Dose Response Modeling of Rabbit Brain 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 dermal exposure.

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% c("HALFBRAIN", "WHOLEBRAIN") & (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:

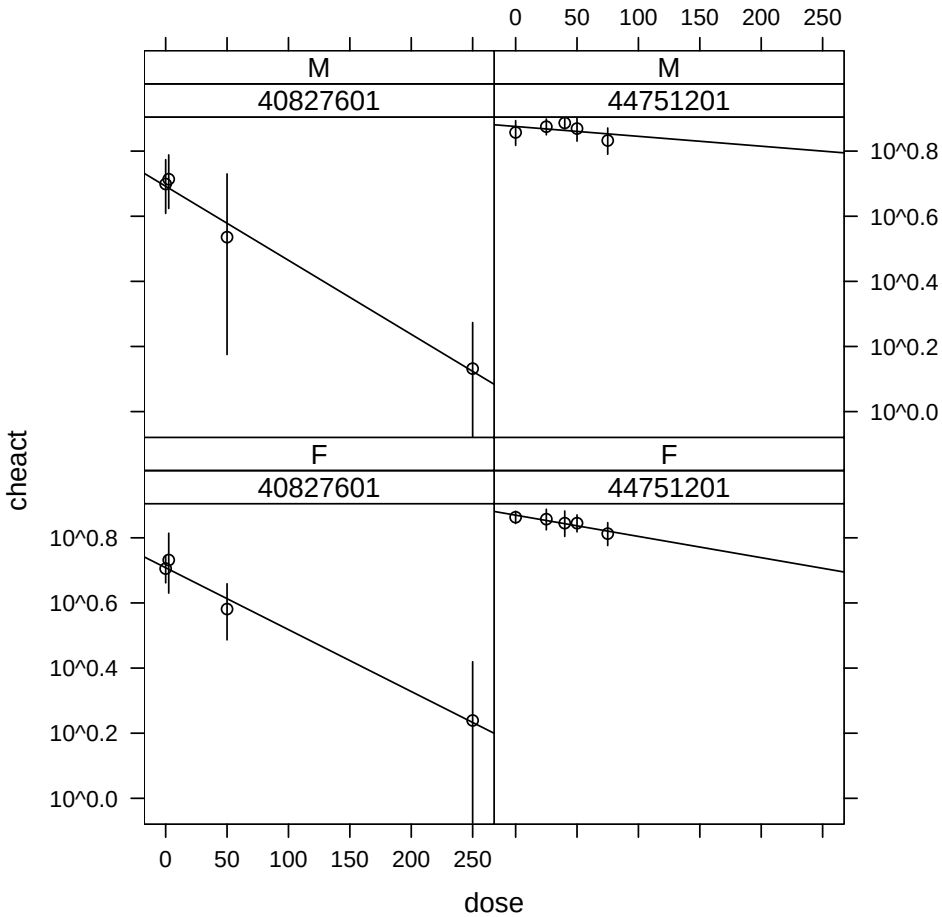
```
> by(dta, dta$mrid, summary)

dta$mrid: 40827601
     cheact            sd               n              dose
 Min.   :1.355   Min.   :0.3280   Min.   :4.000   Min.   :  0.000
 1st Qu.:3.009   1st Qu.:0.5495   1st Qu.:5.000   1st Qu.:  1.875
 Median :4.407   Median :0.7375   Median :5.000   Median : 26.250
 Mean   :3.873   Mean   :0.7555   Mean   :4.875   Mean   : 75.625
 3rd Qu.:5.101   3rd Qu.:0.8117   3rd Qu.:5.000   3rd Qu.:100.000
 Max.   :5.394   Max.   :1.5600   Max.   :5.000   Max.   :250.000
    tmonstdy          sctn     species     tmpstds  sex         mrid
 Min.   :22   HALFBRAIN :0   RABBIT:8   Min.   :1   F:4   40827601:8
 1st Qu.:22   WHOLEBRAIN:8              1st Qu.:1   M:4   44751201:0
```

```
 Median :22                              Median :1
 Mean   :22                              Mean   :1
 3rd Qu.:22                              3rd Qu.:1
 Max.   :22                              Max.   :1
-------------------------------------------------------------
dta$mrid: 44751201
     cheact            sd             n          dose       tmonstdy
 Min.   :6.500   Min.   :0.30   Min.   :6   Min.   : 0   Min.   :21
 1st Qu.:7.000   1st Qu.:0.40   1st Qu.:6   1st Qu.:25   1st Qu.:21
 Median :7.200   Median :0.50   Median :6   Median :40   Median :21
 Mean   :7.160   Mean   :0.48   Mean   :6   Mean   :38   Mean   :21
 3rd Qu.:7.375   3rd Qu.:0.60   3rd Qu.:6   3rd Qu.:50   3rd Qu.:21
 Max.   :7.700   Max.   :0.60   Max.   :6   Max.   :75   Max.   :21

         sctn      species      tmpstds    sex         mrid
 HALFBRAIN :10   RABBIT:10   Min.   : NA   F:5   40827601: 0
 WHOLEBRAIN: 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
  -72.62245 -49.82905 45.31122

Random effects:
 Formula: ~dose - 1 | mrid
               dose Residual
StdDev: 0.002657956 0.263514

Variance function:
 Structure: Different standard deviations per stratum
 Formula: ~1 | mrid
 Parameter estimates:
 40827601  44751201
1.0000000 0.2767349
Fixed effects: lncheact ~ mrid:sex - 1 + dose + sex:dose
                      Value  Std.Error DF  t-value p-value
dose              -0.0031038 0.00191967 92 -1.61684  0.1093
mrid40827601:sexF  1.6516060 0.06972627 92 23.68700  0.0000
mrid44751201:sexF  1.9903778 0.02222238 92 89.56635  0.0000
mrid40827601:sexM  1.5307187 0.06957855 92 21.99987  0.0000
mrid44751201:sexM  2.0279151 0.02223067 92 91.22150  0.0000
sexM:dose          0.0001802 0.00056273 92  0.32031  0.7495
 Correlation:
                  dose   m40827601:F m44751201:F m40827601:M m44751201:M
mrid40827601:sexF -0.090
mrid44751201:sexF -0.131  0.138
mrid40827601:sexM -0.003  0.101      -0.137
mrid44751201:sexM  0.004 -0.132       0.178       0.143
sexM:dose         -0.141  0.280       0.481      -0.291      -0.481

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-2.92362650 -0.51205543  0.04976047  0.66124789  2.32222310

Number of Observations: 99
Number of Groups: 2

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

Note the sex effect on dose is non-significant: P-value = 0.75, 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):

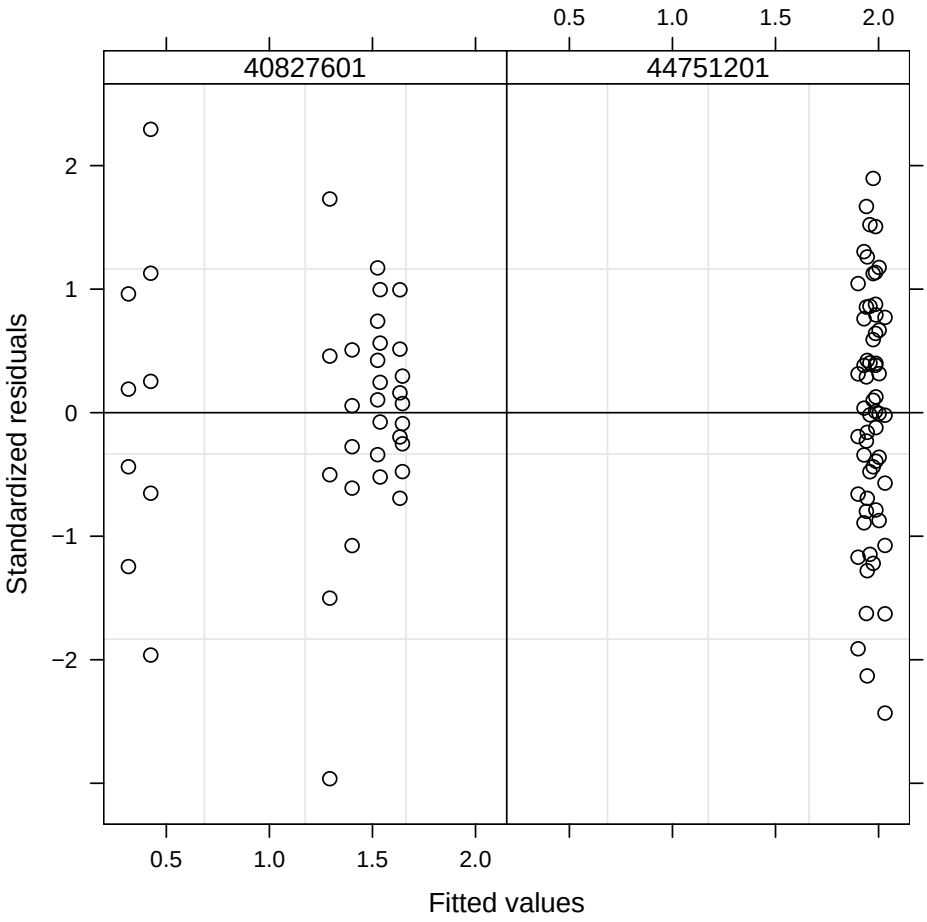
```
> anova(drmod2, drmod3)

       Model df       AIC       BIC   logLik   Test  L.Ratio p-value
drmod2     1  8 -87.65679 -67.31043 51.82840
drmod3     2  7 -58.06036 -40.25730 36.03018 1 vs 2 31.59643  <.0001
```

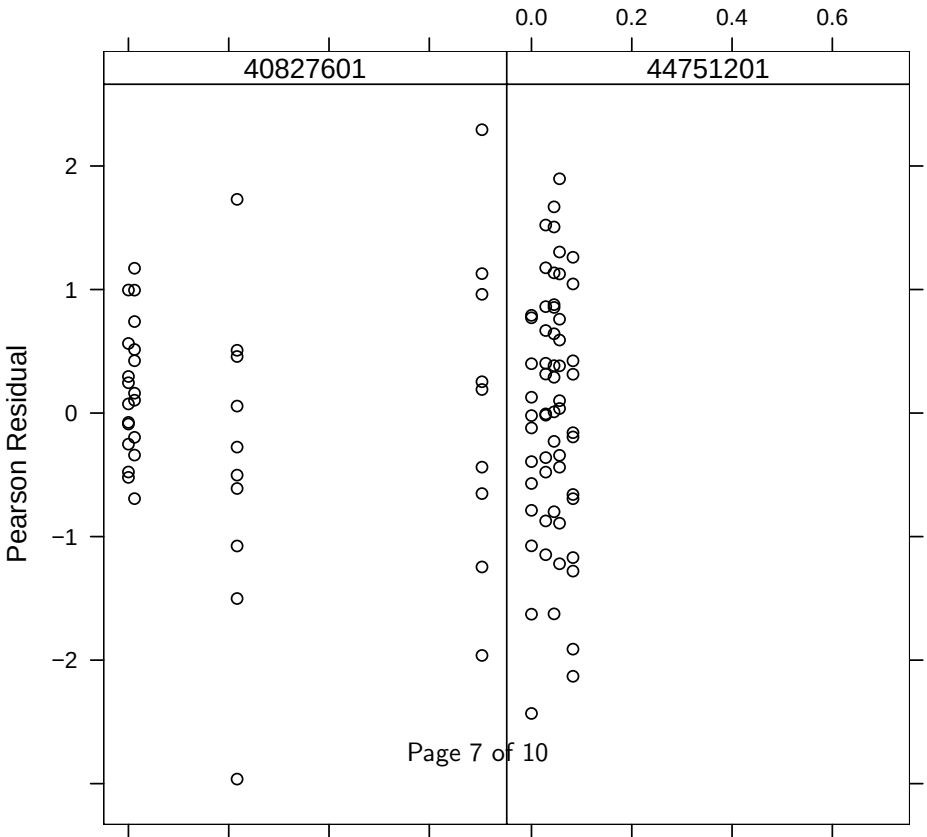
Both AIC is lower for the first model, and the P-value is quite small. These two studies give different estimates of the dose-response slope. The term for the interaction of dose and slope is not significant.

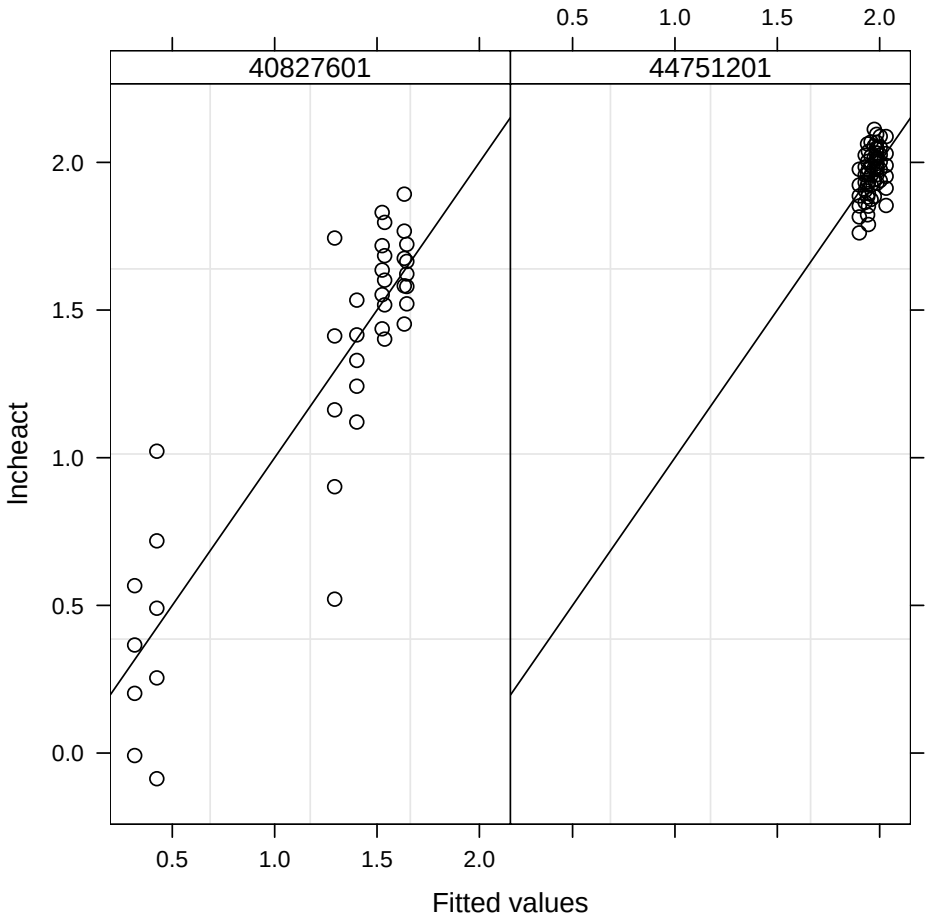
We will use the results in drmod2 to estimate BMDs.

3 Model Summary

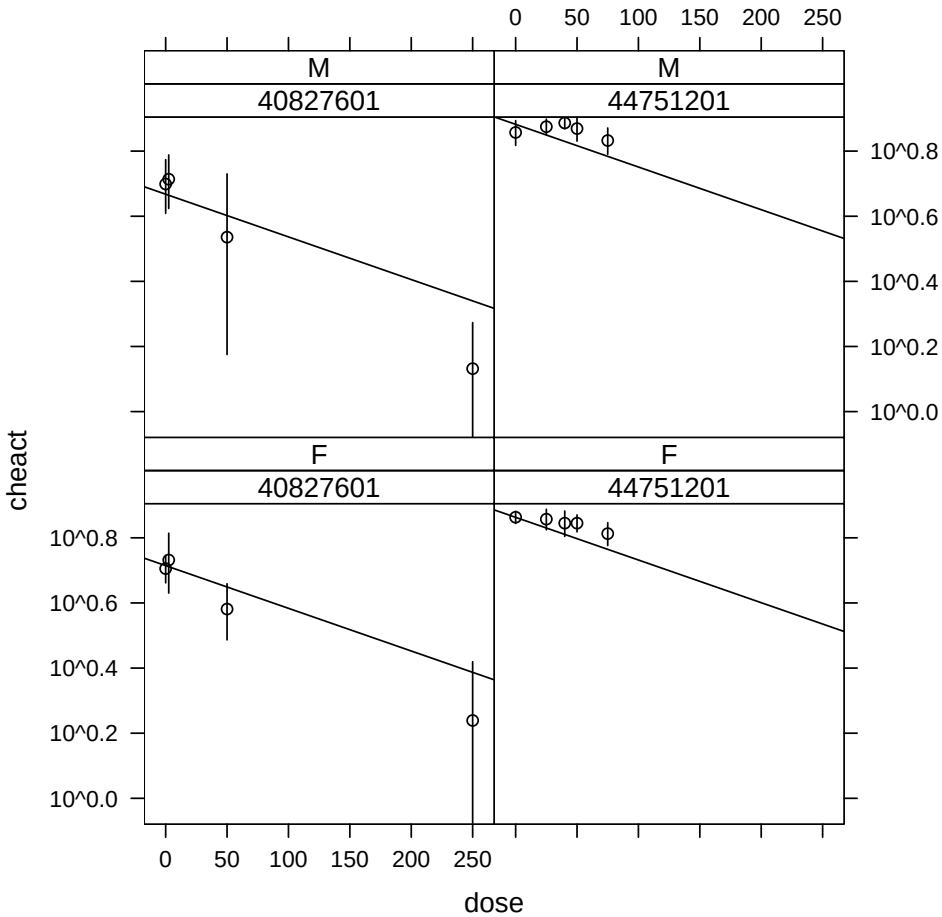
Diagnostic plots for this model:


40827601
44751201
Standardized residuals
Fitted values


40827601
44751201
Pearson Residual



The following figure 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

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

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(drmod2)$tTable
> Ints <- intervals(drmod2, which = "fixed")$fixed
> Ints90 <- intervals(drmod2, which = "fixed", level = 0.9)$fixed
> 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 `[1] "40827601" "44751201"`

`lD` **(standard error)** 3.55283327124806 (-0.630637894406188)

BMD (95% CI) 34.9120929218122,(15.5004900990242, Inf)

BMDL, the one-sided lower 95% CL 17.0490505499596

Save everything:

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

Save the results for incorporating into a database:

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