Dose-Time Response Modeling of Rat RBC AChE Activity: Pirimicarb Gavage Dosing

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` and `repeated_subchronic`. 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
----------------------------------------

> printDataDoc(repeated_subchronic)

----------------------------------------
Data set: repeated_subchronic

Dataset creation date: Wed Jun 22 12:07:28 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 red blood cells. via gavage dosing. It includes acute and subchronic studies.

All the data used for this DR model are from mrids 113638, 44233103, and 44485301, datasets `AggData` and `repeated_subchronic`. `AggData` has aggregated versions of all the data, while `repeated_subchronic` has individual animal versions of 113638 and 44233103, which use a repeated measures design. The data need to be extracted from the appropriate data set, the aggregated data converted to a pseudo-individual dataset,

then all the data combined. The response variable for 113638 needs to be scaled by 600, and that for the other two studies by 1000, to bring the response variable into the approximate range (0,10), which seems to make it somewhat easier to get model convergence.

Now, set up the analysis dataset.

```
> dta <- CleanUp(subset(AggData, mrid == "44485301" & species %in%
+     "RAT" & dsmtd %in% "GAVAGE" & sctn %in% "RBC" & !is.na(cheact) &
+     (n == 1 | !is.na(sd)), select = c("cheact", "sd", "n", "dose",
+     "tmonstdy", "tmpstds", "sex", "mrid")))
> idta <- with(dta, PhonyDF(dose, n, cheact, sd, "dose", "cheact",
+     unclass(dta[, c("tmonstdy", "tmpstds", "sex", "mrid")])))
> idta$id <- seq(along = idta$dose)
> idta$cheact <- idta$cheact/2000
> Idta <- CleanUp(subset(repeated_subchronic, chemical %in% "Primicarb",
+     select = c("dose_mgkg", "id", "sex", "rbc", "time", "mrid")))
> names(Idta) <- c("dose", "id", "sex", "cheact", "tmonstdy", "mrid")
> Idta$tmpstds <- rep(1, nrow(Idta))
> Idta$cheact <- Idta$cheact
> idta <- rbind(idta, Idta[, names(idta)])
> idta <- idta[!is.na(idta$cheact), ]
```

Summary of the relevant variables in this dataset:

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

idta$mrid: 44485301
      dose            cheact          tmonstdy    tmpstds    sex
 Min.   :  0.00   Min.   :0.7737   Min.   :1   Min.   :  3   F:36
 1st Qu.:  0.00   1st Qu.:1.0389   1st Qu.:1   1st Qu.:  3   M:38
 Median : 10.00   Median :1.1020   Median :1   Median :  3
 Mean   : 36.62   Mean   :1.1019   Mean   :1   Mean   :156
 3rd Qu.: 40.00   3rd Qu.:1.1709   3rd Qu.:1   3rd Qu.:336
 Max.   :110.00   Max.   :1.3507   Max.   :1   Max.   :336
       mrid         id
 44485301:74   Length:74
 44233103: 0   Class :character
 113638  : 0   Mode  :character



------------------------------------------------------------
idta$mrid: 44233103
      dose           cheact         tmonstdy         tmpstds  sex
 Min.   : 0.00   Min.   :0.650   Min.   :  0.00   Min.   :1   F:198
 1st Qu.: 6.25   1st Qu.:1.060   1st Qu.: 14.00   1st Qu.:1   M:200
 Median :25.00   Median :1.170   Median : 42.00   Median :1
 Mean   :31.28   Mean   :1.193   Mean   : 51.52   Mean   :1
 3rd Qu.:62.50   3rd Qu.:1.310   3rd Qu.: 84.00   3rd Qu.:1
 Max.   :75.00   Max.   :1.780   Max.   :112.00   Max.   :1
       mrid          id
 44485301:  0   Length:398
 44233103:398   Class :character
 113638  :  0   Mode  :character
```

```
------------------------------------------------------------
idta$mrid: 113638
      dose            cheact          tmonstdy         tmpstds  sex
 Min.   : 0.000   Min.   :0.4600   Min.   :-7.000   Min.   :1   F:76
 1st Qu.: 0.000   1st Qu.:0.6700   1st Qu.:-7.000   1st Qu.:1   M:79
 Median : 3.000   Median :0.7500   Median :-7.000   Median :1
 Mean   : 3.919   Mean   :0.7683   Mean   : 6.955   Mean   :1
 3rd Qu.: 7.750   3rd Qu.:0.8500   3rd Qu.:21.000   3rd Qu.:1
 Max.   :12.500   Max.   :1.2500   Max.   :28.000   Max.   :1
       mrid          id
 44485301:  0   Length:155
 44233103:  0   Class :character
 113638  :155   Mode  :character
```

```
> unique(subset(idta, select = c("mrid", "tmonstdy", "tmpstds")))

         mrid tmonstdy tmpstds
1    44485301        1       3
21   44485301        1     336
712  44233103        0       1
717  44233103        7       1
722  44233103       14       1
727  44233103       28       1
732  44233103       42       1
737  44233103       56       1
742  44233103       70       1
747  44233103       84       1
752  44233103      105       1
757  44233103      112       1
7131   113638       -7       1
7151   113638       14       1
7161   113638       28       1
```

```
> by(idta, idta$mrid, function(x) with(x, table(dose, tmonstdy)))

idta$mrid: 44485301
     tmonstdy
dose  1
  0   20
  10  19
  40  19
  110 16
------------------------------------------------------------
idta$mrid: 44233103
    tmonstdy
dose 0  7  14 28 42 56 70 84 105 112
  0  10 10 10 10 10 10 10 10 10  10
  25 20 20 20 20 20 20 20 20 19  19
  75 10 10 10 10 10 10 10 10 10  10
------------------------------------------------------------
idta$mrid: 113638
      tmonstdy
dose   -7 14 28
  0    39 18 19
```

```
  3    20 10 10
  12.5 19 10 10
```

```
> by(idta, idta$mrid, function(x) with(x, if (as.character(mrid[1]) ==
+     "44485301") table(id, tmpstds) else table(id, tmonstdy)))
```

```
idta$mrid: 44485301
    tmpstds
id   3 336
  1  1 0
  10 1 0
  11 1 0
  12 1 0
  13 1 0
  14 1 0
  15 1 0
  16 1 0
  17 1 0
  18 1 0
  19 1 0
  2  1 0
  20 1 0
  21 0 1
  22 0 1
  23 0 1
  24 0 1
  25 0 1
  26 0 1
  27 0 1
  28 0 1
  29 0 1
  3  1 0
  30 0 1
  31 0 1
  32 0 1
  33 0 1
  34 0 1
  35 0 1
  36 0 1
  37 0 1
  38 0 1
  39 1 0
  4  1 0
  40 1 0
  41 1 0
  42 1 0
  43 1 0
  44 1 0
  45 1 0
  46 1 0
  47 1 0
  48 1 0
  49 1 0
  5  1 0
  50 1 0
```

```
  51 1 0
  52 1 0
  53 1 0
  54 1 0
  55 1 0
  56 1 0
  57 1 0
  58 1 0
  59 0 1
  6  1 0
  60 0 1
  61 0 1
  62 0 1
  63 0 1
  64 0 1
  65 0 1
  66 0 1
  67 0 1
  68 0 1
  69 0 1
  7  1 0
  70 0 1
  71 0 1
  72 0 1
  73 0 1
  74 0 1
  8  1 0
  9  1 0
-------------------------------------------------------------
idta$mrid: 44233103
     tmonstdy
id    0 7 14 28 42 56 70 84 105 112
  1   1 1 1  1  1  1  1  1  1   1
  101 1 1 1  1  1  1  1  1  1   1
  102 1 1 1  1  1  1  1  1  1   1
  103 1 1 1  1  1  1  1  1  1   1
  104 1 1 1  1  1  1  1  1  1   1
  105 1 1 1  1  1  1  1  1  1   1
  136 1 1 1  1  1  1  1  1  1   1
  137 1 1 1  1  1  1  1  1  1   1
  138 1 1 1  1  1  1  1  1  1   1
  139 1 1 1  1  1  1  1  1  1   1
  140 1 1 1  1  1  1  1  1  1   1
  151 1 1 1  1  1  1  1  1  1   1
  152 1 1 1  1  1  1  1  1  1   1
  153 1 1 1  1  1  1  1  1  1   1
  154 1 1 1  1  1  1  1  1  1   1
  155 1 1 1  1  1  1  1  1  1   1
  186 1 1 1  1  1  1  1  1  1   1
  187 1 1 1  1  1  1  1  1  1   1
  188 1 1 1  1  1  1  1  1  1   1
  189 1 1 1  1  1  1  1  1  1   1
  190 1 1 1  1  1  1  1  1  1   1
  2   1 1 1  1  1  1  1  1  1   1
```

```
  3   1 1 1  1  1  1  1  1  1   1
  36  1 1 1  1  1  1  1  1  1   1
  37  1 1 1  1  1  1  1  1  1   1
  38  1 1 1  1  1  1  1  1  1   1
  39  1 1 1  1  1  1  1  1  1   1
  4   1 1 1  1  1  1  1  1  1   1
  40  1 1 1  1  1  1  1  1  1   1
  5   1 1 1  1  1  1  1  1  1   1
  51  1 1 1  1  1  1  1  1  1   1
  52  1 1 1  1  1  1  1  1  1   1
  53  1 1 1  1  1  1  1  1  1   1
  54  1 1 1  1  1  1  1  1  1   1
  55  1 1 1  1  1  1  1  1  1   1
  86  1 1 1  1  1  1  1  1  1   1
  87  1 1 1  1  1  1  1  1  1   1
  88  1 1 1  1  1  1  1  1  0   0
  89  1 1 1  1  1  1  1  1  1   1
  90  1 1 1  1  1  1  1  1  1   1
------------------------------------------------------------
idta$mrid: 113638
    tmonstdy
id   -7 14 28
  1  4  2  2
  10 3  2  2
  11 4  2  2
  12 4  2  2
  13 4  2  2
  14 4  2  2
  15 4  1  2
  16 4  2  2
  17 4  2  1
  18 3  1  2
  19 4  2  2
  2  4  2  2
  20 4  2  2
  3  4  2  2
  4  4  2  2
  5  4  2  2
  6  4  2  2
  7  4  2  2
  8  4  2  2
  9  4  2  2
```

These are all essentially subchronic studies. The study 44485301 has a two week point after the gavage dose. This was an acute neurotox study, and presumably this point was primarily to evaluate residual neurotoxic effects. This is way too far out to evaluate AChE inhibition, and complicates the analysis, so just drop those values:

```
> idta <- CleanUp(subset(idta, !(mrid == "44485301" & tmpstds ==
+     336)))
```

Study 113638 indicates pre-study measurements with non-positive times; set the dose for those to 0:

```
> idta$dose2 <- ifelse(idta$tmonstdy <= 0, 0, dta$dose)
```

Create some new factors, for splitting up the background parameters and allowing groups to have different variances. We are going to allow `lD` to vary among times within studies, so make a factor `ftmonstdy`, and make

sure that the levels that correspond to control doses are combined with a non-control level (dose is set to 0 for those times, so no value of `lD` can be estimated for those times; if we try, we will end up with singularities somewhere, and the optimization will fail).

```
> idta$mridXtmonstdyXsex <- with(idta, interaction(mrid, tmonstdy,
+     sex, drop = TRUE, sep = ":"))
> idta$mridXtmonstdy <- with(idta, interaction(mrid, tmonstdy,
+     drop = TRUE, sep = ":"))
> idta$mridXsex <- with(idta, interaction(mrid, sex, drop = TRUE,
+     sep = ":"))
> idta$ftmonstdy <- factor(idta$tmonstdy)
> lvls <- levels(idta$ftmonstdy)
> lvls[1] <- "14"
> lvls[2] <- "7"
> levels(idta$ftmonstdy) <- lvls
> idta$mridXftmonstdy <- with(idta, interaction(mrid, ftmonstdy,
+     drop = TRUE, sep = ":"))
> idta$id <- factor(idta$id)
```

We have no useful time course data, so `lTr` will be fixed at log(1.5 hours), and `delta` at 0.5 hours, as we have done in all the other chemical.

2 Dose-Response Modeling

2.1 strategy

Use the model with simple exponential recovery (`tcmfn4()`). It looks as if the time to peak effect for all these chemicals is likely to be less than a half-hour (the earliest time point in this dataset), so the exponential recovery model is probably indistinguishable from the one with the more complex time course. Since there is no time course data, this is dose-response modeling, complicated by the fact that the responses are taken at different times after dosing (1 hour in two studies, 3 hours in the other).

Two of these studies looked at the same animals at multiple time points. It would make sense to think of animal and time point as random effects, crossed with each other, and nested within MRID. That model may be fittable with nlme(), but it is complicated. Let's first model just the repeated measures, treating mrid and time in mrid as nested fixed effects. Test whether the differences among times within mrid are different. If they are, then we need to first look to see if there is a trend, and, if not, try to fit the model with nested and crossed effects.

Fitting the model will follow these steps:

1. First, use `GetInitialValues()` to get starting values for the model against these data, and determine how finely we can estimate `lg` and `tz` of the dose-response parameters.

2. Next, fit `tcmfn4()` using the parameterizations determined in the previous step, and a constant variance model, allowing `lD` to vary among mrids.

   Try fitting with other variance models the same model for fixed and random effects, using AIC to identify the best variance model

3. Can we simplify the model for `lD`, that is, does `lD` differ among `tmonstdy` and sexes? Use contrasts in the best model identified in the previous section.

4. Can we simplify the model for `lTr`, first between sexes, then among doses.

2.2 Initial Values

Save the initial values so that we do not need to go through all this to re-run the analysis. Also, set the argument `delta` to 0.5, the earliest non-zero time point.

```
> formals(tcmfn4)$delta <- 0.5
> formals(tcmfn4)$lTr <- log(1.5)
> initfile <- paste("initvals-RBC-DR-1.RData", sep = "")
> if (!file.exists(initfile)) {
+     lA.start <- lm(I(log(cheact)) ~ mridXtmonstdyXsex - 1, data = CleanUp(subset(idta,
+         dose2 %in% 0)))
+     Start <- c(coef(lA.start), rep(log(2), nlevels(idta$mridXftmonstdy)),
+         0, -2, 0, log(1), 0, )
+     init1 <- GetInitialValues(cheact ~ tcmfn4(dose2, tmpstds,
+         lA = lA, tz = tz, lD = lD, lg = lg), data = idta, params = list(lA ~
+         mridXtmonstdyXsex - 1, lD ~ mridXftmonstdy + sex - 1,
+         tz ~ sex, lg ~ sex), start = Start, weights = varComb(varIdent(form = ~1 |
+         mridXsex), varPower(value = 1)))
+     save(init1, file = initfile)
+ } else load(initfile)
> tmp <- t(init1$Redundancy[[1]]$Eigens)
> tmp <- tmp[-grep("^lA", rownames(tmp)), ]
> round(tmp[, 1:7], digits = 2)
```

```
                             [,1] [,2] [,3] [,4] [,5] [,6] [,7]
CondIndex                    5.05 4.55 3.88 3.49 3.07 2.99 2.96
mu                           0.31 0.35 0.41 0.45 0.52 0.53 0.54
lD.mridXftmonstdy44485301:1  0.01 0.02 0.00 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:7  0.06 0.20 0.00 0.00 0.01 0.01 0.03
lD.mridXftmonstdy44233103:14 0.08 0.00 0.02 0.01 0.00 0.00 0.00
lD.mridXftmonstdy44233103:28 0.07 0.29 0.00 0.00 0.06 0.44 0.02
lD.mridXftmonstdy44233103:42 0.02 0.17 0.00 0.00 0.00 0.00 0.01
lD.mridXftmonstdy44233103:56 0.07 0.28 0.00 0.00 0.02 0.17 0.30
lD.mridXftmonstdy44233103:70 0.02 0.10 0.00 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:84 0.07 0.24 0.00 0.00 0.01 0.03 0.31
lD.mridXftmonstdy44233103:105 0.02 0.14 0.00 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:112 0.04 0.20 0.00 0.00 0.63 0.01 0.00
lD.mridXftmonstdy113638:14   0.12 0.00 0.14 0.64 0.00 0.00 0.00
lD.mridXftmonstdy113638:28   0.00 0.15 0.01 0.00 0.00 0.00 0.00
tz.(Intercept)               0.67 0.03 0.15 0.04 0.00 0.00 0.00
tz.sexM                      0.59 0.06 0.29 0.00 0.00 0.00 0.00
lg.(Intercept)               0.09 0.68 0.11 0.03 0.00 0.00 0.00
```

The above shows the results of a redundancy analysis, when sex differences in `lD` and `tz` are NOT calculated. When both are included, the largest condition number is over 700, and the corresponding direction contains only `lD.sexM` and `tz.sexM`. Retry, dropping the sex differences for these two variables:

```
> initfile <- paste("initvals-RBC-DR-2.RData", sep = "")
> if (!file.exists(initfile)) {
+     lA.start <- lm(I(log(cheact)) ~ mridXtmonstdyXsex - 1, data = CleanUp(subset(idta,
+         dose2 %in% 0)))
+     Start <- c(coef(lA.start), rep(3, nlevels(idta$mridXftmonstdy)),
+         -2, 0, log(1))
+     init2 <- GetInitialValues(cheact ~ tcmfn4(dose2, tmpstds,
+         lA = lA, tz = tz, lD = lD, lg = lg), data = idta, params = list(lA ~
+         mridXtmonstdyXsex - 1, lD ~ mridXftmonstdy - 1, tz ~
```

```
+         sex, lg ~ 1), start = Start, weights = varComb(varIdent(form = ~1 |
+         mridXsex), varPower(value = 1)))
+     save(init2, file = initfile)
+ } else load(initfile)
> tmp <- t(init2$Redundancy[[1]]$Eigens)
> tmp <- tmp[-grep("^lA", rownames(tmp)), ]
> round(tmp[, 1:7], digits = 2)
```

```
                              [,1]  [,2] [,3] [,4] [,5] [,6] [,7]
CondIndex                    49.95 34.21 9.79 8.72 4.83 2.99 2.99
mu                            0.04  0.06 0.21 0.23 0.42 0.67 0.67
lD.mridXftmonstdy44485301:1   0.29  0.61 0.02 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:7   0.00  0.00 0.00 0.00 0.00 0.00 0.77
lD.mridXftmonstdy44233103:14  0.00  0.00 0.00 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:28  0.00  0.00 0.00 0.00 0.00 0.77 0.00
lD.mridXftmonstdy44233103:42  0.03  0.00 0.00 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:56  0.96  0.00 0.00 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:70  0.00  0.00 0.00 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:84  0.18  0.77 0.03 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:105 0.72  0.00 0.00 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:112 0.98  0.01 0.00 0.00 0.00 0.00 0.00
lD.mridXftmonstdy113638:14    0.00  0.00 0.00 0.00 0.91 0.00 0.00
lD.mridXftmonstdy113638:28    0.24  0.71 0.03 0.02 0.00 0.00 0.00
tz.(Intercept)                0.01  0.92 0.00 0.07 0.00 0.00 0.00
tz.sexM                       0.00  0.66 0.13 0.20 0.00 0.00 0.00
lg                            0.93  0.07 0.00 0.00 0.00 0.00 0.00
```

The inverse unscaled covariance matrix is numerically singular. Although the nominal estimate of `tz` is around 6 (!), adjusting it manually to lower values does not change the fit very much. Try again, setting `tz` to -10, fitting the model above:

```
> formals(tcmfn4)$tz <- -10
> initfile <- paste("initvals-RBC-DR-3.RData", sep = "")
> if (!file.exists(initfile)) {
+     lA.start <- lm(I(log(cheact)) ~ mridXtmonstdyXsex - 1, data = CleanUp(subset(idta,
+         dose2 %in% 0)))
+     Start <- c(coef(lA.start), rep(3, nlevels(idta$mridXftmonstdy)),
+         log(1))
+     init3 <- GetInitialValues(cheact ~ tcmfn4(dose2, tmpstds,
+         lA = lA, lD = lD, lg = lg), data = idta, params = list(lA ~
+         mridXtmonstdyXsex - 1, lD ~ mridXftmonstdy - 1, lg ~
+         1), start = Start, weights = varComb(varIdent(form = ~1 |
+         mridXsex), varPower(value = 1)))
+     save(init3, file = initfile)
+ } else load(initfile)
> tmp <- t(init3$Redundancy[[1]]$Eigens)
> tmp <- tmp[-grep("^lA", rownames(tmp)), ]
> round(tmp[, 1:7], digits = 2)
```

```
                              [,1] [,2] [,3] [,4] [,5] [,6] [,7]
CondIndex                     5.04 4.10 3.74 2.44 2.44 2.44 2.38
mu                            0.31 0.38 0.42 0.64 0.64 0.64 0.65
lD.mridXftmonstdy44485301:1   0.08 0.07 0.00 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:7   0.00 0.00 0.00 0.06 0.73 0.00 0.00
lD.mridXftmonstdy44233103:14  0.00 0.00 0.00 0.00 0.00 0.00 0.00
```

```
lD.mridXftmonstdy44233103:28  0.00 0.00 0.00 0.00 0.00 0.80 0.00
lD.mridXftmonstdy44233103:42  0.00 0.00 0.00 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:56  0.00 0.00 0.00 0.73 0.06 0.00 0.00
lD.mridXftmonstdy44233103:70  0.00 0.00 0.00 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:84  0.21 0.20 0.00 0.00 0.00 0.00 0.19
lD.mridXftmonstdy44233103:105 0.00 0.00 0.00 0.00 0.00 0.00 0.00
lD.mridXftmonstdy44233103:112 0.36 0.31 0.00 0.00 0.00 0.00 0.25
lD.mridXftmonstdy113638:14    0.00 0.00 0.91 0.00 0.00 0.00 0.00
lD.mridXftmonstdy113638:28    0.78 0.18 0.00 0.00 0.00 0.00 0.00
lg                            0.53 0.36 0.00 0.00 0.00 0.00 0.00
```

Most of the estimates of `lD` are quite large, ranging from about 5 to about 15 (one estimate in 113638 after 28 days is only 2.44), after preliminary optimization. This corresponds to doses yielding 10% inhibition ranging from about 150 to over 3 million (in study 113638 after 28 days, the BMD is about 11). The highest dose in any of these studies is 110 mg/kg (in 44485301).

We may end up being interested mainly in the lower bound of the `lD` estimate. Fit the simplest version of the model possible:

```
> formals(tcmfn4)$lg <- 0
> Parm <- init3$start$beta
> Start0 <- c(getParms("^lA", Parm), getParms("^lD", Parm))
> Start <- list(fixed = Start0)
> icnt <- 1
> Maxcnt <- 20
> repeat {
+     drmod1 <- try(nlme(cheact ~ tcmfn4(dose2, tmpstds, lA = lA,
+         lD = lD), data = idta, fixed = list(lA ~ mridXtmonstdyXsex -
+         1, lD ~ mridXftmonstdy - 1), start = Start, random = lA ~
+         1 | id), silent = TRUE)
+     if (!inherits(drmod1, "try-error") || icnt > Maxcnt) {
+         if (icnt <= Maxcnt)
+             writeLines(paste("Success after", icnt, "trie(s)."))
+         else writeLines(paste("Maxcnt,", Maxcnt, "exceeded"))
+         break
+     }
+     Start <- list(fixed = Start0 * (1 + rnorm(length(Start0),
+         sd = 0.2)))
+     icnt <- icnt + 1
+ }
```

That fails with the error:

```
Error: Singularity in backsolve at level 0, block 1
```

It seems likely that there is simply no dose-response here. To check this, fit an exponential model, with no transformation or reparameterization to force the slope to be negative:

```
> drfn <- deriv(~exp(lA) * exp(-m * x), c("lA", "m"), c("x", "lA",
+     "m"))
> Parm <- init3$start$beta
> Start0 <- c(getParms("^lA", Parm), rep(0, nlevels(idta$mridXftmonstdy)))
> Start <- list(fixed = Start0)
> icnt <- 1
> Maxcnt <- 20
> repeat {
```

```
+     drmod2 <- try(nlme(cheact ~ drfn(dose2, lA = lA, m = m),
+         data = idta, fixed = list(lA ~ mridXtmonstdyXsex - 1,
+             m ~ mridXftmonstdy - 1), start = Start, weights = varIdent(form = ~1 |
+             mrid), random = lA ~ 1 | id), silent = TRUE)
+     if (!inherits(drmod2, "try-error") || icnt > Maxcnt) {
+         if (icnt <= Maxcnt)
+             writeLines(paste("Success after", icnt, "trie(s)."))
+         else writeLines(paste("Maxcnt,", Maxcnt, "exceeded"))
+         break
+     }
+     Start <- list(fixed = Start0 * (1 + rnorm(length(Start0),
+         sd = 0.2)))
+     icnt <- icnt + 1
+ }

Success after 1 trie(s).
```

Are the estimates of m different from each other?

```
> Parms <- getParms("^m", fixed.effects(drmod2))
> L <- diag(length(Parms)) - matrix(1, nrow = length(Parms), ncol = length(Parms))
> colnames(L) <- names(Parms)
> L <- L[-nrow(L), ]
> anova(drmod2, L = L)

F-test for linear combination(s)
   m.mridXftmonstdy44485301:1 m.mridXftmonstdy44233103:7
1                           0                         -1
2                          -1                          0
3                          -1                         -1
4                          -1                         -1
5                          -1                         -1
6                          -1                         -1
7                          -1                         -1
8                          -1                         -1
9                          -1                         -1
10                         -1                         -1
11                         -1                         -1
   m.mridXftmonstdy44233103:14 m.mridXftmonstdy44233103:28
1                           -1                          -1
2                           -1                          -1
3                            0                          -1
4                           -1                           0
5                           -1                          -1
6                           -1                          -1
7                           -1                          -1
8                           -1                          -1
9                           -1                          -1
10                          -1                          -1
11                          -1                          -1
   m.mridXftmonstdy44233103:42 m.mridXftmonstdy44233103:56
1                           -1                          -1
2                           -1                          -1
3                           -1                          -1
4                           -1                          -1
```

```
5                            0                           -1
6                           -1                            0
7                           -1                           -1
8                           -1                           -1
9                           -1                           -1
10                          -1                           -1
11                          -1                           -1
   m.mridXftmonstdy44233103:70 m.mridXftmonstdy44233103:84
1                           -1                           -1
2                           -1                           -1
3                           -1                           -1
4                           -1                           -1
5                           -1                           -1
6                           -1                           -1
7                            0                           -1
8                           -1                            0
9                           -1                           -1
10                          -1                           -1
11                          -1                           -1
   m.mridXftmonstdy44233103:105 m.mridXftmonstdy44233103:112
1                            -1                            -1
2                            -1                            -1
3                            -1                            -1
4                            -1                            -1
5                            -1                            -1
6                            -1                            -1
7                            -1                            -1
8                            -1                            -1
9                             0                            -1
10                           -1                             0
11                           -1                            -1
   m.mridXftmonstdy113638:14 m.mridXftmonstdy113638:28
1                         -1                        -1
2                         -1                        -1
3                         -1                        -1
4                         -1                        -1
5                         -1                        -1
6                         -1                        -1
7                         -1                        -1
8                         -1                        -1
9                         -1                        -1
10                        -1                        -1
11                         0                        -1
  numDF denDF  F-value p-value
1    11   486 1.080941  0.3746
```

No. Is the average value of `m` different from 0?

```
> L <- matrix(1, nrow = 1, ncol = length(Parms))
> colnames(L) <- names(Parms)
> anova(drmod2, L = L)
```

```
F-test for linear combination(s)
  m.mridXftmonstdy44485301:1   m.mridXftmonstdy44233103:7
                           1                            1
```

```
 m.mridXftmonstdy44233103:14  m.mridXftmonstdy44233103:28
                           1                            1
 m.mridXftmonstdy44233103:42  m.mridXftmonstdy44233103:56
                           1                            1
 m.mridXftmonstdy44233103:70  m.mridXftmonstdy44233103:84
                           1                            1
m.mridXftmonstdy44233103:105 m.mridXftmonstdy44233103:112
                           1                            1
   m.mridXftmonstdy113638:14    m.mridXftmonstdy113638:28
                           1                            1
  numDF denDF   F-value p-value
1     1   486 0.4942634  0.4824
```

No. Refit the model with a single value of m. Calculate upper confidence bounds on `m` to give lower confidence bounds on BMD.

```
> Parm <- fixed.effects(drmod2)
> Start0 <- c(getParms("^lA", Parm), mean(getParms("^m", Parm)))
> Start <- list(fixed = Start0)
> icnt <- 1
> Maxcnt <- 20
> repeat {
+     drmod3 <- try(nlme(cheact ~ drfn(dose2, lA = lA, m = m),
+         data = idta, fixed = list(lA ~ mridXtmonstdyXsex - 1,
+             m ~ 1), start = Start, weights = varIdent(form = ~1 |
+             mrid), random = lA ~ 1 | id), silent = TRUE)
+     if (!inherits(drmod3, "try-error") || icnt > Maxcnt) {
+         if (icnt <= Maxcnt)
+             writeLines(paste("Success after", icnt, "trie(s)."))
+         else writeLines(paste("Maxcnt,", Maxcnt, "exceeded"))
+         break
+     }
+     Start <- list(fixed = Start0 * (1 + rnorm(length(Start0),
+         sd = 0.2)))
+     icnt <- icnt + 1
+ }
```

```
Success after 1 trie(s).
```

There is a simple relationship between the parameter `m` in the previous model and the BMD for a $100 \times BMR\%$ inhibition of AChE:

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

If we substitute an upper confidence limit for `m` in this formula, the result will be a *lower* confidence limit for `BMD`. Thus, the lower end of a one-sided 95% confidence bound for BMD is:

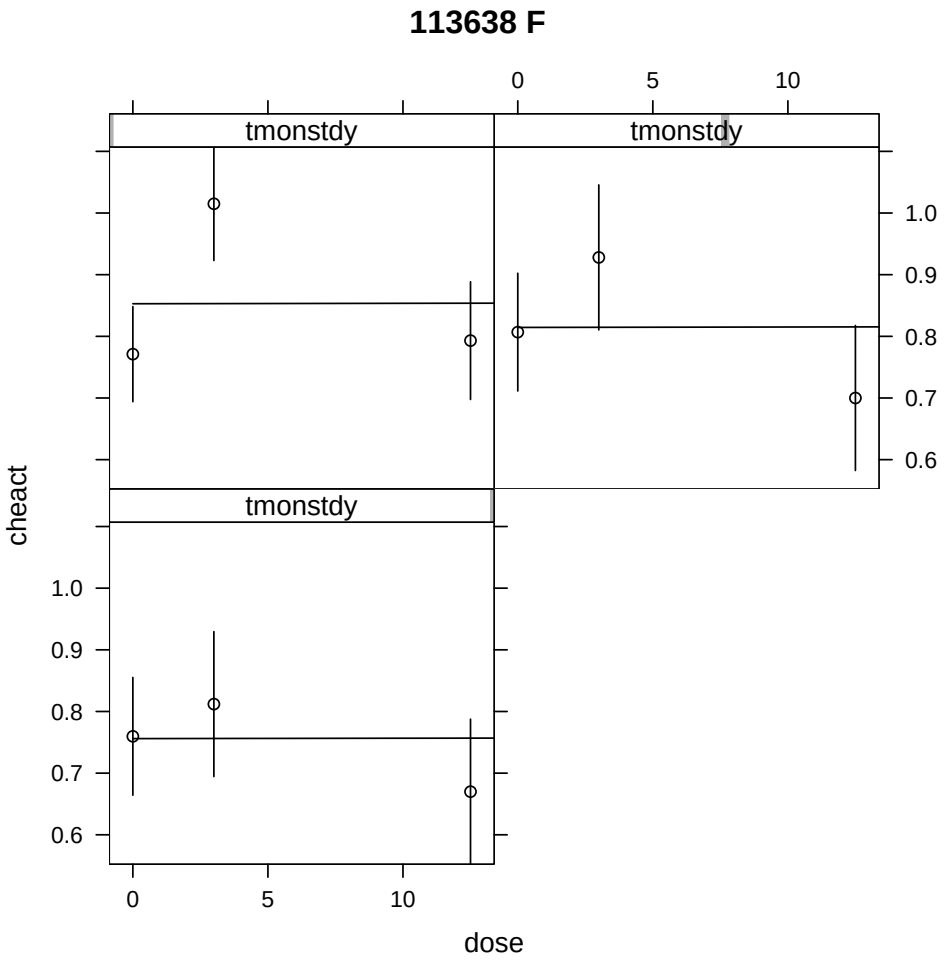
```
> Ints <- intervals(drmod3, type = "fixed", level = 0.9)$fixed
> BMDL <- -log(0.9)/Ints["m", "upper"]
```

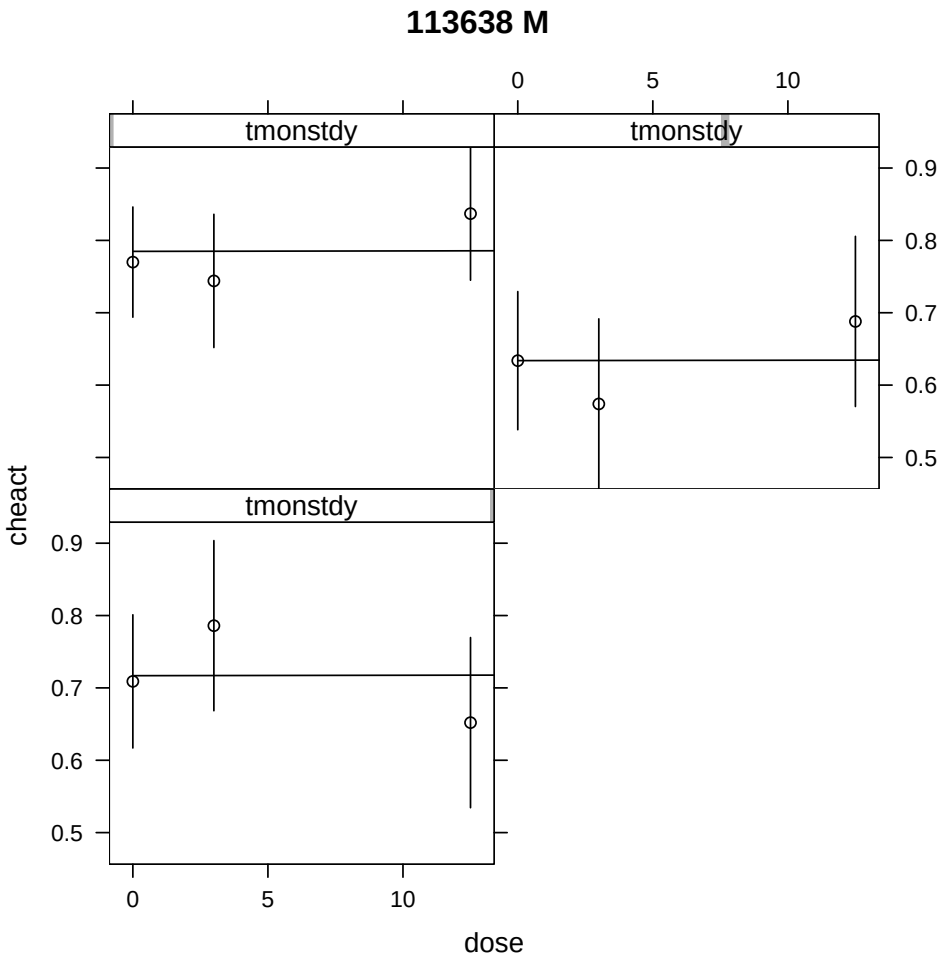
This gives a BMDL of 930.

Diagnostic plots for this model:

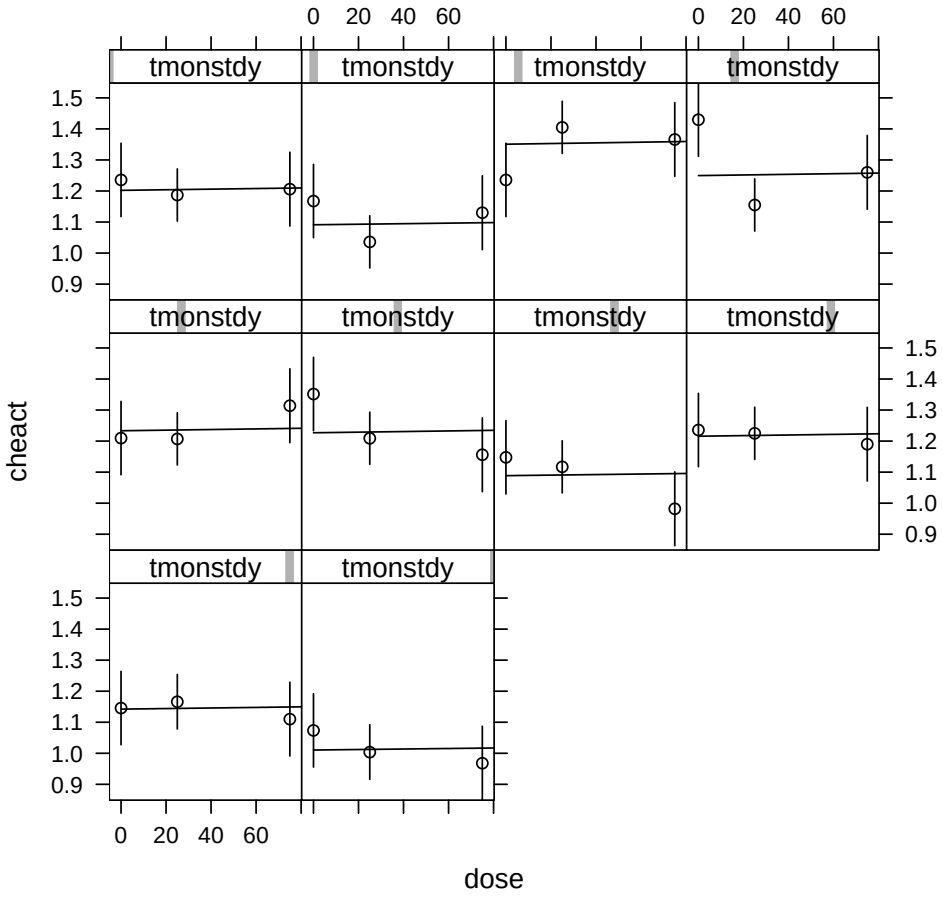
Data and dose-response curve (each panel reflects a different mrid by sex combination, labeled on the top margin). First, set up the fitted values for the curve plots:

```
> tdta <- with(idta, {
+     mridXtmonstdyXsexXdose <- interaction(mridXtmonstdyXsex,
+         dose, drop = TRUE, sep = ":")
+     tmp <- lme(cheact ~ mridXtmonstdyXsexXdose - 1, random = ~1 |
+         id, weights = varIdent(form = ~1 | mrid))
+     Ints <- intervals(tmp, which = "fixed")$fixed
+     nms <- gsub("mridXtmonstdyXsexXdose", "", rownames(Ints))
+     sstr <- strsplit(nms, ":")
+     data.frame(cheact = Ints[, "est."], llim = Ints[, "lower"],
+         ulim = Ints[, "upper"], mrid = factor(sapply(sstr, function(x) x[1])),
+         tmonstdy = as.numeric(sapply(sstr, function(x) x[2])),
+         sex = factor(sapply(sstr, function(x) x[3])), dose = as.numeric(sapply(sstr,
+             function(x) x[4])))
+ })
> tdta$mridXtmonstdyXsex <- with(tdta, interaction(mrid, tmonstdy,
+     sex, drop = TRUE, sep = ":"))
> tmp <- drmod3
> tmp$contrasts <- NULL
> ndta <- expand.grid(mridXtmonstdyXsex = levels(tdta$mridXtmonstdyXsex),
+     dose2 = seq(0, max(tdta$dose), length = 101))
> sstr <- strsplit(levels(ndta$mridXtmonstdyXsex), ":")
> ndta$mrid <- factor(sapply(sstr, function(x) x[1]))
> ndta$tmonstdy <- as.numeric(sapply(sstr, function(x) x[2]))
> ndta$sex <- factor(sapply(sstr, function(x) x[3]))
> ndta$pred <- predict(tmp, newdata = ndta, level = 0)
```

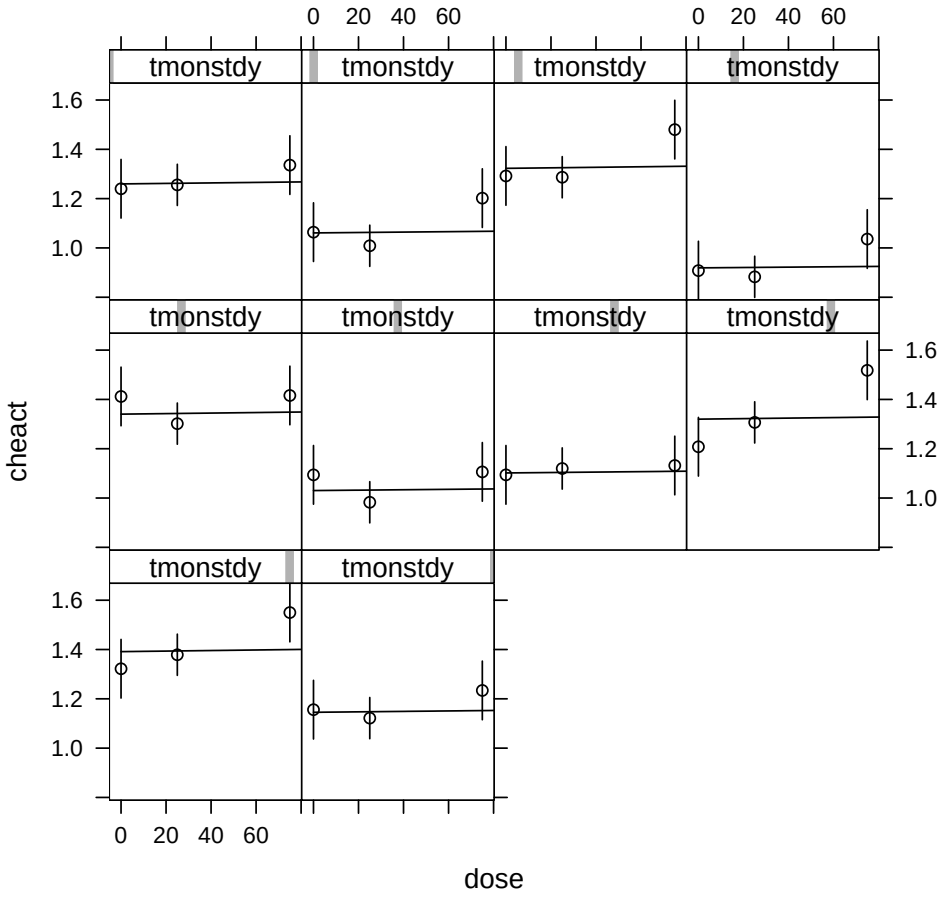

113638 F
tmonstdy
tmonstdy
tmonstdy
cheact
dose
0
5
10
1.0
0.9
0.8
0.7
0.6


113638 M
tmonstdy
tmonstdy
tmonstdy
cheact
dose
0
5
10
0.5
0.6
0.7
0.8
0.9

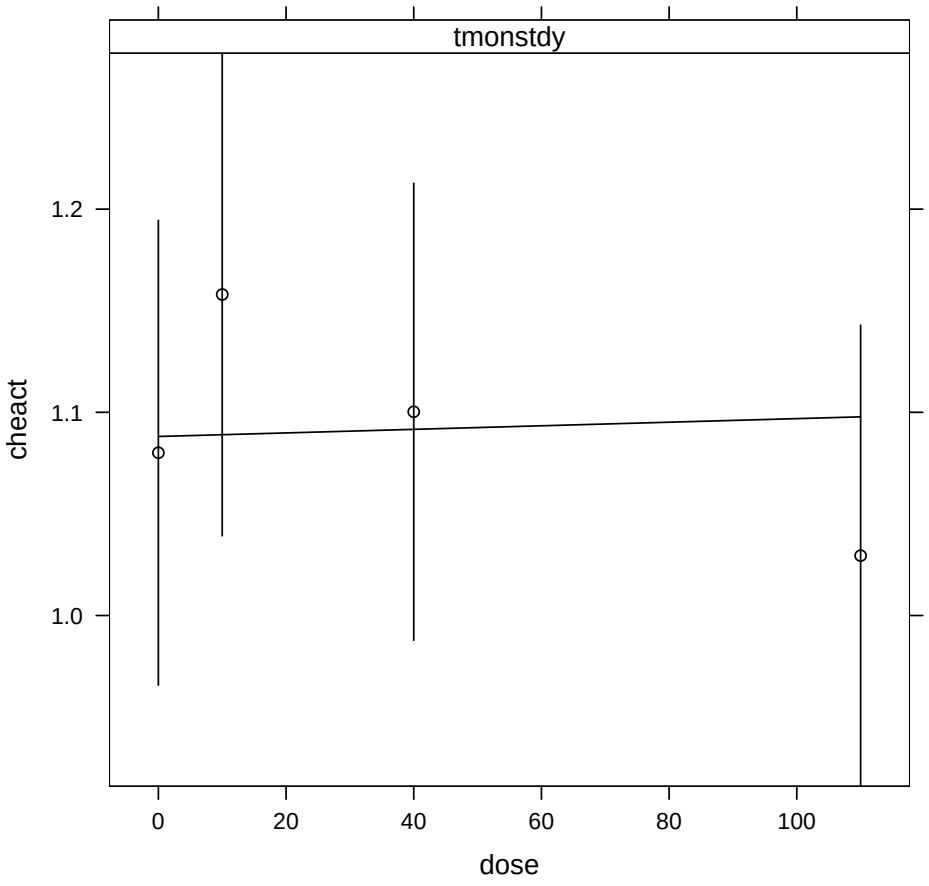
44233103 F



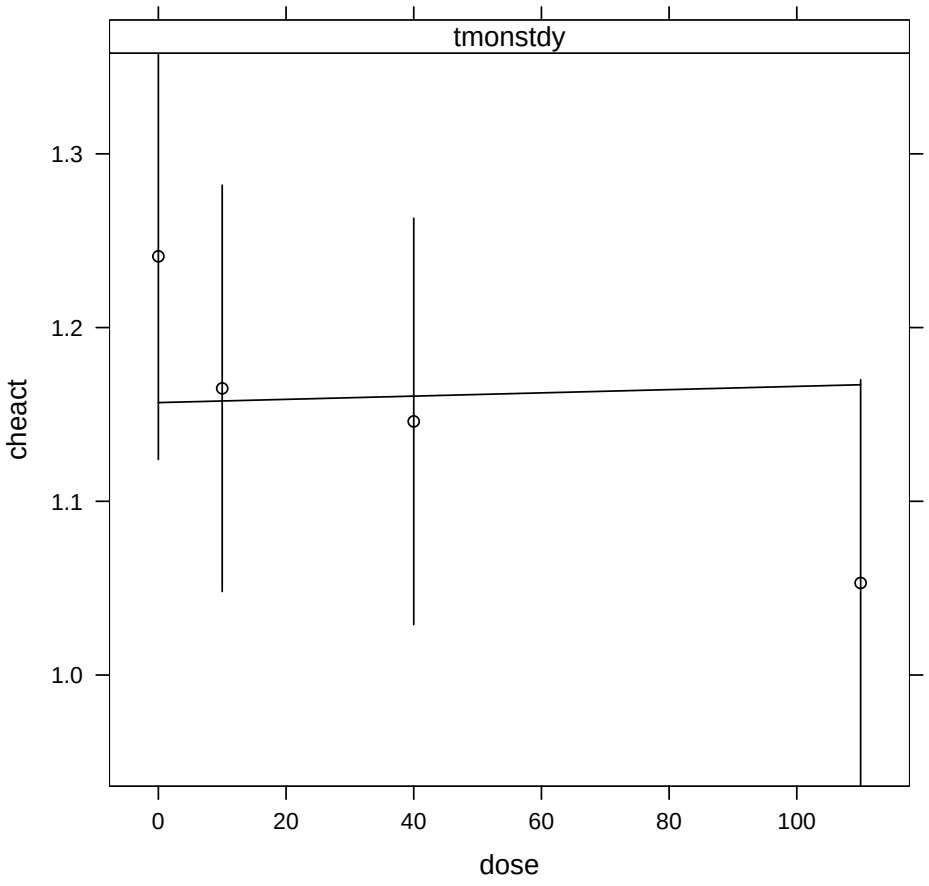
44233103 M

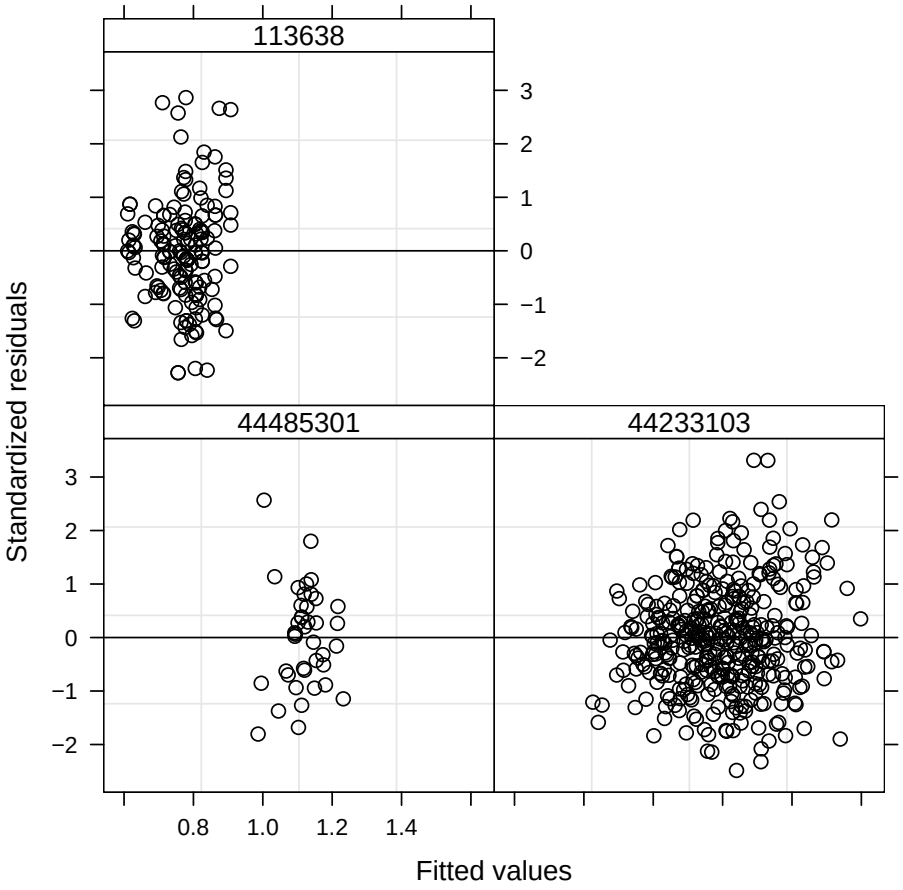


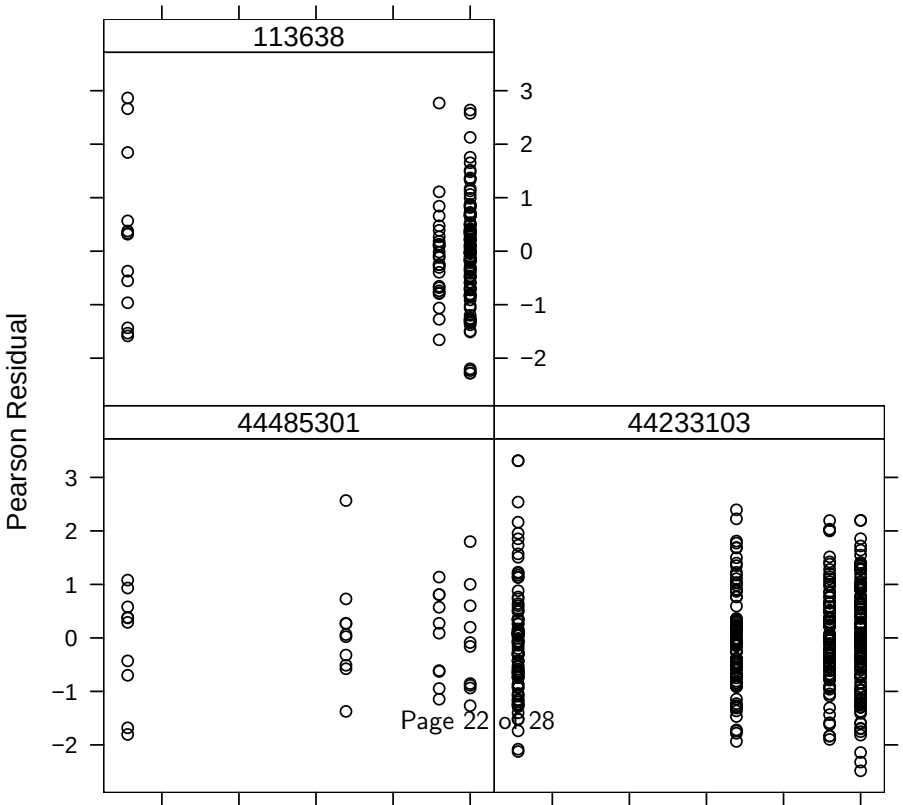
44485301 F

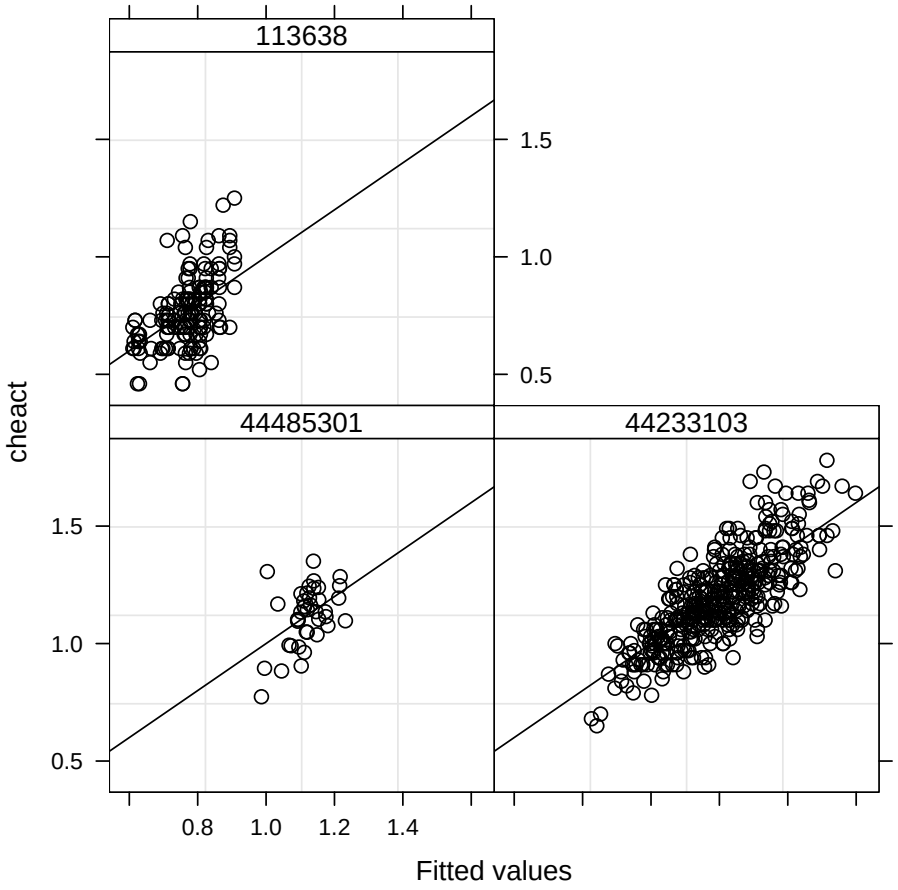


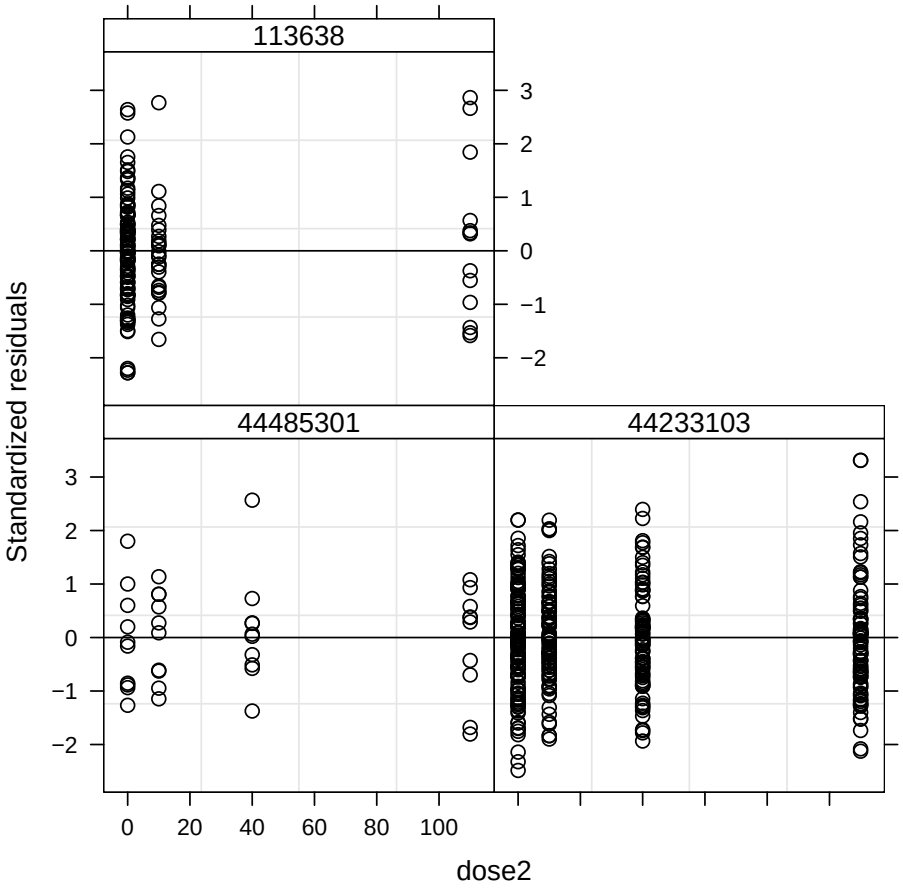
44485301 M

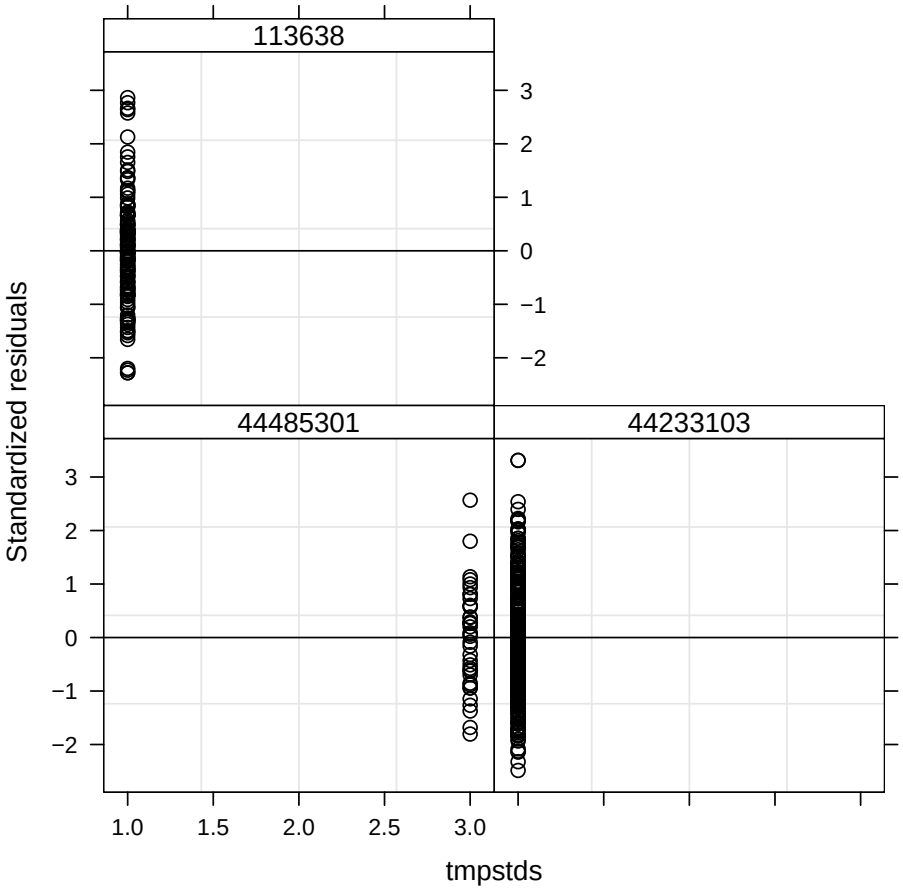



113638
44485301
44233103
Standardized residuals
Fitted values
0.8
1.0
1.2
1.4
3
2
1
0
−1
−2


113638
44485301
44233103
Pearson Residual
3
2
1
0
−1
−2

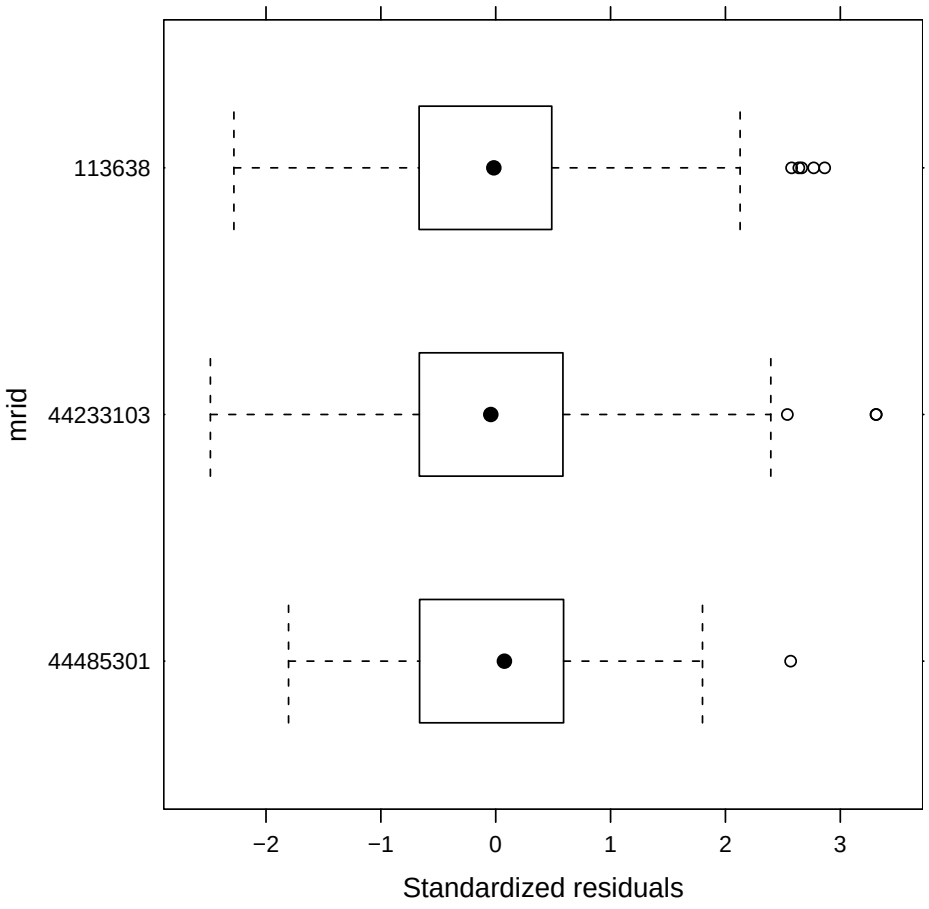

113638
44485301
44233103
cheact
Fitted values
0.5
1.0
1.5
0.8
1.2
1.4

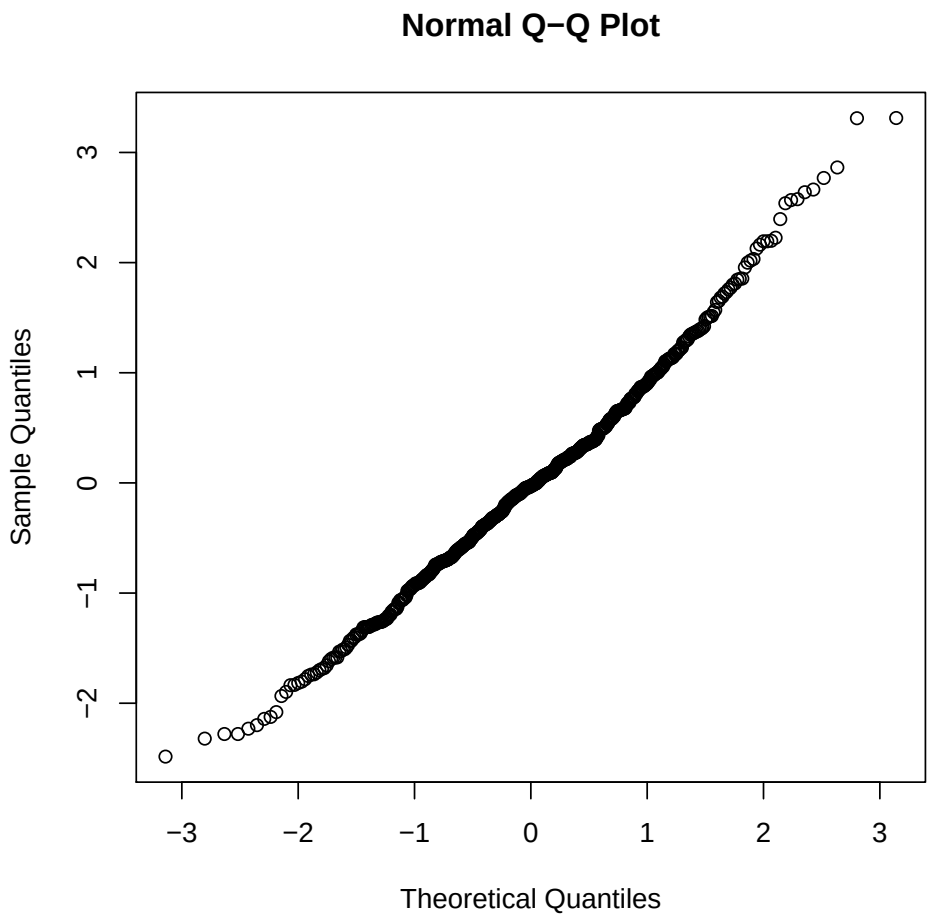

113638
44485301
44233103
Standardized residuals
dose2
3
2
1
0
−1
−2
0
20
40
60
80
100

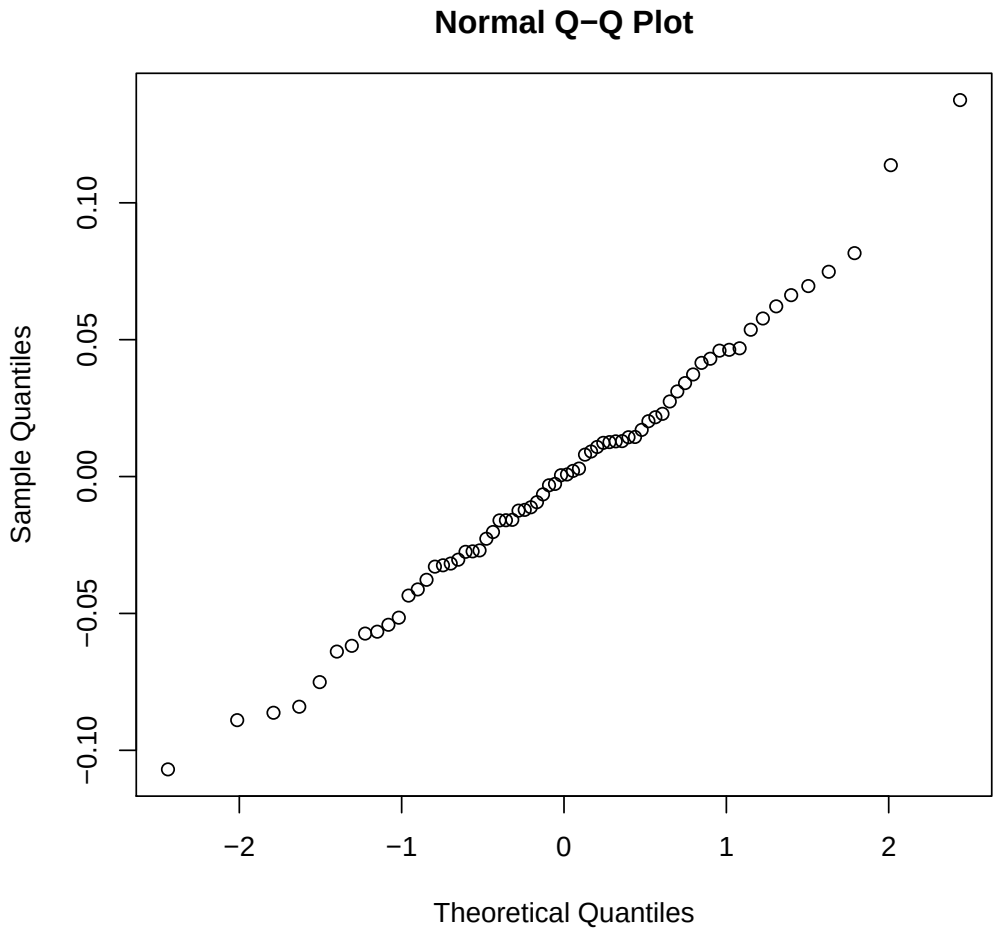


Some more graphs:

Pearson residuals split by mrid and sex, and QQ plots for the Pearson residuals and random effects:


113638
44233103
44485301
mrid
−2
−1
0
1
2
3
Standardized residuals


Normal Q−Q Plot
Sample Quantiles
Theoretical Quantiles
−3
−2
−1
0
1
2
3



3 Summary

There is no relationship in these studies between RBC AChE activity and primicarb dose. The 95% lower bound on the benchmark dose for 10% RBC AChE inhibition is (with lots of superfluous significant digits) 929.648594472188.

species RAT

mrid [1] "44485301" "44233103" "113638"

Save the results:

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

Save the results for incorporating into a database:

```
> primicarb.oral.rbc <- list(mrid = levels(idta$mrid), species = "RAT",
+     BMDs = list(combined = list(BMDL = BMDL)))
> save(primicarb.oral.rbc, file = file.path("..", "..", "01Summaries",
+     "primicarb.oral.rbc.RData"))
```