Dose Response Modeling of Rat Brain AChE Activity: Oxamyl Inhalation Exposure

June 24, 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. Per an email from Anna Lowit, 6/17/2005, there is a concurrent control for each treatment group in this study, and the controls and treatment groups are interleaved in this file, so a block variable needs to be created to account for the different groups:

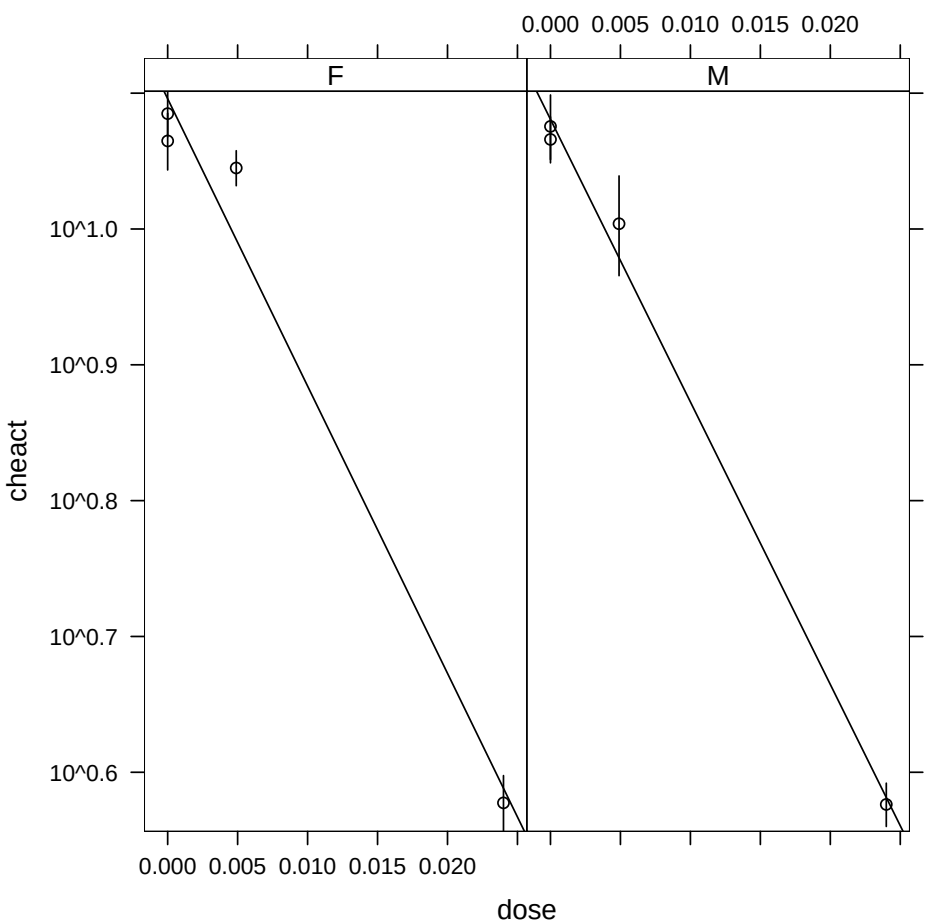
```
> dta <- CleanUp(subset(AggData, chemical %in% "OXAMYL" & dsmtd %in%
+     "INHALATION" & sctn %in% "WHOLEBRAIN", select = c("cheact",
+     "sd", "n", "dose", "tmonstdy", "sctn", "species", "tmpstds",
+     "sex", "mrid")))
> dta$block <- factor(rep(rep(c(1, 2), c(2, 2)), 2))
> idta <- with(dta, PhonyDF(dose, n, cheact, sd, "dose", "cheact",
+     Avals = dta[, c("sex", "block")]))
> idta$lncheact <- log(idta$cheact)
```

Summary of the relevant variables in this dataset:

```
> summary(dta)

     cheact             sd               n              dose
 Min.   : 3.770   Min.   :0.1800   Min.   : 9.00   Min.   :0.000000
 1st Qu.: 8.512   1st Qu.:0.4075   1st Qu.: 9.75   1st Qu.:0.000000
 Median :11.350   Median :0.6250   Median :10.00   Median :0.002450
```

```
 Mean   : 9.505   Mean   :0.6275   Mean   : 9.75   Mean   :0.007225
 3rd Qu.:11.705   3rd Qu.:0.8125   3rd Qu.:10.00   3rd Qu.:0.009675
 Max.   :12.160   Max.   :1.1900   Max.   :10.00   Max.   :0.024000

    tmonstdy         sctn    species    tmpstds    sex         mrid    block
 Min.   :1   WHOLEBRAIN:8   RAT:8   Min.   : NA   F:4   45155801:8   1:4
 1st Qu.:1                          1st Qu.: NA   M:4                 2:4
 Median :1                          Median : NA
 Mean   :1                          Mean   :NaN
 3rd Qu.:1                          3rd Qu.: NA
 Max.   :1                          Max.   : NA
                                    NA's   :  8

> with(dta, table(dose, tmpstds))

  0
  0.0049
  0.024
```

Plots of RBC AChE activity versus dose, by 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 figure describe the overall trend of the data, but there is a suggestion that the slope at lower doses differs from that at the higher doses, especially in the female data. Some preliminary analysis, repeated here:

```
> tmp <- lm(lncheact ~ block:sex - 1 + block:dose + block:sex:dose,
+     data = idta)
> tmp2 <- lm(lncheact ~ block:sex - 1 + sex:dose, data = idta)
> tmp3 <- lm(lncheact ~ block:sex - 1 + block:dose, data = idta)
> anova(tmp, tmp2)

Analysis of Variance Table

Model 1: lncheact ~ block:sex - 1 + block:dose + block:sex:dose
Model 2: lncheact ~ block:sex - 1 + sex:dose
  Res.Df      RSS Df Sum of Sq      F    Pr(>F)
1     70  0.33880
2     72  0.44768 -2  -0.10888 11.248 5.807e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> anova(tmp, tmp3)

Analysis of Variance Table

Model 1: lncheact ~ block:sex - 1 + block:dose + block:sex:dose
Model 2: lncheact ~ block:sex - 1 + block:dose
  Res.Df      RSS Df Sum of Sq      F Pr(>F)
1     70  0.33880
2     72  0.35371 -2  -0.01491 1.5403 0.2215

> summary(tmp3)

Call:
lm(formula = lncheact ~ block:sex - 1 + block:dose, data = idta)

Residuals:
      Min        1Q    Median        3Q       Max
-0.214180 -0.043839  0.001953  0.046057  0.174054

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
block1:sexF   2.51609    0.01919 131.082  < 2e-16 ***
block2:sexF   2.45106    0.01936 126.588  < 2e-16 ***
block1:sexM   2.45464    0.01919 127.880  < 2e-16 ***
block2:sexM   2.45195    0.02005 122.262  < 2e-16 ***
block1:dose -26.54866    4.52333  -5.869 1.24e-07 ***
block2:dose -46.85525    0.94750 -49.451  < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.07009 on 72 degrees of freedom
Multiple R-Squared: 0.9991,        Adjusted R-squared: 0.999
F-statistic: 1.297e+04 on 6 and 72 DF,  p-value: < 2.2e-16
```

says that the slopes differ across blocks, but not between sexes. In order to capture the curvature, ignore the blocking (the control values are quite similar) since there are not enough doses otherwise to fit other than a linear model. That the confidence intervals do not seem to vary much in length across the doses suggests we can model log(cheact) as a power of dose.

Fitting the model will follow these steps:

1. Create a pseudo-individual dataset, and log the `cheact` variable (already done).
2. Use gnls to fit a power model, where the intercept and coefficient differs across `sex`, but power is a constant (to borrow information across the sexes about the power).
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.

Fit the model:

```
> drmod1 <- gnls(lncheact ~ lA + m * dose^g, data = idta, params = list(lA ~
+     sex - 1, m ~ sex, g ~ 1), start = c(2.5, 2.5, -49, 0, 1))
> summary(drmod1)

Generalized nonlinear least squares fit
  Model: lncheact ~ lA + m * dose^g
  Data: idta
        AIC       BIC   logLik
  -181.6935 -167.5532 96.84674

Coefficients:
                   Value Std.Error   t-value p-value
lA.sexF          2.48759   0.01500 165.86411  0.0000
lA.sexM          2.44873   0.01525 160.57050  0.0000
m.(Intercept) -266.63740 106.48655  -2.50395  0.0145
m.sexM           7.02774   9.48834   0.74067  0.4613
g                1.45864   0.10802  13.50342  0.0000

 Correlation:
              lA.sxF lA.sxM m.(In) m.sexM
lA.sexM        0.171
m.(Intercept)  0.385  0.411
m.sexM         0.205 -0.460 -0.336
g             -0.415 -0.412 -0.998  0.298

Standardized residuals:
        Min          Q1         Med          Q3         Max
-3.14691856 -0.62880724  0.05722224  0.66361988  2.22557979

Residual standard error: 0.07226332
Degrees of freedom: 78 total; 73 residual

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

Note the sex effect on dose is non-significant: P-value = 0.46, so refit with a single slope for dose:

```
> drmod2 <- gnls(lncheact ~ lA + m * dose^g, data = idta, params = list(lA ~
+     sex - 1, m ~ 1, g ~ 1), start = c(2.5, 2.5, -49, 1))
> summary(drmod2)
```

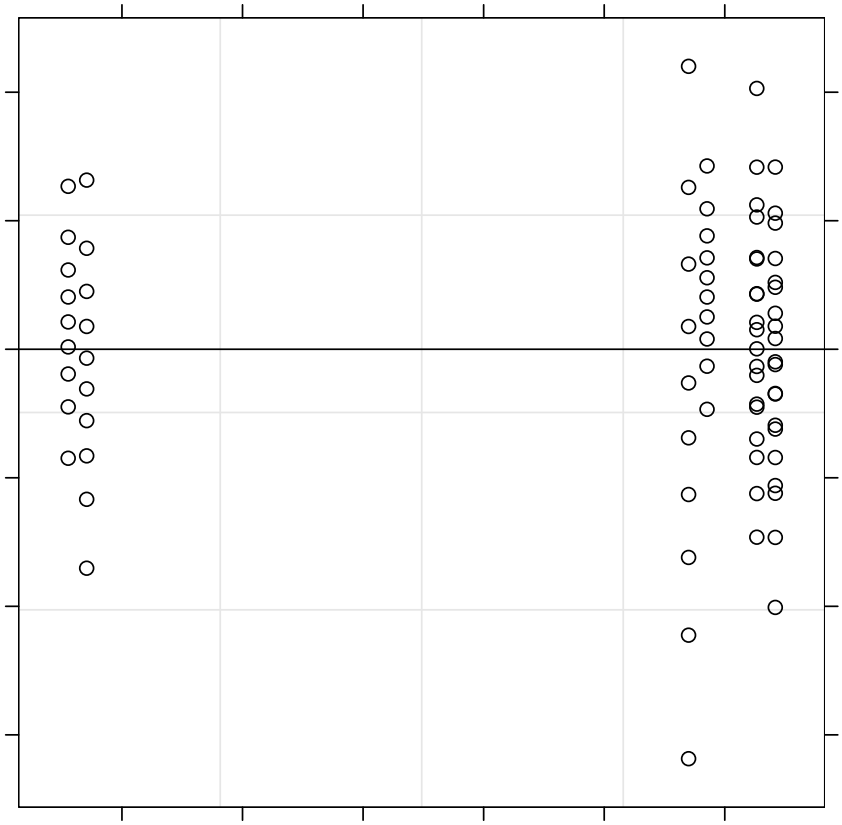
```
Generalized nonlinear least squares fit
  Model: lncheact ~ lA + m * dose^g
  Data: idta
        AIC       BIC   logLik
  -183.0540 -171.2705 96.52701

Coefficients:
             Value Std.Error   t-value p-value
lA.sexF    2.48380   0.01402 177.20855  0.0000
lA.sexM    2.45313   0.01426 172.05260  0.0000
m       -259.79541 102.71338  -2.52932  0.0136
g          1.45503   0.10704  13.59380  0.0000

 Correlation:
        lA.sxF lA.sxM m
lA.sexM  0.333
m        0.421  0.428
g       -0.438 -0.444 -0.999

Standardized residuals:
       Min         Q1        Med         Q3        Max
-3.1854580 -0.5821632  0.0818568  0.6927751  2.2015860

Residual standard error: 0.0720682
Degrees of freedom: 78 total; 74 residual
```

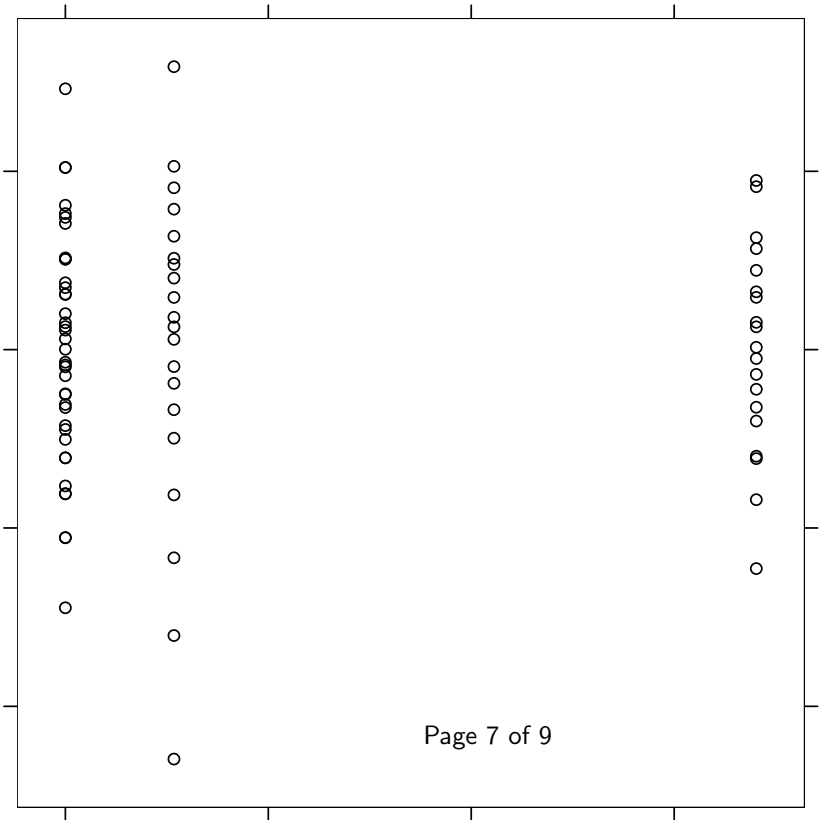
Note that the power, g, is significantly greater than 1, so this model is more appropriate than the strictly linear model.

We will use the results in `drmod2` to estimate BMDs.

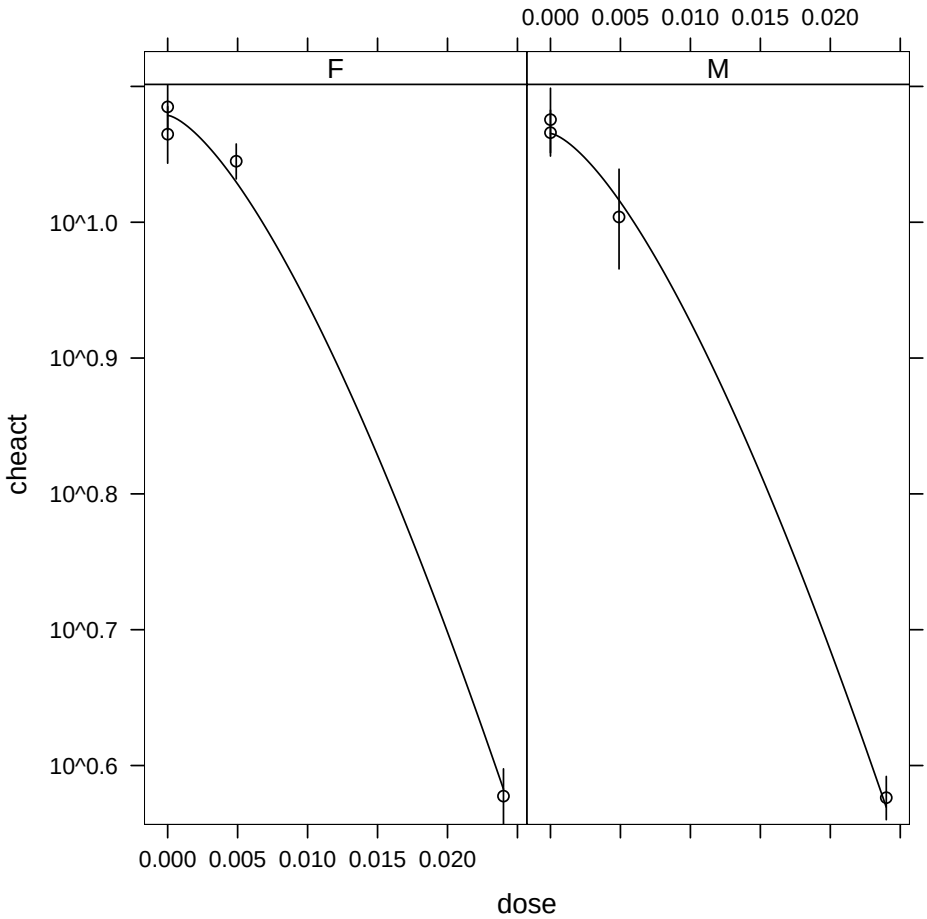
3 Model Summary

Diagnostic plots for this model:





shows the mean AChE values and their 95% confidence intervals, and the fitted line from `drmod2`.



4 Calculating BMDs

We have essentially fit the model

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

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:

$$\left(\frac{log(1 - BMR)}{m}\right)^{\frac{1}{g}}$$

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:

$$\hat{\sigma}_{lD} = \frac{\hat{\sigma}_m}{|gm|}$$

The following code calculates the BMDs, etc.

```
> tTab <- summary(drmod2)$tTable
> Ints <- intervals(drmod2, which = "coef")$coef
> Ints90 <- intervals(drmod2, level = 0.9, which = "coef")$coef
```

```
> BMD <- log(1 - 0.1)/tTab["m", "Value"]
> BMDL <- log(1 - 0.1)/Ints90["m", "lower"]
> BMD.CI <- log(1 - 0.1)/Ints["m", c("lower", "upper")]
> if (BMD.CI[2] < 0) BMD.CI[2] <- Inf
> lD <- log(BMD)
> lD.se <- tTab["m", "Std.Error"]/abs(tTab["g", "Value"] * tTab["m",
+     "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/L.

species RAT

mrid `[1] "45155801"`

`lD` **(standard error)** -7.81026177484887 (0.271721474820111)

BMD (95% CI) 0.000405551870643857,(0.000226846999132681, 0.00191097131334738)

BMDL, the one-sided lower 95% CL 0.000244520743414806

Save everything:

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

Save the results for incorporating into a database:

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