Dose Response Modeling of Rabbit RBC AChE Activity: Oxamyl Dermal Dosing

June 28, 2005

1 Preamble

Here is some code to set up the analysis: loading required libraries and datasets, and defining some functions.

First, `CarbamateData` loads the full dataset for this risk assessment, and causes the library `DRUtils` to be loaded.

```
> library(CarbamateData)

Loading required package: DRUtils
Loading required package: nlme
Loading required package: tcltk
```

Set up lattice to use B&W instead of color:

```
> library(lattice)
> ltheme <- canonical.theme(color = FALSE)
> ltheme$strip.background$col <- "transparent"
> lattice.options(default.theme = ltheme)
```

Use package `Hmisc` for some formatting support.

```
> library(Hmisc)

Hmisc library by Frank E Harrell Jr

Type library(help='Hmisc'), ?Overview, or ?Hmisc.Overview')
to see overall documentation.

NOTE:Hmisc no longer redefines [.factor to drop unused levels when
subsetting.  To get the old behavior of Hmisc type dropUnusedLevels().

Attaching package 'Hmisc':


        The following object(s) are masked from package:stats :

         ecdf
```

All the rat gavage data for this analysis are in `AggData`. The following code prints out documentation for the datasets in use:

```
> printDataDoc(AggData)
```

```
----------------------------------------
Data set: AggData

Dataset creation date: Wed Jun 22 12:07:24 2005
Script name: /home/setzer/tasks/CarbamateCRA/EmpiricalDoseResponsesFinal/Data/getdata.R
Script last modified: 2005-06-22 12:05:47
sysname: Linux
release: 2.4.24-openmosix2
version: #1 Wed Jul 28 14:33:52 CEST 2004
nodename: node24.cluster2.org
machine: i686
login: unknown
user: setzer
----------------------------------------
```

The following function turns out to be quite useful on subsetted dataframes. It just eliminates unused levels of all factors in the data frame:

```
> CleanUp <- function(x) {
+     for (nm in names(x)) {
+         if (is.factor(x[, nm]))
+             x[, nm] <- factor(x[, nm])
+     }
+     x
+ }
```

To get starting values, we often have to extract values from a previously fit model. The following function simplifies that. The argument `what` is a regular expression:

```
> getParms <- function(what, Par) {
+     Par[grep(what, names(Par))]
+ }
```

This script is for modeling the dose-time response for rat brain. via gavage dosing. It includes acute, subchronic, and chronic studies.

All the data used for this DR model are in `AggData`.

Now, set up the analysis dataset.

```
> dta <- CleanUp(subset(AggData, chemical %in% "OXAMYL" & dsmtd %in%
+     "DERMAL" & sctn %in% "RBC" & (is.na(tmpstds) | tmpstds <
+     300), select = c("cheact", "sd", "n", "dose", "tmonstdy",
+     "sctn", "species", "tmpstds", "sex", "mrid")))
```

Summary of the relevant variables in this dataset:

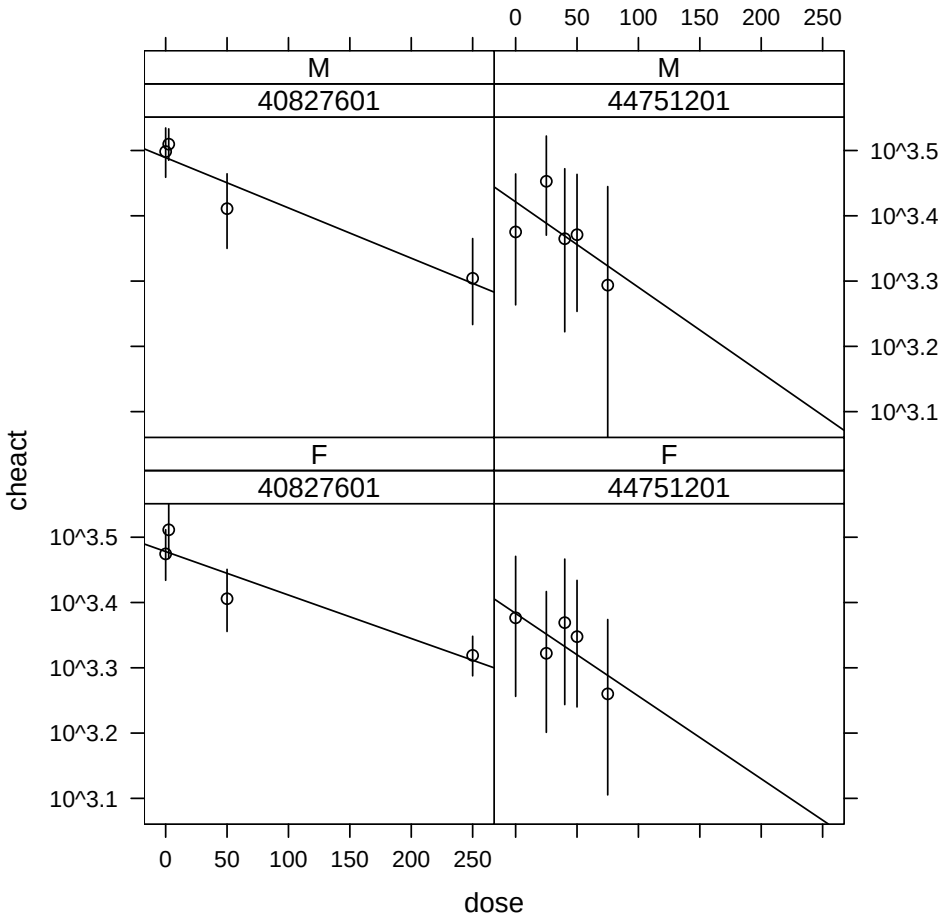
```
> by(dta, dta$mrid, summary)

dta$mrid: 40827601
     cheact           sd              n               dose            tmonstdy
 Min.   :2015   Min.   :144.1   Min.   : 5.000   Min.   :  0.000   Min.   :22
 1st Qu.:2431   1st Qu.:218.6   1st Qu.: 5.000   1st Qu.:  1.875   1st Qu.:22
 Median :2779   Median :261.5   Median : 6.000   Median : 26.250   Median :22
 Mean   :2729   Mean   :271.9   Mean   : 7.125   Mean   : 75.625   Mean   :22
 3rd Qu.:3172   3rd Qu.:338.6   3rd Qu.:10.000   3rd Qu.:100.000   3rd Qu.:22
 Max.   :3246   Max.   :382.3   Max.   :10.000   Max.   :250.000   Max.   :22
  sctn     species     tmpstds  sex         mrid
 RBC:8   RABBIT:8   Min.   :1   F:4   40827601:8
```

```
                    1st Qu.:1   M:4   44751201:0
                    Median :1
                    Mean   :1
                    3rd Qu.:1
                    Max.   :1
------------------------------------------------------------
dta$mrid: 44751201
     cheact           sd              n          dose       tmonstdy   sctn
 Min.   :1820   Min.   :466.0   Min.   :6   Min.   : 0   Min.   :21   RBC:10
 1st Qu.:2132   1st Qu.:493.5   1st Qu.:6   1st Qu.:25   1st Qu.:21
 Median :2328   Median :525.5   Median :6   Median :40   Median :21
 Mean   :2271   Mean   :549.1   Mean   :6   Mean   :38   Mean   :21
 3rd Qu.:2367   3rd Qu.:557.2   3rd Qu.:6   3rd Qu.:50   3rd Qu.:21
 Max.   :2837   Max.   :779.0   Max.   :6   Max.   :75   Max.   :21

   species      tmpstds    sex         mrid
 RABBIT:10   Min.   : NA   F:5   40827601: 0
             1st Qu.: NA   M:5   44751201:10
             Median : NA
             Mean   :NaN
             3rd Qu.: NA
             Max.   : NA
             NA's   : 10
```

> by(dta, dta$mrid, function(x) with(x, table(dose, tmpstds)))

```
dta$mrid: 40827601
     tmpstds
dose  1
  0   2
  2.5 2
  50  2
  250 2
------------------------------------------------------------
dta$mrid: 44751201

  0
  25
  40
  50
  75
```

Plots of RBC AChE activity versus dose, by mrid and sex. There is only one time point in each of these two studies. The fitted line is the best fitting line to the log of the mean values within each panel.



2 Dose-Response Modeling

The fitted lines in the above figures seem to describe the data pretty well, and, given the log-scale of the y-axis, indicate that the simplest version of the exponential model describes these data adequately. That the confidence intervals do not seem to vary much in length across the doses suggests we can model log(cheact) as a linear function of dose in a mixed model where slope is treated as a random coefficent that varies between mrids.

Fitting the model will follow these steps:

1. Create a pseudo-individual dataset, and log the `cheact` variable.
2. Use lme to fit a random slope model, where the intercept differs across `mrid:sex` and slope differs between sexes, and varies randomly among mrids.
3. Test whether slopes differ between sexes; if not, refit without the sex difference.
4. Construct logBMDs, BMDs, standard errors, and confidence intervals using these estimates and the delta method.

The individual data-set:

```
> idta <- with(dta, PhonyDF(dose, n, cheact, sd, "dose", "cheact",
+     Avals = dta[, c("mrid", "sex")]))
> idta$lncheact <- log(idta$cheact)
```

Fit the model:

```
> drmod1 <- lme(lncheact ~ mrid:sex - 1 + dose + sex:dose, data = idta,
+     random = ~dose - 1 | mrid, weights = varIdent(form = ~1 |
+         mrid))
> summary(drmod1)

Linear mixed-effects model fit by REML
 Data: idta
        AIC      BIC   logLik
  -10.96252 13.42326 14.48126

Random effects:
 Formula: ~dose - 1 | mrid
                dose  Residual
StdDev: 0.0007376331 0.1198951

Variance function:
 Structure: Different standard deviations per stratum
 Formula: ~1 | mrid
 Parameter estimates:
40827601 44751201
1.000000 2.125238
Fixed effects: lncheact ~ mrid:sex - 1 + dose + sex:dose
                      Value  Std.Error  DF   t-value p-value
dose              -0.001817 0.00068541 110  -2.65084  0.0092
mrid40827601:sexF  8.001722 0.02818730 110 283.87684  0.0000
mrid44751201:sexF  7.736519 0.05626695 110 137.49667  0.0000
mrid40827601:sexM  8.036494 0.02820721 110 284.90916  0.0000
mrid44751201:sexM  7.827415 0.05634714 110 138.91414  0.0000
sexM:dose         -0.000328 0.00029048 110  -1.13086  0.2606
 Correlation:
                  dose   m40827601:F m44751201:F m40827601:M m44751201:M
mrid40827601:sexF -0.160
mrid44751201:sexF -0.357  0.066
mrid40827601:sexM  0.017  0.001      -0.016
mrid44751201:sexM -0.319 -0.016       0.298       0.068
sexM:dose         -0.191  0.417       0.091      -0.430      -0.105

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-2.75720486 -0.73079277  0.06270251  0.70367544  2.13080467

Number of Observations: 117
Number of Groups: 2

> pv <- summary(drmod1)$tTable["sexM:dose", "p-value"]
```

Note the sex effect on dose is non-significant: P-value = 0.26, so refit with a single slope for dose:

```
> drmod2 <- lme(lncheact ~ mrid:sex - 1 + dose, data = idta, random = ~dose -
+     1 | mrid, weights = varIdent(form = ~1 | mrid))
> drmod3 <- gls(lncheact ~ mrid:sex - 1 + dose, data = idta, weights = varIdent(form = ~1 |
+     mrid))
```

The second model, above (drmod3) can be used to test whether there is really much evidence that the slopes differ among the mrids (really a test of whether the among-slope variance component is significantly different from 0):

```
> anova(drmod2, drmod3)

       Model df       AIC       BIC   logLik   Test   L.Ratio p-value
drmod2     1  8 -26.13452 -4.386532 21.06726
drmod3     2  7 -27.98513 -8.955641 20.99257 1 vs 2 0.1493901  0.6991
```

Both AIC is lower for the second model, and the P-value is large. These two studies agree on their estimate of the dose-response slope. Another way to confirm this is to directly test the differences between the two slopes:

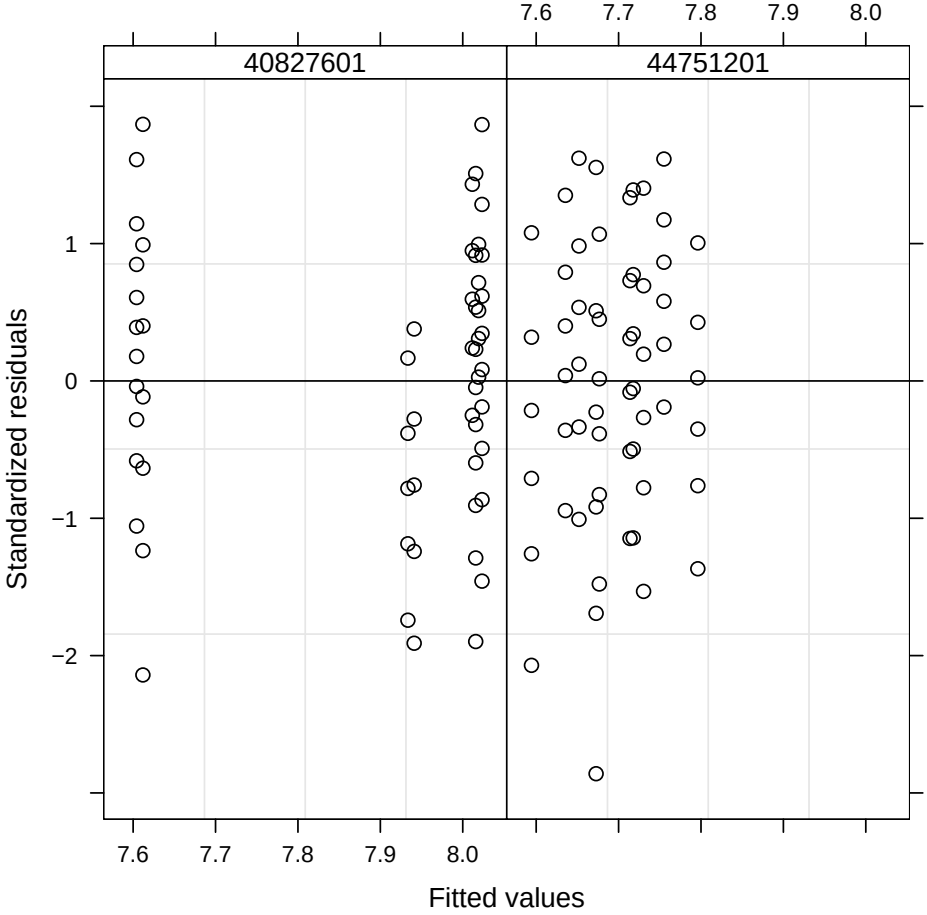
```
> drmod4 <- gls(lncheact ~ mrid:sex - 1 + dose + mrid:dose, data = idta,
+     weights = varIdent(form = ~1 | mrid))
> summary(drmod4)

Generalized least squares fit by REML
  Model: lncheact ~ mrid:sex - 1 + dose + mrid:dose
  Data: idta
        AIC      BIC   logLik
  -16.22339 5.452853 16.11169

Variance function:
 Structure: Different standard deviations per stratum
 Formula: ~1 | mrid
 Parameter estimates:
 44751201  40827601
1.0000000 0.4714197

Coefficients:
                      Value  Std.Error   t-value p-value
dose              -0.001626 0.00014536 -11.18381  0.0000
mrid40827601:sexF  8.013856 0.02569743 311.85437  0.0000
mrid44751201:sexF  7.781750 0.06828219 113.96456  0.0000
mrid40827601:sexM  8.021831 0.02553522 314.14765  0.0000
mrid44751201:sexM  7.860164 0.06828219 115.11293  0.0000
mrid44751201:dose -0.001704 0.00132308  -1.28826  0.2003

 Correlation:
                  dose   m40827601:F m44751201:F m40827601:M m44751201:M
mrid40827601:sexF -0.521
mrid44751201:sexF  0.000  0.000
mrid40827601:sexM -0.424  0.221       0.000
mrid44751201:sexM  0.000  0.000       0.536       0.000
mrid44751201:dose -0.110  0.057      -0.727       0.047      -0.727

Standardized residuals:
        Min          Q1         Med          Q3         Max
-2.63144750 -0.67797228  0.09412481  0.72825339  1.87933750

Residual standard error: 0.2548651
Degrees of freedom: 117 total; 111 residual
```

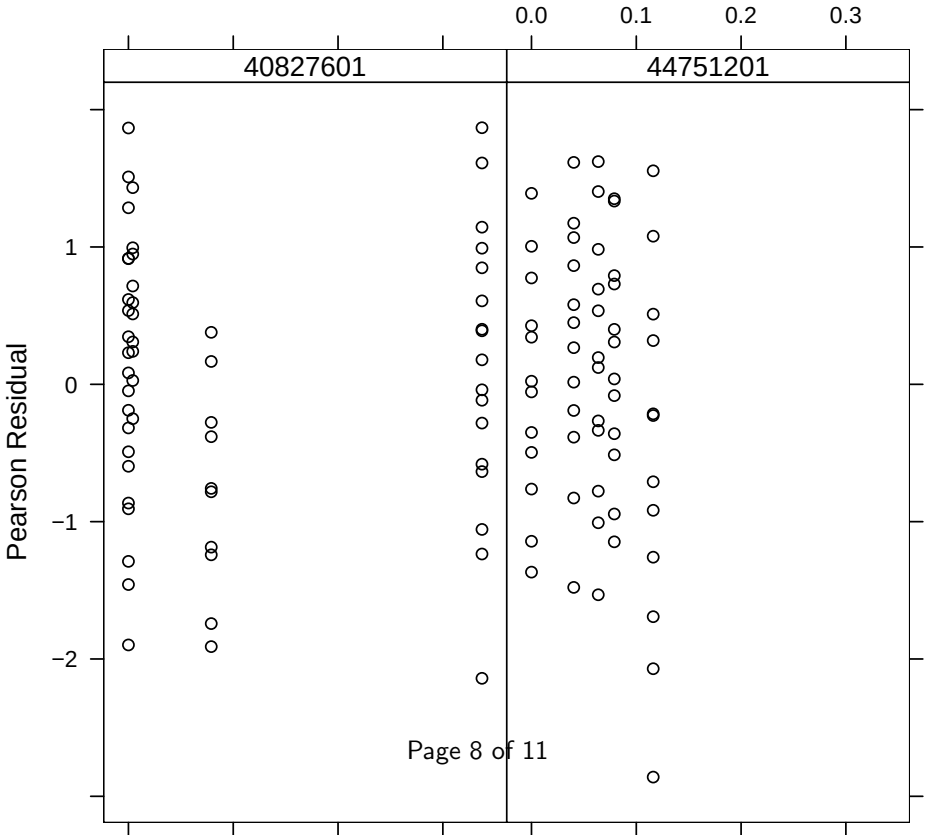
The term for the interaction of dose and slope is not significant.

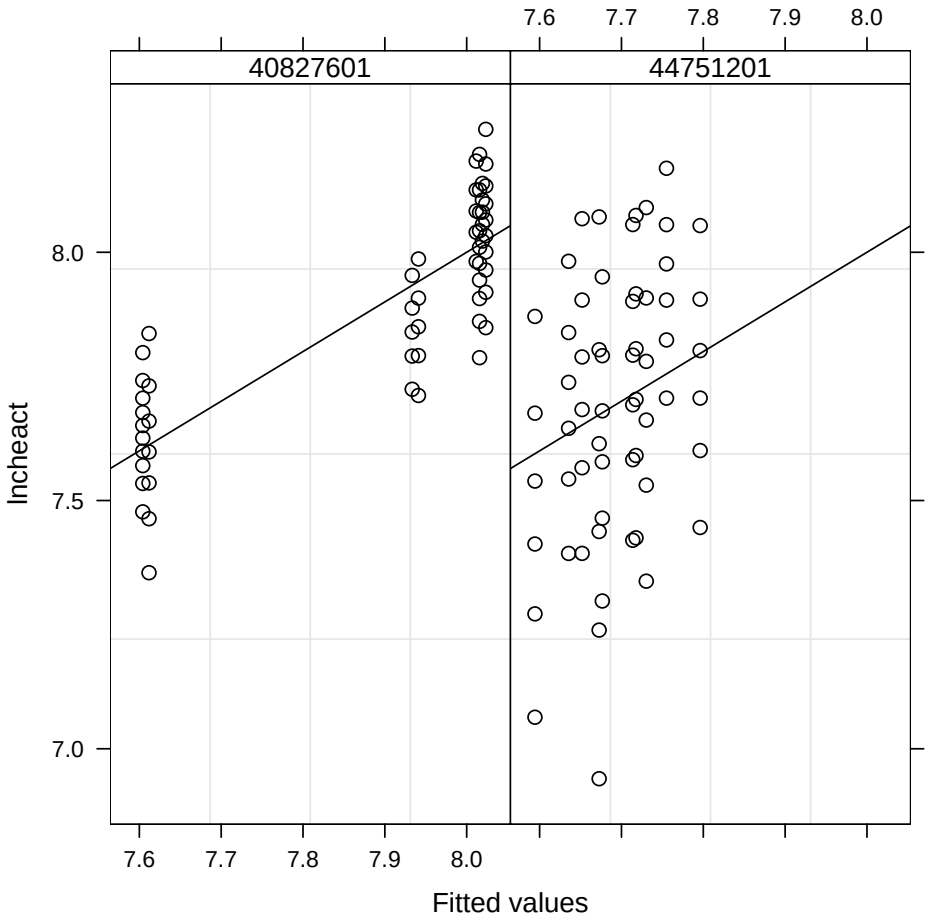
We will use the results in drmod3 to estimate BMDs.

3 Model Summary

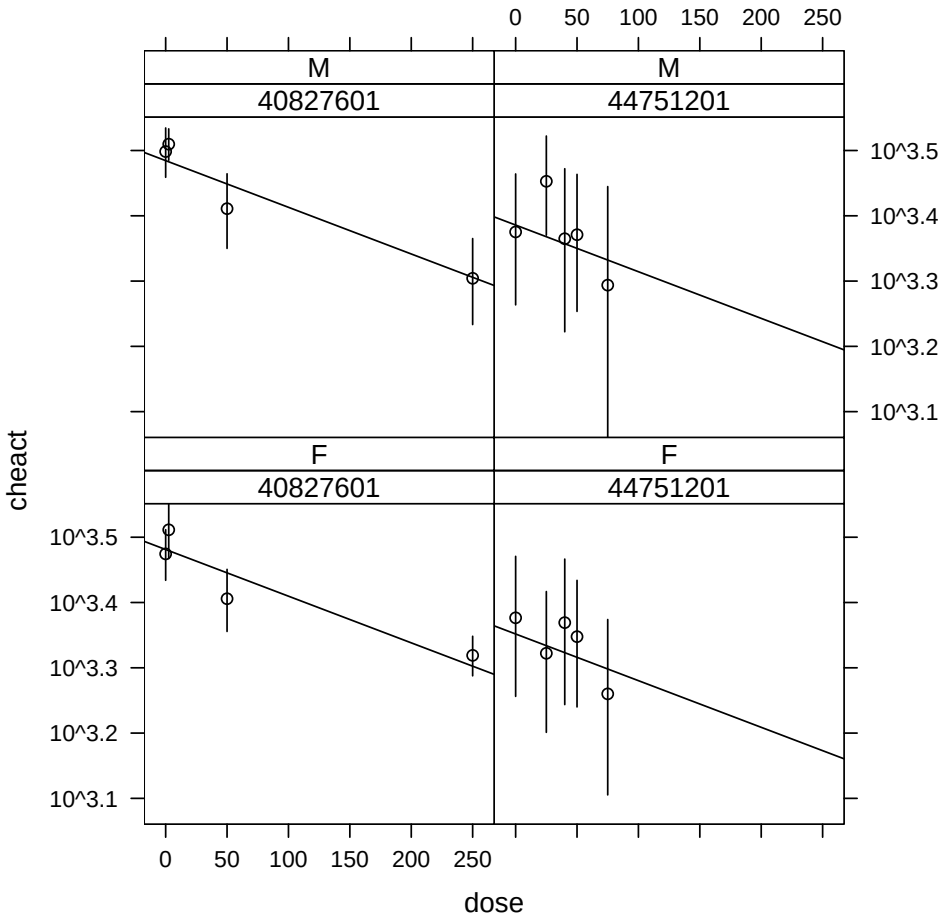
Diagnostic plots for this model:


40827601
44751201
7.6
7.7
7.8
7.9
8.0
Standardized residuals
1
0
−1
−2
Fitted values


40827601
44751201
0.0
0.1
0.2
0.3
Pearson Residual
1
0
−1
−2



The following figure shows the mean AChE values and their 95% confidence intervals, and the fitted line from `drmod3`.



4 Calculating BMDs

We have essentially fit the model

$$Y = Ae^{m \times dose}$$

with a constant coefficient of variation model for the error variance. In this model, for a BMD that results in BMR$\times$100% inhibition, the BMD is calculated as:

$$\frac{log(1 - BMR)}{m}$$

For consistency with the other endpoints and models, and to be able to calculate RPFs with confidence intervals, we need log BMD and its standard error. log BMD is just the log of the above quantity, and its standard error is, based on the delta method:

$$\hat{\sigma}_{lD} = \frac{\hat{\sigma}_m}{m}$$

The following code calculates the BMDs, etc.

```
> tTab <- summary(drmod3)$tTable
> Ints <- intervals(drmod3, which = "coef")$coef
> Ints90 <- intervals(drmod3, which = "coef", level = 0.9)$coef
> BMD <- log(1 - 0.1)/Ints["dose", "est."]
> BMDL <- log(1 - 0.1)/Ints90["dose", "lower"]
```

```
> BMD.CI <- log(1 - 0.1)/Ints["dose", c("lower", "upper")]
> if (BMD.CI[2] < 0) BMD.CI[2] <- Inf
> lD <- log(BMD)
> lD.se <- tTab["dose", "Std.Error"]/tTab["dose", "Value"]
```

5 Summary

The critical estimates from this analysis are listed below. They are printed with greater than usual precision, in case they are to be used in further computation. For reporting, round to two or three significant digits. BMD has units mg/kg.

species RABBIT

mrid `> levels(dta$mrid)`

```
[1] "40827601" "44751201"
```

`lD` **(standard error)** 4.15900670986409 (-0.0877869227009205)

BMD (95% CI) 64.0079125853754,(54.524074908726, 77.4856536073198)

BMDL, the one-sided lower 95% CL 55.8727805779971

Save everything:

```
> save.image(file = "DermalRBCDR.RData")
```

Save the results for incorporating into a database:

```
> oxamyl.dermal.rbc <- list(mrid = levels(dta$mrid), species = levels(dta$species),
+     BMD = list(combined = list(lD = lD, lD.se = lD.se, BMD = BMD,
+         BMD.CI = BMD.CI, BMDL = BMDL)))
> save(oxamyl.dermal.rbc, file = file.path("..", "..", "01Summaries",
+     "oxamyl.dermal.rbc.RData"))
```