= 0.1$

```
bmd(kelp.m1,
    bmr = 0.1,
    backgType = "absolute",
    backg = 14,
    def = "hybridAdd")
```

```
##        BMD      BMDL
##   12.30724 5.248608
```

Estimating the BMD - a less efficient use of a cut-off

Fitting a dose-response model for a dichotomized response variable

```
KelpNew <- GiantKelp
KelpNew$lengthBin <- ifelse(KelpNew$tubeLength < 14, 1, 0)

kelp.m2 <- drm(lengthBin ~ dose,
               data = KelpNew,
               fct = LL.4(),
               type = "binomial")
```

BMD (from binomial data) based on the additional definition with BMR = 0.1

```
bmd(kelp.m2,
    bmr = 0.1,
    backgType = "modelBased",
    def = "additional")
```

```
##        BMD       BMDL
##  9.278657 -53.68048
```

The negative value of BMDL means no information can be taken from this analysis; any dose could result in a 10% increase in the risk of an adverse event

Estimating the BMD - the hybrid approach with less knowledge

If there is no clear-cut definition of an adverse event, it can be based on the distribution of the control group instead.

Estimating BMD based on the hybrid approach, defining an adverse event based on 2 SDs for the control group, with BMR = 0.1

```
bmd(kelp.m1,
       bmr = 0.1,
       backgType = "hybridSD",
       def = "hybridAdd",
       backg = 2)
```

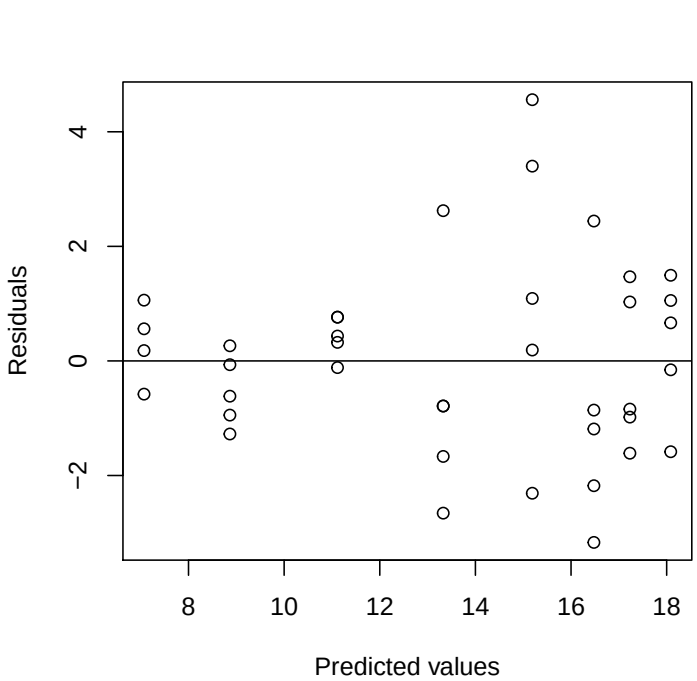
```
##        BMD      BMDL
##  8.878006 2.542278
```

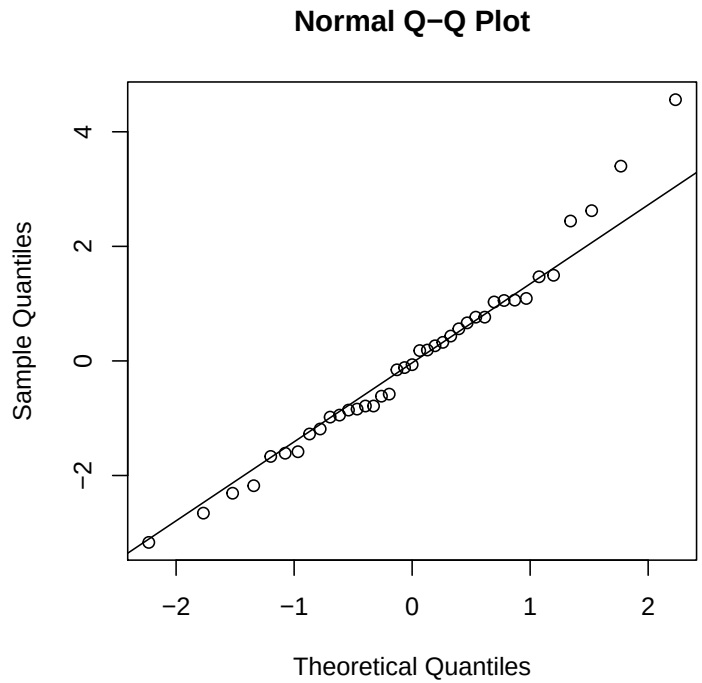
Checking model assumptions

A residual plot reveals a deviation from the assumption of variance homogeneity

```
plot(resid(kelp.m1) ~ predict(kelp.m1),
     xlab = "Predicted values", ylab = "Residuals")
abline(h = 0)

qqnorm(resid(kelp.m1))
qqline(resid(kelp.m1))
```





Dealing with the model misspecification

Addressing the model misspecification, using robust standard errors (for BMDL)

```
library(sandwich)

bmd(kelp.m1,
        bmr = 0.1,
        backgType = "hybridSD",
        def = "hybridAdd",
        backg = 2,
        sandwich.vcov = TRUE)
```

```
##        BMD     BMDL
##  8.878006 1.275386
```

Benchmark doses for count (and continuous) response data

Three definitions

Added response:

$$BMR = f(BMD, \beta) - p_0$$

Extra response:

$$BMR = \frac{f(BMD, \beta) - p_0}{f(\infty, \beta) - p_0}$$

Relative response (critical effect size)

$$BMR = \frac{f(BMD, \beta) - p_0}{p_0}$$

Example: Daphnids expsosed to copper under varying temperatures

- 12 nematode worms were exposed to each of 5 concentrations of copper
- The daily temperatures fluctuated ± 4 degrees around a mean temperature of 20 °C
- 36 worms (the controls) were exposed to the same temperature fluctuations but not copper
- Total number of offsprings were considered as response

Importing data, VarTemp.csv

```
file.choose()
CopperTemp <- read.csv2(...)
```

```
head(CopperTemp,3)
```

```
##   Temperature Variation Replicate Conc Offspring Lifespan
## 1          20  _+/-_4_C         1    0       227       15
## 2          20  _+/-_4_C         2    0       279       21
## 3          20  _+/-_4_C         3    0       254       19
```

Cedergreen et al. (2016)

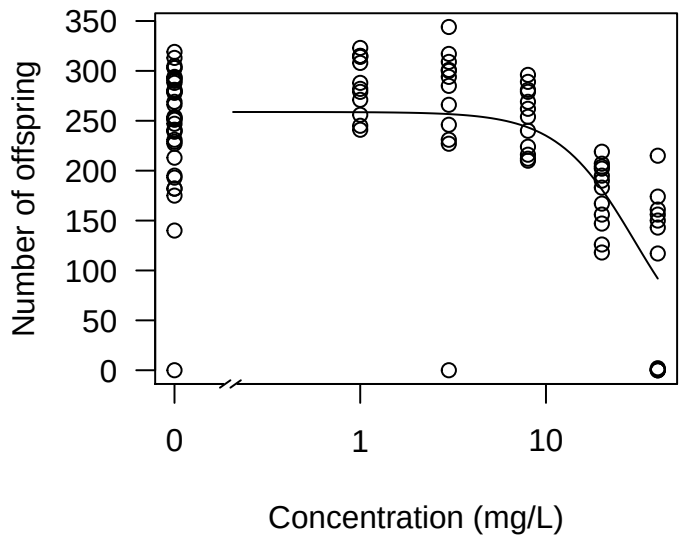
Model fitting

A three-parameter log-logistic model is fitted to data

```
CopperTemp.m1 <- drm(Offspring ~ Conc,
                     data = CopperTemp,
                     type = "Poisson",
                     fct = LL.3())
```

Plotting the fitted model

```
plot(CopperTemp.m1, broken = TRUE, type = "all",
  ylab = c("Number of offspring"), xlab = c("Concentration (mg/L)"))
```



Estimating the BMD

Estimating BMD using the relative definition and BMR = 0.1

```
bmd(CopperTemp.m1,
    bmr = 0.1,
    backgType = "modelBased",
    def = "relative")
```

```
##        BMD      BMDL
##   10.47006 9.403057
```

A weighted dose-response analysis

Taking into account that some worms die before end of experiment

```
CopperTemp.m2 <- drm(Offspring ~ Conc,
                     data = CopperTemp,
                     weights = Lifespan,
                     type = "Poisson",
                     fct = LL.3())
```

Estimating BMD

```
bmd(CopperTemp.m2,
    bmr = 0.1,
    backgType = "modelBased",
    def = "relative")
```

```
##        BMD     BMDL
##   31.16192 28.89062
```

Accounting for overdispersion in the BMD analysis

Estimating BMD with sandwich variance-covariance estimates

```
library(sandwich)

bmd(CopperTemp.m2,
    bmr = 0.1,
    backgType = "modelBased",
    def = "relative",
    sandwich.vcov = TRUE)
```

```
##        BMD      BMDL
##   31.16192 18.62904
```

Note: The BMD remains the same, only the BMDL changes as this is now based on adjusted standard errors.

Accounting for overdispersion with an alternative model

Fitting a dose-response model assuming an underlying negative binomial distribution

```
CopperTemp.m3 <- drm(Offspring ~ Conc,
                     data = CopperTemp,
                     weights = Lifespan,
                     type = "negbin2",
                     fct = LL.3())
```

Estimating BMD

```
bmd(CopperTemp.m3,
    bmr = 0.1,
    backgType = "modelBased",
    def = "relative")
```

```
##        BMD      BMDL
##   27.98813 15.99371
```

Example: A fish test

- A 21 day fish test following OECD guidelines, using Rainbow trout *Oncorhynchus mykiss*
- 7 (6 + control) concentrations of an unknown agent
- After 28 days the wet weight was registered
- Up till 10 replicates per concentration, with a total of 61 observations

Data are found in *drcData* with the name: O.mykiss

```
head(O.mykiss)
```

```
##   conc weight
## 1    0    2.8
## 2    0    3.0
## 3    0    2.7
## 4    0    3.9
## 5    0    3.1
## 6    0    1.8
```

OECD (2006)

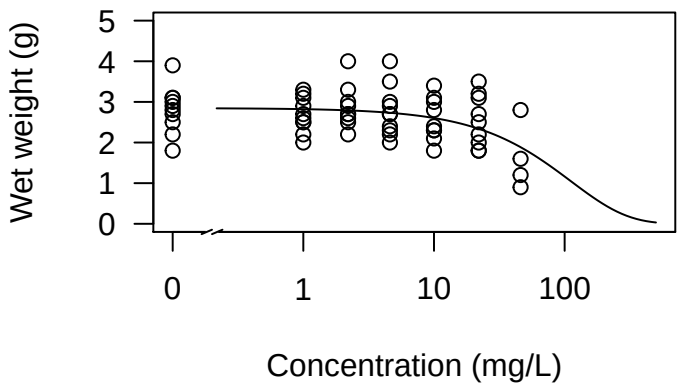
Fitting the model

Fitting a two-parameter exponential decay model.

```
O.mykiss.c <- na.omit(O.mykiss)
O.mykiss.EXD.2 <- drm(weight ~ conc,
                      data = O.mykiss.c,
                      fct = EXD.2())
```

Plotting the fitted model

```
plot(O.mykiss.EXD.2, broken = TRUE, type = "all",
     xlim = c(0, 500), ylim = c(0, 5),
     xlab = "Concentration (mg/L)", ylab = "Wet weight (g)")
```



Estimating the BMD

A BMD associated with a BMR=0.05 using the hybrid approach with 2 SDs as the cutoff

```
bmd(O.mykiss.EXD.2,
    bmr = 0.05,
    backgType = "hybridSD",
    def = "hybridAdd",
    backg = 2)
```

```
##        BMD      BMDL
##   12.65354 6.371416
```

Alternative models

Fitting three other models to data; a three-parameter log-logistic and 2 different three-parameter Weibull models

```
O.mykiss.LL.3<-drm(weight ~ conc,
                   data = O.mykiss,
                   fct = LL.3(),
                   na.action = na.omit)

O.mykiss.W1.3<-drm(weight ~ conc,
                   data = O.mykiss,
                   fct = W1.3(),
                   na.action = na.omit)

O.mykiss.W2.3<-drm(weight ~ conc,
                   data = O.mykiss,
                   fct = W2.3(),
                   na.action = na.omit)
```

Estimating BMD for the new models

```
bmd(O.mykiss.LL.3, bmr = 0.05,
    backgType = "hybridSD",
    backg = 2,
    def = "hybridAdd")
```

```
##       BMD     BMDL
##  22.91039 9.046736
```

```
bmd(O.mykiss.W1.3, bmr = 0.05,
    backgType = "hybridSD",
    backg = 2,
    def = "hybridAdd")
```

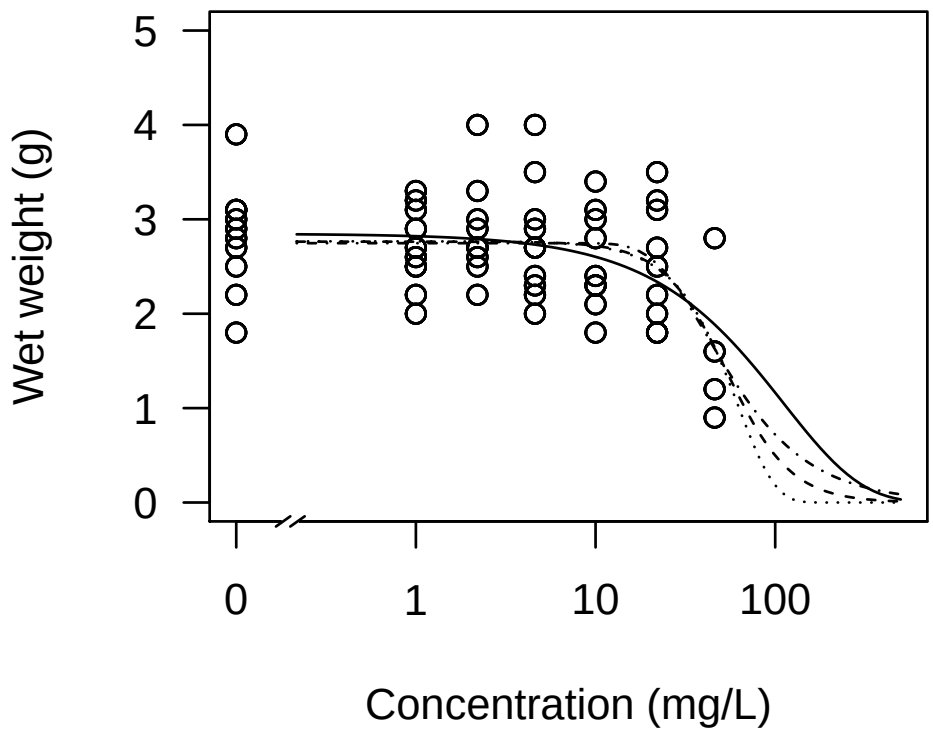
```
##       BMD     BMDL
##  22.71909 8.124225
```

```
bmd(O.mykiss.W2.3, bmr = 0.05,
    backgType = "hybridSD",
    backg = 2,
    def = "hybridAdd")
```

```
##       BMD    BMDL
##  24.25913 14.2518
```

Comparing models graphically

```
plot(O.mykiss.EXD.2, broken = TRUE, type = "all", xlim = c(0, 500),
     ylim = c(0, 5), xlab="Concentration (mg/L)", ylab="Wet weight (g)")
plot(O.mykiss.LL.3, broken = TRUE, type = "all",
     xlim = c(0, 500), ylim = c(0, 5), add = TRUE, lty=2)
plot(O.mykiss.W1.3, broken = TRUE, type = "all",
     xlim = c(0, 500), ylim = c(0, 5), add = TRUE, lty=3)
plot(O.mykiss.W2.3, broken = TRUE, type = "all",
     xlim = c(0, 500), ylim = c(0, 5), add = TRUE, lty=4)
```



Comparing models using AIC

```
AIC(O.mykiss.EXD.2,
    O.mykiss.LL.3,
    O.mykiss.W1.3,
    O.mykiss.W2.3)
```

```
##                  df      AIC
## O.mykiss.EXD.2    3 106.3066
## O.mykiss.LL.3     4 106.6501
## O.mykiss.W1.3     4 106.5823
## O.mykiss.W2.3     4 106.9406
```

BMD estimation using model averaging

Using the Buckland definition to estimate BMDL

```
modelList <- list(O.mykiss.EXD.2, O.mykiss.LL.3,
                  O.mykiss.W1.3, O.mykiss.W2.3)

bmdMA(modelList, modelWeights = "AIC", bmr = 0.05,
      backgType = "hybridSD", def = "hybridAdd", backg = 2,
      type = "Buckland")
```

```
##     BMD_MA   BMDL_MA
##   19.68024 5.804238
```

Using bootstrap to estimate BMDL (may take some time to run)

```
bmdMA(modelList, modelWeights = "AIC", bmr = 0.05,
      backgType = "hybridSD", def = "hybridAdd", backg = 2,
      type = "bootstrap", R = 1000)
```

```
##     BMD_MA   BMDL_MA
##   19.68024 8.590238
```

Example: An acute toxicity test on daphnids

- Nine independent experiments conducted at different times
- D. magma neonates were exposed to different concentrations of α-cypermethrin for 48 hours
- The number of concentrations used in each sub-experiment varied from 6 to 7
- Four replicates of five organisms were used for each concentration

Importing data, Alpha_cyp.csv

```
file.choose()
alpha.cyp <- read.csv2(...)
```

```
head(alpha.cyp, 3)
```

```
##   Dose Total Mobile Immobile  Exp
## 1    0     5      5         0 Exp1
## 2    0     5      4         1 Exp1
## 3    0     5      5         0 Exp1
```

Gottardi and Cedergreen (2019)

Estimating the models

Defining a function that fits a two-parameter log-logistic model to a dataset

```
fitFct.LL.2 <- function(dataSet){
                    drm(Immobile/Total ~ Dose,
                    weights = Total,
                    data=dataSet,
                    type = "binomial",
                    fct = LL.2())
}
```

Applying the model to each subset of the data set alpha.cyp defined by experiment

```
library(plyr)

modelFits <- dlply(alpha.cyp, .(Exp), fitFct.LL.2)
```

Estimating BMD for each model

Applying bmd() to each of the fitted models

```
BMDList <- lapply(modelFits,
                  FUN = function(x){
                          bmd(x,
                              bmr=0.05,
                              backgType = "modelBased",
                              def = "excess",
                              display = FALSE)
                    }
                  )
```

Collecting all information in a data set

Extracting information about BMD, BMDL and experiment

```
BMDVec <- sapply(BMDList, FUN = function(x) {x$Results[1]})
SEVec <- sapply(BMDList, FUN = function(x) {x$SE})
ExpVec <- names(BMDVec)
```

Collecting all informations in a data frame

```
step2Data<-data.frame(BMD = BMDVec,
                      SE = SEVec,
                      Exp = ExpVec)
```

Fitting a meta-analytic model

```
library(metafor)

meta.m1 <- rma(BMD,
               SE^2,
               data = step2Data,
               level = 0.9)
```

Estimated BMD

```
meta.m1$beta
```

```
##                [,1]
## intrcpt 0.07052855
```

Estimated BMDL (lower bound of the 90% confidence interval)

```
meta.m1$ci.lb
```

```
## [1] 0.05137664
```

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